# Age at Establishment of Chronic Hepatitis B Infection as a Risk Factor for Persistent Viral Replication, Liver Fibrosis and Hepatocellular Carcinoma in The Gambia, West Africa

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I, Yusuke Shimakawa, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Date 30 November 2014

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# Abstract

Early age at hepatitis B virus (HBV) infection is known to increase the risk of chronic HBV (CHB) infection. This thesis investigated whether, in addition to increasing the risk of chronicity, early age at HBV infection further increases the risk of hepatocellular carcinoma (HCC) by maintaining high viral replication. A systematic review of observational studies suggested that early age at HBV infection might increase the risk of sequelae of CHB infection. However, there was no data from Africa. A project was therefore designed to explore the effect of age at HBV infection on HCC and its predictors in The Gambia, West Africa, using two proxy variables for the age at infection: birth order and maternal HBV sero-status.

A historical cross-sectional study of children born to HBeAg-positive mothers and HBeAg-negative mothers found that having an HBeAg-positive mother is associated with higher risk of positive HBeAg in children. The distribution of birth order was compared between HBV-related HCC cases and HBsAg-positive controls using a historical case-control study and data from the PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa) project. The former study did not find statistically significant association whilst the latter found an inverse association between birth order and HCC, suggesting that chronic carriers with low birth order might have an increased risk of HCC. Finally, an open community cohort study of HBsAg-positive people in three Gambian villages found that having an HBsAg-positive mother is associated with a number of factors predictive of disease (delayed HBeAg seroclearance, high HBV DNA and alanine transaminase levels over time, active CHB disease, significant liver fibrosis and condition requiring antiviral treatment). These findings suggest that interrupting

perinatal mother-to-infant transmission might significantly reduce the burden of liver disease associated with CHB infection in The Gambia.

# Table of contents

**Chapter 1. Background: epidemiology of chronic hepatitis B virus infection** ..... 12

**1.1. Global burden of hepatitis B virus infection, liver cirrhosis and liver cancer**..... 12

**1.2. Serological markers of HBV infection** ..... 15

**1.3. Modes of transmission** ..... 16

**1.4. Natural history of chronic hepatitis B infection** ..... 32

**1.5. Hepatitis B vaccines** ..... 47

**1.6. Factors modifying the progression of liver disease in individuals with chronic hepatitis B infection** ..... 61

**1.7. The effect of early age at hepatitis B virus infection on the risk of hepatocellular carcinoma and its predictors**..... 69

**Chapter 2. The use of birth order in the epidemiology** ..... 72

**2.1. Introduction**..... 72

**2.2. Definition of birth order** ..... 73

**2.3. Design of birth order study** ..... 77

**2.4. Potential confounding factors for the association of birth order with a disease** ..... 77

**2.5. Association between higher birth order and lower age at infection with common pathogens** ..... 83

**2.6. Use of birth order in cancer epidemiology**..... 85

**2.7. Birth order and the epidemiology of hepatitis B virus infection in The Gambia**..... 87

**2.8. Summary**..... 89

**Chapter 3. Association of early age at establishment of chronic hepatitis B infection with persistent viral replication, liver cirrhosis and hepatocellular carcinoma: a systematic review** ..... 90

**3.1. Abstract**..... 90

**3.2. Introduction**..... 92

**3.3. Methods**..... 92

**3.4. Results** ..... 95

**3.5. Discussion**..... 108
**3.6. Summary**..... 112
**Chapter 4. Association between perinatal mother-to-infant transmission of hepatitis B virus and the risk of hepatitis B e antigenaemia in children: a cross-sectional study in The Gambia, West Africa** ..... 113
**4.1. Abstract**..... 113
**4.2. Introduction**..... 115
**4.3. Methods**..... 116
**4.4. Results**..... 125
**4.5. Discussion**..... 131
**4.6. Summary**..... 134
**Chapter 5. The association of birth order with hepatocellular carcinoma and liver cirrhosis in people with chronic hepatitis B infection: a secondary data analysis of the Gambia Liver Cancer Study (GLCS), a case-control study in the Gambia, West Africa** 136
**5.1. Abstract**..... 136
**5.2. Introduction**..... 138
**5.3. Methods**..... 138
**5.4. Results**..... 144
**5.5. Discussion**..... 154
**5.6. Summary**..... 156
**Chapter 6. The natural history of chronic hepatitis B infection in The Gambia, West Africa: a longitudinal population-based study of chronic carriers in three rural villages**157
**6.1. Abstract**..... 157
**6.2. Introduction**..... 159
**6.3. Methods**..... 163
**6.4. Results**..... 178
**6.5. Discussion**..... 217
**6.6. Summary**..... 221
**Chapter 7. The association between birth order and hepatocellular carcinoma in people with chronic hepatitis B infection: a case-control study in the Gambia, West Africa**..... 223

**7.1. Abstract** ... 223
**7.2. Introduction** ... 226
**7.3. Methods** ... 228
**7.4. Results** ... 252
**7.5. Discussion** ... 295
**7.6. Summary** ... 310
**Chapter 8. Discussion** ... 312
**8.1. Key results** ... 312
**8.2. Replication-infection cycle** ... 314
**8.3. Limitations in the interpretation** ... 314
**8.4. Public health implications** ... 316
**References** ... 317
**Appendix 1. Search strategy** ... 349
**Appendix 2. Protocol for the systematic review** ... 361
**Appendix 3. Information included in the Standardised Data Extraction Sheet** ... 365
**Appendix 4. Framework for assessing the risk of bias in individual studies** ... 367
**Appendix 5. Description of risk of bias in included studies** ... 369
**Appendix 6. Poster used for the community sensitisation** ... 375
**Appendix 7. Questionnaire for birth order** ... 376

# List of abbreviations

| | |
|---|---|
| ALT | Alanine aminotransferase |
| Anti-HBc | Antibody against hepatitis B core antigen |
| Anti-HBe | Antibody against hepatitis B e antigen |
| Anti-HBs | Antibody against hepatitis B surface antigen |
| BMI | Body mass index |
| CHB | Chronic hepatitis B |
| DNA | Deoxyribo Nucleic Acid |
| DSS | Demographic Surveillance System |
| DTP | Diphtheria-tetanus-pertussis |
| EASL | European Association for the Study of the Liver |
| EIA | Enzyme immunoassay |
| ENCHB | Hepatitis B e antigen-negative chronic hepatitis B disease |
| EPCHB | Hepatitis B e antigen-positive chronic hepatitis B disease |
| ESLD | End-stage liver disease |
| GLCS | Gambia Liver Cancer Study |
| HBeAg | Hepatitis B e antigen |
| HBIG | Hepatitis B immunoglobulin |
| HBsAg | Hepatitis B surface antigen |
| HBV | Hepatitis B virus |
| HCC | Hepatocellular carcinoma |
| IARC | International Agency for Research on Cancer |
| IC | Inactive HBsAg carrier |

| | |
|---|---|
| IC | Immunochromatography |
| IT | Immune toleratnt phase |
| LC | Liver cirrhosis |
| LOWESS | Locally weighted regression |
| LSM | Liver stiffness measurement |
| MEIA | Microparticle enzyme immunoassay |
| MRC | Medical Research Council |
| OR | Odds ratio |
| PCR | Polymerase chain reaction |
| PROLIFICA | Prevention of Liver Fibrosis and Cancer in Africa |
| q-PCR | Quantitative real-time polymerase chain reaction |
| RIA | Radioimmunoassay |
| RCT | Randomised-controlled trial |
| RPHA | Reverse passive haemagglutination assay |
| RR | Relative risk |
| sSA | Sub-Saharan Africa |
| VL | Viral load |
| WHO | World Health Organisation |
| 95% CI | 95% confidence interval |

# Chapter 1. Background: epidemiology of chronic hepatitis B virus infection

## 1.1. Global burden of hepatitis B virus infection, liver cirrhosis and liver cancer

Globally, hepatitis B virus (HBV) infection is one of the most prevalent viral infections. More than two billion people who are currently alive have been infected,[1] of whom 240 million are chronically infected.[2] The global prevalence of chronic HBV infection is estimated to be 3.9% in males and 3.5% in females in 2005.[2]

The Global Burden of Disease, Injuries, and Risk Factors Study estimated that 1,030,800 and 752,100 people died of liver cirrhosis and liver cancer in 2010, respectively.[3] The age-standardised death rate of liver cirrhosis was 15.6 per 100,000 and liver cancer was 11.5 per 100,000. Liver cirrhosis ranks 12$^{th}$ and liver cancer 16$^{th}$ amongst 235 causes of death, and the latter is the 3$^{rd}$ commonest cause of cancer death, after lung and stomach.[3]

HBV infection accounts for 30.3% of liver cirrhosis deaths and 45.4% of liver cancer deaths. Overall, 786,000 people were estimated to have died in 2010 from HBV-related liver diseases including acute/fulminant hepatitis, liver cirrhosis and liver cancer, which makes HBV infection as a 15$^{th}$ ranked cause of death.[3]

The prevalence of chronic hepatitis B (CHB) infection in adults in 2005 varies considerably between countries (figure 1.1); low prevalence (<2%) is seen in North America and Western Europe, low intermediate (2-4%) in the Middle East, the Indian subcontinent and Japan, high intermediate (5-7%) in China, Southeast Asia, Eastern and Southern Africa and high (≥8%) in Middle and Western Africa.[2] Because CHB infection is responsible for about half of liver cancer worldwide, the prevalence of CHB infection and incidence of liver cancer are positively correlated (figure 1.1 and 1.2).[2,4] Low incidence is seen in the USA and Western Europe while the incidence is high in Eastern and Southeast Asia, and Middle and Western Africa. In Africa, liver cancer is the leading cause of cancer death in men and the third most frequent cause in women.[5]

Figure 1.1 Prevalence of hepatitis B infection, adults 19-49 years, 2005[2]

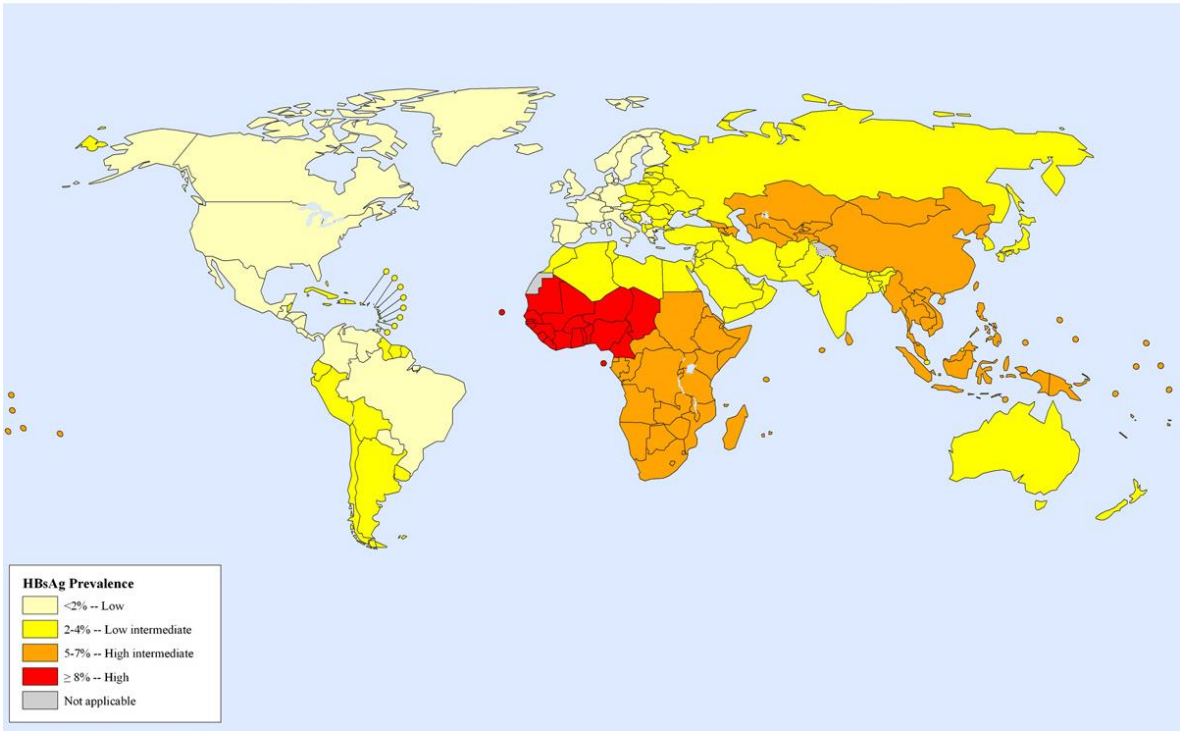
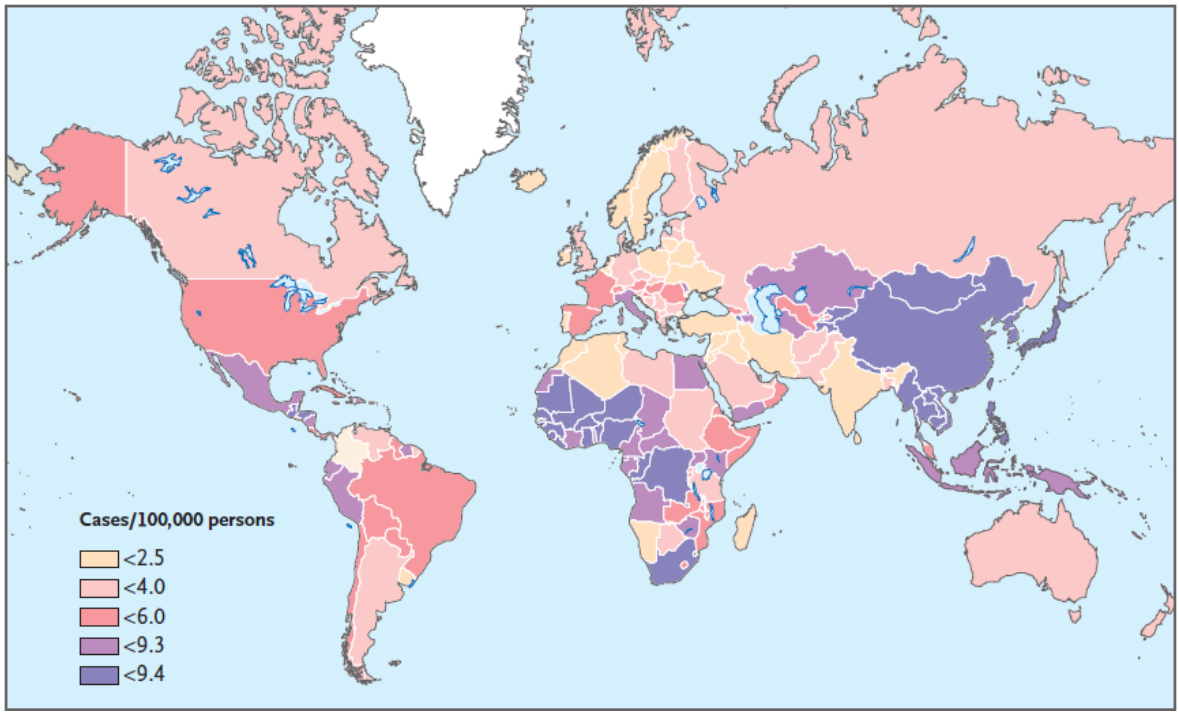



Figure 1.2 Age-standardised incidence rates of liver cancer, 2008[4]



## 1.2. Serological markers of HBV infection

There are six important serological markers of HBV infection: hepatitis B surface antigen (HBsAg) and its antibody (anti-HBs), hepatitis B e antigen (HBeAg) and its antibody (anti-HBe), and two classes of antibody against hepatitis B core antigen (anti-HBc IgG and anti-HBc IgM).[6] A positive serum HBsAg indicates an active HBV infection. The infection is chronic if HBsAg persists for longer than six months, otherwise the infection is acute. HBsAg is the first serological marker that appears during acute HBV infection and it is followed by anti-HBc IgM, which usually disappears several months after the acute infection and therefore indicates recent infection. After the clearance of HBsAg, anti-HBs appears, which usually confers a good prognosis with lifelong immunity. HBeAg is present early during acute infection, and disappears before the HBsAg seroclearance. It is associated with high viral replication (i.e., high HBV DNA levels). HBeAg loss is often followed by the appearance of anti-HBe. As anti-HBc IgG persists from the acute infection throughout one's life, this is a marker of past or present infection.

Chronic infection with HBV (CHB infection) differs from CHB disease. The latter is defined as "chronic necroinflammatory disease of the liver caused by persistent infection with HBV".[7] The terms: CHB, chronic HBV infection, hepatitis B carrier, and chronic carrier of HBsAg are used interchangeably in the literatures.

## 1.3. Modes of transmission

HBV is highly infectious and can be transmitted through skin or mucous membrane by exposure to infected blood or other body fluids (wound secretions, semen, vaginal fluid, or saliva).[8] Major modes of transmission include mother-to-infant, child-to-child, sexual, and contaminated needles, syringe or blood products.[8]

### 1.3.1. Mother-to-infant/child transmission

There are three mechanisms of transmission from infectious mothers to infants and children, two in the perinatal period and one in postnatal period: i) transplacental intrauterine transmission; ii) transmission during delivery through contact with infectious maternal fluids in the birth canal; and iii) postnatal transmission through a close contact between mothers and children.[9,10] Perinatal transmission usually occurs at the time of delivery and intrauterine transmission is relatively infrequent.[8,10] The term "vertical transmission" is defined as a transmission through germ cell lines, although it has sometimes been incorrectly used to indicate perinatal mother-to-infant transmission of HBV.[10]

#### *1.3.1.1. Intrauterine transmission*

An indicator of intrauterine transmission has not yet been established. Theoretically, the presence of HBV DNA in the liver tissues of newborns should accurately reflect intrauterine transmission. Indeed, a Chinese study reported that 8.3% (4/48) of foetuses from terminated pregnancies in HBsAg-positive mothers had evidence of intrauterine HBV infection (two with positive HBV DNA in liver tissue, one with HBsAg-positive liver tissue and one with HBsAg-positive cardiac blood).[11] However, liver tissue is

rarely obtained from newborns.[12] Studies often examine HBsAg or HBV DNA in either umbilical cord blood at delivery or peripheral blood of neonate at birth as an indicator of intrauterine transmission. These proxy measures are not perfect; the former has a risk of being contaminated by maternal blood,[12–14] and the latter may give a false-positive because the HBsAg particle can cross the placenta.[15] The risk of intrauterine transmission, indicated by positive-HBsAg in peripheral blood of newborns at birth, is 0-6% in neonates born to HBsAg-positive HBeAg-negative mothers and 7-15% in those born to HBeAg-positive mothers, after excluding extreme values reported in early studies[16–19] (table 1.1-a). Table 1.1-b also shows that higher levels of maternal HBV DNA are associated with higher chance of intrauterine transmission.

Positive HBeAg without HBsAg in cord blood or peripheral blood at birth is common in neonates born to HBeAg-positive mothers (90% in cord blood studies[20,21] and 40-60% in neonatal peripheral blood studies[22,23]). This condition usually represents transplacental passage of HBeAg. A spontaneous clearance of HBeAg usually occurs within 6 months after birth without resulting in infection, if the risk of postpartum transmission is eliminated by passive and active immunoprophylaxis (i.e., HBV vaccine and immunoglobulin).[23,12]

Table 1.1 Frequency of intrauterine transmission by maternal HBeAg status and maternal HBV DNA levels in infants born to HBsAg-positive mothers (case-control study was not included)

Table 1.1-a According to maternal HBeAg

| Author, year | Country | Study design | Type of sample | Type of outcome | Type of exposure | Prevalence of intrauterine transmission in exposed | Prevalence of intrauterine transmission in unexposed | Other factors associated with intrauterine infection |
|---|---|---|---|---|---|---|---|---|
| Wong VCW, 1980[16] | Hong Kong | Co | Cord blood | HBsAg | HBeAg | 46.8% (22/47) | 24.0% (12/50) | Longer 1st stage of labour associated, HBsAg in amniotic fluid not associated |
| Barin F, 1981[17] | Senegal | CS | Peripheral | HBsAg | HBeAg | 66.7% (4/6) | 7.1% (1/14) | N/R |
| Goudeau A, 1983[19] | Senegal | CS | Peripheral within 6 days | HBsAg | HBeAg | 30.7% (4/13) | 5.3% (2/38) | N/R |
| Marinier E, 1985[13] | Senegal | Co | Cord blood | HBsAg | HBeAg | 0% (0/26)[a] | 0% (0/106)[a] | N/R |
| Roingeard P, | Senegal | CS | Peripheral | HBsAg | HBeAg | 100% (2/2) | 10.5% (2/19)[b] | N/R |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1993[18] | | | | | | | | |
| Wang JS, 2000[22] | China | CS | Peripheral | HBsAg | HBeAg | 6.7% (1/15) | 0% (0/18) | N/R |
| Xu DZ, 2002[24] | China | CS | Peripheral | HBsAg | HBeAg | 9.8% (13/133) | 0.7% (2/269) | Maternal age not associated, threatened preterm labour associated |
| Wang Z, 2003[23] | China | Co | Peripheral | HBsAg | HBeAg | 9.1% (3/33) | 0% (0/21) | N/R |
| Shao ZJ, 2007,[25] 2011[26] | China | CS | Peripheral | HBsAg | HBeAg | 14.6% (6/41) | 2.5% (4/163) | Maternal age not associated, sexual intercourse in the 2nd trimester associated |
| Guo Z, 2013[27] | China | CS | Peripheral | HBsAg and/or HBV DNA | HBeAg | 13.9% (57/410) | 5.7% (36/633) | Maternal age not associated, menstrual irregularity, severe nausea in the 1st trimester and vaginal delivery associated |

Table 1.1-b According to maternal HBV DNA levels

| Author, year | Country | Study design | Type of sample | Type of outcome | Type of exposure ($log_{10}$ copies/ml) | Prevalence of intrauterine transmission in exposed | Prevalence of intrauterine transmission in unexposed | Other factors associated with intrauterine infection |
|---|---|---|---|---|---|---|---|---|
| Xu DZ, 2002[24] | China | CS | Peripheral | HBsAg | ≤5.8 | 0% (0/39) | N/A | Maternal age not associated, threatened preterm labour associated |
| | | | | | 5.8 – 6.8 | 1.4% (1/72) | | |
| | | | | | 6.8 – 7.5 | 3.5% (1/29) | | |
| | | | | | 7.5 – 8.5 | 0% (0/18) | | |
| | | | | | ≥8.5 | 22.2% (6/27) | | |
| Candotti D, 2007[28] | Ghana | CS | Cord blood | HBV DNA | Detection (≥5 $log_{10}$ copies/ml) | 10.8% (13/120) | 0% (0/7) | Pre-core wild-type at position 1896 associated |
| Shao ZJ, 2007,[25] 2011[26] | China | Co | Peripheral | HBsAg | Detection | 12.2% (7/57) | 2.1% (3/145) | Maternal age not associated, sexual intercourse in the 2nd trimester associated |
| | | | | | 3-5 | 5.0% (1/20) | N/A | |
| | | | | | 5-8 | 18.5% (5/27) | | |
| | | | | | >8 | 10.0% (1/10) | | |
| Xu WM, 2009[29] | China | RCT | Peripheral | HBsAg | ≥9 | 25.5% (14/55) | N/A | N/R |
| Guo Z, 2013[27] | China | CS | Peripheral | HBsAg and/or HBV DNA | 3-5 | 12.3% (64/518) | 6.1% (32/528) | Maternal age not associated, menstrual irregularity, severe |
| | | | | | ≤6 | 8.5% (15/177) | N/A | |
| | | | | | 6-8 | 13.9% (41/296) | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | >8 | 17.8% (8/45) | | nausea in the 1st trimester and vaginal delivery associated |

[a] Number of children exposed (having HBeAg-positive mothers) and unexposed (having HBeAg-negative HBsAg-positive mothers) was extrapolated from the prevalence of HBeAg reported in the same study.

[b] Excluding four subjects with suspected contamination at birth.

Abbreviations: CS, cross-sectional study; Co, cohort study; RCT, randomised-controlled trial; RIA, radioimmunoassays; RPHA, reverse passive haemagglutination; qPCR, real-time quantitative polymerase chain reaction; N/R, not reported.

*1.3.1.2. Transmission during delivery*

The risk of perinatal mother-to-infant transmission (including intrauterine infection) is largely determined by the maternal HBeAg status. The risk of infection (indicated by a positive-HBsAg at 3-12 months after the birth) ranges from 15-35% in children born to HBsAg-positive HBeAg-negative mothers to 80-100% in children born to HBeAg-positive mothers in East Asia.[16,30–33] Subsequent studies have confirmed that maternal HBV DNA levels are a more important risk factor for perinatal transmission than maternal HBeAg.[34,35]

In some countries, an elective caesarean section has been performed before the onset of labour or the rupture of membranes to prevent the mother-to-infant transmission. A recent meta-analysis of four randomised controlled trials of elective caesarean section versus vaginal delivery in HBsAg-positive pregnant women with positive HBV DNA ($>10^3$ copies/ml) confirmed that the risk of infection in their babies (indicated by a positive HBV-DNA in cord blood or neonatal peripheral blood at birth) was significantly lower in children born with caesarean section (10.5%) than in those born with vaginal delivery (28.0%).[36]

*1.3.1.3. Postnatal transmission from infectious mothers*

Beasley and Hwang prospectively followed up a cohort of Taiwanese children born to HBsAg-positive mothers. The children were given hepatitis B immunoglobulin at birth (which reduced the risk of perinatal transmission) but not hepatitis B vaccine (which made them susceptible to subsequent infection), and were not yet infected at 12 months of age. After an average of 17.5 months of additional follow-up, 67.6% (25/37) of children born to HBeAg-positive mothers became infected while 22.1% (15/68) of children born to

HBeAg-negative HBsAg-positive mothers infected. The association remained statistically significant after adjusting for the presence of an HBsAg-positive older sibling (a potential source of infection), whilst the association between having HBV infected older siblings and postnatal infection disappeared after controlling for the presence of an HBeAg-positive mother.[9] The study illustrated high postnatal infectivity of HBeAg-positive mothers in Taiwan. Nevertheless, in the absence of immunoprophylaxis, most of the children (>70-90%) born to HBeAg-positive mothers become HBsAg-positive by 3 months old.[16,37–39] Therefore, the role of postnatal transmission through HBeAg-positive mother is not large in East Asia.

Although the exact mechanism of postnatal maternal transmission is not known, the authors suggested that maternal saliva was a source of infection because the infant's food is often premasticated by the mother in Taiwan.[9] Breastfeeding was shown not to be a route of HBV transmission in a recent systematic review of intervention studies.[40]

1.3.2. Horizontal transmission during childhood

HBV can be horizontally transmitted during childhood in the absence of perinatal, sexual or parenteral transmission.[41] Horizontal transmission mainly occurs within the household from infectious mothers[9] or siblings,[42] however, it can also occur in preschool day-care facilities and schools.[41,43–45] For example, in Okinawa, Japan, 10 of 15 children identified to carry HBsAg by a sero-survey in nursery schools did not have any family member who carried HBsAg, but all 10 children had HBeAg-positive playmates in the classroom, suggesting that they might have acquired the infection at the nurseries.[43] Another study found HBV transmission between children attending the same day-care centre based on molecular evidence.[46]

The exact mechanism of horizontal transmission during childhood is not completely understood.[8,47] The most probable route includes exposure to blood or wound secretions through cutaneous sores, abrasions or mucous membranes.[8,47] Transmission can also occur following the contact with contaminated saliva through bites,[48,49] skin cuts[50] or skin that is not intact (e.g., chapped hands).[51] It is also possible that the transmission occurs through sharing objects such as towels, chewing gum, or toothbrushes,[52] because the virus can be found on objects without visible blood,[53] and can survive outside the body for at least one week.[54] It has also been proposed that blood-feeding arthropods might act as vectors for HBV transmission.[55] However, a randomised controlled trial of an intervention aimed at bedbug elimination in rural villages in The Gambia failed to reduce the incidence of HBV infection.[56]

### 1.3.3. Major mode of transmission and its geographical distribution

The prevalence of HBV infection is correlated with the major mode of HBV transmission in an area and therefore with the predominant age at which infection occurs.[8] In areas with the low prevalence, most HBV infections occur in young adults as a result of intravenous drug use or unprotected sex. In high-endemic areas such as sub-Saharan Africa (sSA) or Asia, transmission is predominantly in the perinatal period from infectious mother or during early childhood from one child to another.

#### *1.3.3.1. HBV transmission in East Asia*

In East Asia, perinatal maternal transmission is relatively common compared to other regions. In the pre-vaccine era 15% of pregnant women in Taiwan had HBsAg,[31,57] and 40% of children

born to these mothers became HBsAg-positive by the age of 3 months (table 1.2).[57] The risk of transmission was especially high when the mothers also carried HBeAg; in this case >70-90% of their children became sero-positive for HBsAg by 3 months in Taiwan[37,38] and in Hong Kong.[16,39]

*1.3.3.2. HBV transmission in sub-Saharan Africa*

Perinatal transmission plays less of a role in sSA than in East Asia, and most HBV infections occur during early childhood. In Senegal, Marinier *et al*. tested mothers for HBV sero-markers and followed their newborn infants unvaccinated for HBV and examined the risk of infection (positive HBsAg) at birth (cord blood), 1 week to 5 months, 5-12 months, 12-24 months and 24-38 months.[13] In mothers, the prevalence of HBsAg was 9.8% (141/1442), and 19.8% (23/116) of HBsAg-positive mothers also carried HBeAg. At birth, none of 132 children born to HBsAg-positive mother had HBsAg in their cord blood. The subsequent risks of the infection in those born to HBsAg-positive mothers were 0% (0/88), 3.8% (3/78), 10.9% (6/55), and 35.3% (6/17), in 1 week to 5 months, 5-12 months, 12-24 months and 24-38 months, respectively (table 1.2). In those born to HBsAg-negative mothers, the risks were 0% (0/374), 2.2% (4/186), 6.2% (6/97) and 14.3% (5/35), respectively. These imply that in Senegal most transmission occurs after 5 months of age, and the risk of postnatal transmission is higher in children born to HBsAg-positive mothers.

Likewise, a cohort study in Kenya followed 51 unvaccinated infants born to HBsAg-positive mothers (of whom 8% were also HBeAg-positive and 26% were HBV DNA positive);[58] by the age of 3 months, no infants were infected. By the age of 9 months, 25.0% (1/4) and 4.3% (2/47)

were infected in those born to HBeAg-positive and HBeAg-negative mothers, respectively. When stratified by maternal HBV DNA detection, 7.7% (1/13) born to mothers with positive HBV DNA and 5.3% (2/38) born to mothers with negative HBV DNA were infected by the age of 9 months. A cohort study in Tanzania also found that the infection by the age of 8 months was infrequent: 20.0% (3/15) in children born to HBeAg-positive mothers and 2.6% (1/38) in children born to HBsAg-positive HBeAg-negative mothers.[59]

The findings from these cohort studies are supported by a number of cross-sectional sero-surveys in sSA conducted before the introduction of hepatitis B vaccine. In West Africa (The Gambia, Senegal and Liberia), the HBsAg seroprevalence during the first 6 months of life was only 0-3%, but this sharply increased by the age of 5 years when the prevalence reached 14-35%, indicating the predominance of postnatal transmission during early childhood.[17,42,60,61] In rural Senegal, the second peak in HBsAg prevalence was noted in the age of 6-7 years (32.0%), suggesting that in addition to the transmission during early childhood, entry to the primary school might also increase the risk of infection.[17] By the age of 12-13 years, almost all children (91.2%) had been exposed to HBV (indicated by presence of any HBV sero-marker), implying that transmission during puberty and adulthood is uncommon. Similar patterns of age-specific HBsAg prevalence were found in Keneba and Manduar, two rural villages in The Gambia; a steep increase in HBsAg from 0% in infants aged less than 6 months[42] to 10-30% in children aged 2-4 years was followed by a second peak at the school entry (7-8 years old), and by the age of 13 years >90% showed the past exposure to HBV.[60] In Namibia, Southern Africa, where 11% of mothers carry HBsAg (of whom 15% also carry HBeAg), the prevalence of HBsAg in infants aged <6 months was only 1% (4/314) whilst the prevalence in children ≥1

year old was 13% (81/604),[14] confirming the predominance of postnatal transmission. Based on these data the proportion of HBsAg-positive individuals who acquired the infection perinatally in the pre-vaccine era was estimated at 40% in East Asia[62] and <10% in sSA.[63]

A different pattern of age-specific prevalence was observed in one cohort study in Senegal. In this study, two neonates born to HBeAg-positive mothers were both tested positive for HBsAg at birth (peripheral blood) and one remained positive at 6 months (another lost HBsAg). Two of 19 infants (10.5%) born to HBsAg-positive HBeAg-negative mothers were HBsAg-positive at birth and remained positive at 6 months old.[18] A study in Ghana found that 10.8% of cord blood from 120 neonates born to mothers with positive HBsAg and positive HBV DNA were positive for HBV DNA.[28] The study found that maternal HBeAg positivity, high viral load, and a wild-type HBV genome were associated with higher risk of intrauterine transmission. A recent study in Malawi which assessed the risk of intrauterine transmission in neonates of mothers co-infected with HIV and HBV found that 3% (1/34) of children were HBV DNA positive two weeks after the birth.[64] These studies confirm that maternal-foetal transmission occurs in sSA although it is less frequent than in East Asia.

*1.3.3.3. The reason for the difference between Asia and sub-Saharan Africa*

The main reason for the lower frequency of perinatal mother-to-infant transmission in sSA than in Asia is the difference in prevalence of maternal HBeAg. Although the prevalence of HBsAg in pregnant women is similar between sSA and Asia (10-15%), the prevalence of HBeAg among HBsAg-positive mothers is 10% in sSA and 40% in Asia.[63] Moreover, the risk of

perinatal transmission from HBeAg-positive mother to infant is also lower in sSA;[10] positive HBsAg by the age of 3 months is 0% in Senegal[13] and Kenya[58] whilst it is >90% in Taiwan.[37,38]

*1.3.3.4. Sibling-to-sibling transmission in sub-Saharan Africa*

The HBV infection in sSA occurs mainly within the household and most often between siblings during early childhood. In Ghana, HBsAg-positive individuals in the household, rather than within the larger domestic compound, were the main source of HBV infection for children.[52] A phylogenetic analysis in The Gambia support this hypothesis.[65]

As discussed above, the risk of postnatal transmission is generally higher in children born to HBsAg-positive (or HBeAg-positive) mothers than in those born to negative mothers.[13,42,61] But infectious mothers are not the main source of postnatal transmission in Africa. HBV infection tends to cluster within families,[66] and other family members act as a source of infection.[41] In The Gambia, the risk of the youngest child in each household being sero-positive for HBsAg was strongly associated with the number of HBsAg-positive older siblings after adjusting for age, sex, family size and maternal HBsAg status; the relative risks were 1.0 (reference), 3.3 (95% CI: 1.2-9.2) and 9.3 (2.6-33.0) in children with no, one and more than one older sibling with positive HBsAg, respectively.[60]

Child-to-child transmission is frequent in sSA because infected children are highly infectious to others. The prevalence of HBeAg in HBsAg-positive mothers and HBsAg-positive children aged less than 5 years are 13% and 86% (children aged <5 years) in The Gambia,[60] and 10% and 57% (children aged <5 years) in South Africa,[67] respectively.

Table 1.2 Risk of HBV infection (positive HBsAg) in unvaccinated infants and children according to age and maternal HBV status in longitudinal cohort studies in East Asia and sub-Saharan Africa

| Author, country, year | Maternal serostatus | At birth | 3 months | 6 months | 9 months | 12 months | 24 months | >24 months |
|---|---|---|---|---|---|---|---|---|
| **Asian studies** | | | | | | | | |
| Stevens, Taiwan, 1975[57,a] | HBsAg(+) | 20% (21/103)[c] | 41% (30/74) | 34% (18/53) | 37% (26/71) | 36% (25/69) | | |
| Okada, Japan, 1976[30] | HBsAg(+), HBeAg(+) | | | | | 100% (10/10) | | |
| | HBsAg(+), HBeAg(-) | | | | | 15% (2/13) | | |
| Beasley, Taiwan, 1977[31] | HBsAg(+), HBeAg(+) | | | | | 85% (17/20) | | |
| | HBsAg(+), HBeAg(-) | | | | | 31% (13/42) | | |
| Beasley, Taiwan, 1979[32] | HBsAg(+), HBeAg(+) | | | | | 96% (45/47) | | |
| | HBsAg(+), HBeAg(-) | | | | | 17% (3/18) | | |
| Wong, Hong Kong, 1980[16,33,a] | HBsAg(+), HBeAg(+) | 47% (22/47)[c] | 81% (39/48) | 79% (37/47)[d] | | | | |
| | HBsAg(+), HBeAg(-) | 24% (12/50)[c] | 17% (7/42) | 3% (1/30)[d] | | | | |
| Beasley, Taiwan, 1983[37,38,a] | HBsAg(+), HBeAg(+) | 20% (N/R) | 93% (57/61) | 95% (58/61) | 95% (58/61) | 95% (58/61) | | |
| Wong/Ip, Hong Kong, 1984/89[39,35,a] | HBsAg(+), HBeAg(+) | 3% (1/34)[e] | 74% (25/34) | 79% (27/34) | | | | 64% (30/47) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **African studies** | | | | | | | | |
| Marinier, Senegal, 1985[13,b] | HBsAg(+), HBeAg(+) | 0% (0/26)[c,f] | 0% (0/16) | | 20% (3/15) | | 0% (0/8) | 33% (1/3) |
| | HBsAg(+), HBeAg(-) | 0% (0/106)[c,f] | 0% (0/71) | | 0% (0/62) | | 13% (6/46) | 31% (4/13) |
| | HBsAg(+) | 0% (0/132)[c] | 0% (0/88) | | 4% (3/78) | | 11% (6/55) | 35% (6/17) |
| | HBsAg(-) | 0% (0/1221)[c] | 0% (0/374) | | 2% (4/186) | | 6% (6/97) | 14% (5/35) |
| Greenfield, Kenya, 1986[58] | HBsAg(+), HBeAg(+) | | 0% (0/4) | | 25% (1/4) | | | |
| | HBsAg(+), HBeAg(-) | | 0% (0/47) | | 4% (2/47) | | | |
| Menendez, Tanzania, 1999[59,a] | HBsAg(+), HBeAg(+) | | | | 20% (3/15)[g] | | 40% (6/15)[h] | |
| | HBsAg(+), HBeAg(-) | | | | 3% (1/38)[g] | | 16% (6/38)[h] | |
| | HBsAg(-) | | | | 2% (1/42)[g] | | 21% (9/42)[h] | |
| Candotti, Ghana, 2007[28] | HBsAg(+), DNA(+) | 11% (13/120)[c] | | | | | | |
| | HBsAg(+), DNA(-) | 0% (0/7)[c] | | | | | | |
| | HBsAg(-), DNA(+) | 0% (0/20)[c;] | | | | | | |
| Chasela, Malawi, 2014[64,a,i] | HBsAg(+), DNA(+) | 3% (1/34)[j] | | | | 17% (5/30) | | |
| | HBsAg(+), DNA(-) | 0% (0/21)[j] | | | | 0% (0/12) | | |
| | HBsAg(-), DNA(+) | 0% (0/2)[j] | | | | 0% (0/9) | | |

[a] Number of children with positive HBsAg includes those who already tested positive in previous tests.

[b] Number of children with positive HBsAg excludes those who already tested positive in previous tests.

[c] Test using cord blood

[d] Bleeding by 5 months.

[e] Definition of intrauterine transmission is positive anti-HBc IgM in cord blood or in the 3rd-day sample or increment of HBsAg level from sample in cord blood to the 3rd-day sample.

[f] Number of children having HBeAg-positive mothers and having HBeAg-negative HBsAg-positive mothers was extrapolated from the prevalence of HBeAg reported in the same study.

[g] Bleeding at 8 months.

[h] Bleeding at 18 months.

[i] Study included children born to HIV-positive mothers and excluded children who are infected with HIV during the follow-up. Hepatitis B vaccine was started from 6 weeks of age and hepatitis B immunoglobuling was not given. All mothers received antiviral regimen including lamivudine for 7 days from the onset of labour, and similarly all children received regiment including lamivuding for 7 days after birth as a prophylaxis against HIV.

[j] Bleeding at 2 weeks.

## 1.4. Natural history of chronic hepatitis B infection

1.4.1. Establishment of chronic hepatitis B infection following an acute infection

After exposure to HBV, a person can develop a symptomatic illness called acute hepatitis B or remain asymptomatic. Except in those rare individuals (<1%) who develop fulminant hepatitis, the acute phase does not result in severe morbidity.[6] After acute infection, some individuals acquire lifelong immunity,[8] while others develop a chronic infection which may eventually lead to liver cirrhosis or HCC.

The major determinant of an individual's risk of developing chronic infection is the age at HBV infection.[68] Infection persists in 80-90%, 20-30%, <10%, and <5% of people infected perinatally, in early childhood, adolescence, and adulthood, respectively.[68,69]

1.4.2. Natural history after establishing the chronic hepatitis B infection

According to the international guidelines, the natural course of chronic infection consists of five phases of variable duration, which are not necessarily sequential: the immune tolerant phase, the HBeAg-positive CHB disease, the HBeAg-negative CHB disease, the inactive HBsAg carrier phase and resolved hepatitis B (figure 1.3).[7,70,71] It should be emphasised that this is based on observations from Asia, Europe and North America, and to date there has not been any study of the natural history of CHB infection in sSA.[72,73]

*1.4.2.1. Immune tolerant phase*

Persons infected via the perinatal route frequently experience a period of immune tolerance for 10-30 years, during which there is no or minimal disease progression despite high viral

replication.[74,72] This is thought to be related to the transplacental passage of HBeAg or HBeAg-derived peptides from mother to foetus, which induces a tolerance in newborns against the virus.[75] The observed association between perinatal transmission and higher risk of chronic infection may also be explained by this mechanism.[6] This phase is usually short-lived or even absent in people who acquired the infection during childhood or adulthood.[72]

*1.4.2.2. HBeAg-positive chronic hepatitis B disease*

Individuals infected through perinatal transmission may advance to HBeAg-positive CHB disease (also called as the immune active phase or HBeAg clearance phase) years after the start of the immune tolerant period. In contrast, those infected later in adulthood who could not clear the infection may transition into this phase shortly after the acute infection without experiencing the immune tolerant phase.[74] In this phase, HBV is recognised as being foreign by the immune system of the host, which tries to clear infected hepatocytes. This is characterised by an active hepatic inflammation associated with elevated serum alanine aminotranspherase (ALT) and reduced HBV DNA levels. This acute exacerbation is often followed by seroconversion from HBeAg-positive to anti-HBe-positive status. For example, a cohort study of 58 Taiwanese children who had HBeAg seroconversion (78% of them have HBsAg-positive mothers and therefore considered as having been perinatally infected) found that the mean ages at peak HBV DNA levels, peak ALT and HBeAg seroconversion were 13.4 ± 5.8, 16.3 ± 6.0, and 17.2 ± 5.8 years, respectively.[76]

Table 1.3-a presents the rates of spontaneous HBeAg loss reported from cohort studies of chronic HBV carriers. The annual rates ranged from 3.2% to 9.6% in studies including subjects

in the immune tolerant phase and those with HBeAg-positive CHB disease. The annual loss was 13.9% in studies restricted to those with HBeAg-positive CHB disease.[77–84] HBeAg seroclearance is slower in men, younger age groups, and in individuals without symptoms, who have an HBV-infected mother (as a proxy for mother-to-infant transmission), and who are infected with HBV genotype C (rather than B).[77–84]

*1.4.2.3. Inactive HBsAg carrier and HBeAg-negative chronic hepatitis B disease*

There are three possible scenarios after HBeAg seroconversion.[85] First, many people will be in an inactive HBsAg carrier state in which there is usually no progress to liver injury with normal serum ALT and low HBV DNA levels. HBsAg clearance can be expected (2%/year), but some may experience reactivation of hepatitis B.[71] Second, a few seroconverted persons will experience HBeAg reversion and develop HBeAg-positive CHB disease again. Third, some patients develop HBeAg-negative CHB disease. This is associated with the precore or basal core promoter mutations of the virus, which prevents HBeAg formation without compromising replication of the virus.[71] Similar to HBeAg-positive CHB, serum ALT levels in this immune active phase can be increased because of active hepatic inflammation. Serum HBV DNA levels are usually lower in this phase than in the HBeAg-positive CHB disease ($2 \times 10^3$-$10^7$ *versus.* $2 \times 10^5$-$10^9$ IU/ml).[8] A longitudinal study in Taiwan showed that during a median follow-up of 9 years following spontaneous HBeAg seroconversion, 67% remained in inactive HBsAg carrier, 4% experienced HBeAg reversion and 24% developed HBeAg-negative CHB disease; cirrhosis developed in 0.5%, 55% and 23% of subjects in each category, respectively.[85]

Table 1.3-b presents the rate of HBsAg loss reported in cohort studies of chronic HBV carriers. The annual rate of HBsAg loss ranged from 0.4% to 1.0% in carriers in immune tolerant phase or HBeAg-positive CHB disease and from 1.0% to 1.7% in inactive HBsAg carriers. Male sex, younger age, delayed HBeAg seroconversion, low viral load and low HBsAg levels over time and having an HBV-positive mothers were associated with a delay in HBsAg loss.[77,80–82,86–90] The rate of progression from inactive HBsAg carriers to HBeAg-negative CHB disease is between 1.4-4.4% per annum in Taiwan.[91,92]

*1.4.2.4. Resolved hepatitis B and occult hepatitis B*

The inactive HBsAg carrier state followed by a resolution of infection through HBsAg seroclearance usually confers a favourable prognosis in the absence of pre-existing cirrhosis ("resolved hepatitis B").[93] This condition is characterised by undetectable HBV DNA in serum while anti-HBc and anti-HBs are often detectable.[70] In contrast, an increase in duration and frequency of the CHB disease episodes leads to repetitive liver injury and regeneration, resulting in hepatic necroinflammation/fibrosis and an increased risk of cirrhosis and HCC.[8] Occult HBV infection, defined as negative-HBsAg with detectable HBV DNA in serum or liver tissue, was associated with chronic liver disease including HCC in two recent meta-analyses.[94,95] Of note, neither review included any African studies.

Figure 1.3 Natural history of CHB infection (adapted from Chen et al., 2007)[93]

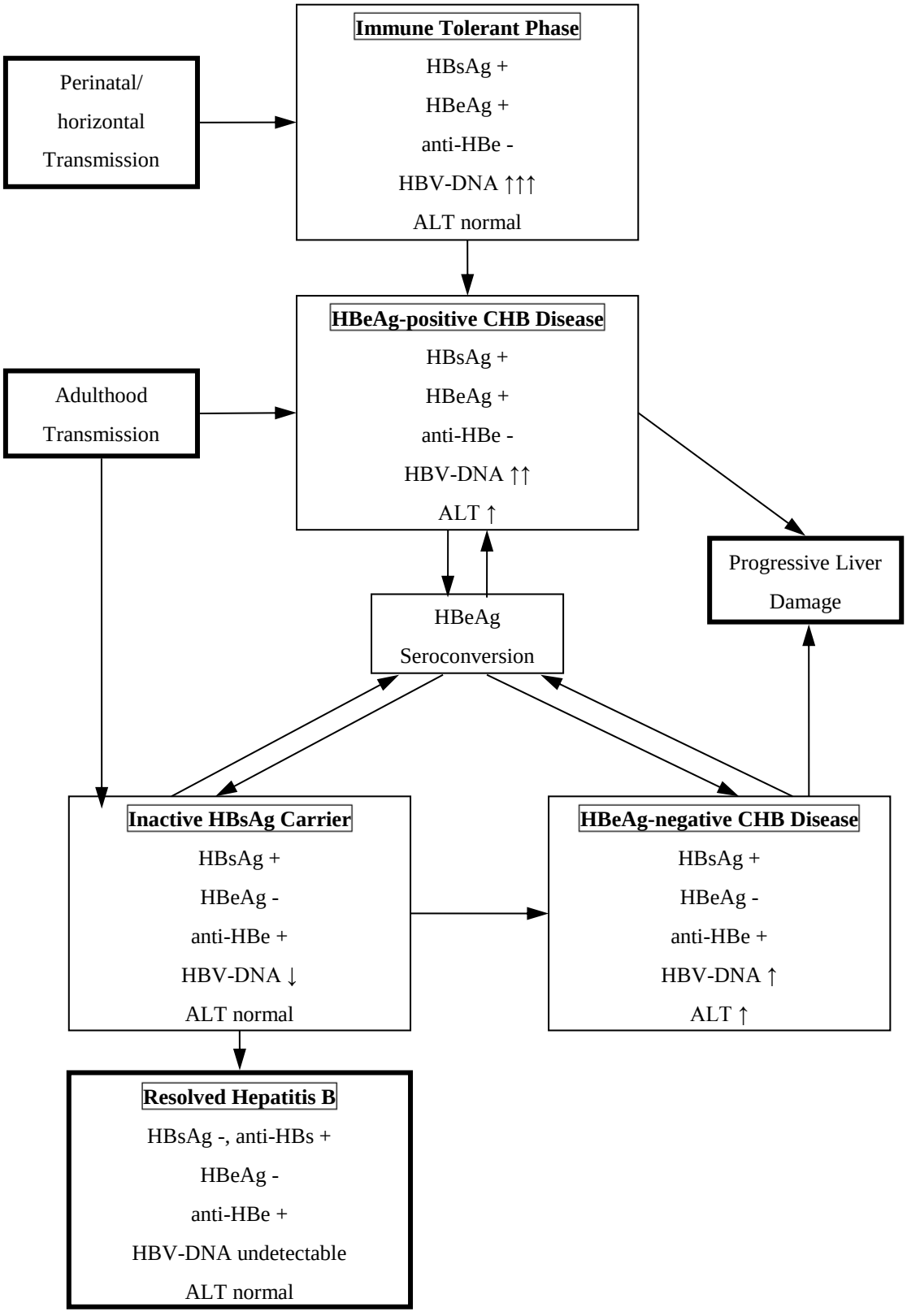


Table 1.3 Annual rate of HBeAg seroclearance, HBsAg seroclearance and HCC in cohort studies of treatment naïve individuals with CHB infection

Table 1.3-a Annual rate of HBeAg seroclearance

| Author, year | Country, settings | Baseline stage of cohort | Mean age at baseline (years, range) | Recruitment period | Frequency of F/U | N | Mean length of F/U (years, range) | No. with outcome | Annual rate (%) | Factors associated with outcome | Other risk factors examined in the study |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Alward WLM, 1985[77] | Alaska, population-based | IT & EPCHB | 20.5 (0-68) | 1971-73 | ≥6 mo | 102 | 6.1 ± 2.3 | Overall:60 (loss) | 9.6* | Female, older age | N/R |
| | | | | | | 64 | 6.1 | Men: 30 | 7.7* | | |
| | | | | | | 38 | 6.1 | Women: 30 | 12.9* | | |
| Chang MH, 1989[78] | Taiwan, hospital/school-based | IT & EPCHB | 4.8 (0-15) | N/R | 6 mo | 228 | 3.4 | 48 (loss) | 6.2* | Older age at baseline, presence of symptom, maternal HBsAg(-), origin of participants (univariable) | Sex |
| Bortolotti | Italy | EPCHB | 7.2 ± | 1976-8 | N/R | 76 | 5.0 ± | 53 | 13.9* | Male sex, previous | Age, ALT, liver |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| F, 1990[79] | (Padova), hospital-based | | 4.2 | 7 | | | 2.6 | (seroconversion) | | acute hepatitis (univariable) | histology |
| Bortolotti F, 1998[80] | Italy (Padya) & Spain (Madrid), hospital-based | IT & EPCHB | 5.5 ± 3.4 | 1975-85 | ≤12 mo | 112 | 12.8 ± 3.2 | 108 (seroconversion) | 7.5* | N/R | N/R |
| McMahon BJ, 2001[81] | Alaska, population-based | IT & EPCHB | Median 19.9 (1-87) | N/R | 6 mo | 532 | 5 | 218 | 8.2* | Older age | Sex, ethnicity |
| Iorio R, 2007[82] | Italy (Naples), hospital-based | IT & EPCHB | Median 6.7 (1-13) | 1981-2005 | ≤6 mo | 62 | Median 10.0 (5-23) | 46 (loss) | 7.4* | None | Age, sex, ALT |
| Tseng YR, 2011[83] | Taiwan, hospital/school-based | IT & EPCHB | Median 5.7 (0-16) | 1984-2004 | 6 mo | 185 | 19.7 | 121 (seroconversion) | 3.3 | Maternal HBeAg(-), early maternal HBeAg | Age, sex, maternal HBsAg, ALT |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | seroconversion, genotype B (vs. C) | |
| Roushan MRH, 2012[84] | Iran, hospital-based | IT & EPCHB, all born to HBsAg(+) mothers | <10 | N.R | 6 mo | 139 | 18.2 (2-29) | 82 (seroconversion) | 3.2 | Longer length of F/U, having HB vaccine and HBIG | Sex, maternal HBeAg |

Table 1.3-b Annual rate of HBsAg seroclearance

| Author, year | Country, settings | Baseline stage of cohort | Mean age at baseline (years, range) | Recruitment period | Frequency of F/U | N | Mean length of F/U (years, range) | No. with outcome | Annual rate (%) | Factors associated with outcome | Other risk factors examined in the study |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Alward WLM, 1985[77] | Alaska, population-based | Mixed (HBeAg(+) 69%) | 20.5 (0-68) | 1971-73 | ≥6 mo | 150 | 6.1 ± 2.3 | Overall:9 (loss) | 0.98* | Female | Age |
| | | | | | | 90 | 6.1 | Men: 2 | 0.36* | | |
| | | | | | | 60 | 6.1 | Women: 7 | 1.9* | | |
| Hsu HY, | Taiwan, | IT & EPCHB | 5.5 ± | N/R | ≤6 mo | 375 | 4.3 ± | 10 (loss) | 0.43* | Older age, | Sex, origin of |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1992[86] | hospital/school-based | | 4.2 | | | | 2.5 | | | anti-HBe(+), chronic active hepatitis, maternal HBsAg(-) (univariable) | participants, ALT |
| | | IC & ENCHB | 9.2 ± 4.4 | N/R | ≤6 mo | 45 | 4.0 ± 1.9 | 3 (loss) | 1.7* | | |
| Bortolotti F, 1998[80] | Italy (Padya) & Spain (Madrid), hospital-based | IT & EPCHB | 5.5 ± 3.4 | 1975-85 | ≤12 mo | 112 | 12.8 ± 3.2 | 7 (loss) | 0.49* | N/R | N/R |
| McMahon BJ, 2001[81] | Alaska, population-based | Mixed (HBeAg(+) 42%) | Median 19.9 (1-87) | N/R | 6 mo | 1536 | 12.6 | 106 | 0.54 | Older age, ethnicity, HBeAg(-) | Sex |
| Manno M, 2004[87] | Italy (Modena), blood donors | IC | 36 ± 11 | 1972-77 | 12-24 mo | 183 | N/R | 59 (loss) | 1.0 | Older age | N/R |
| Bortolotti F, 2006[88] | Italy (Padova), | IC (except one with | 11.4 ± 5.1 | 1975-85 | ≤12 mo | 64 | 14.8 ± 5.7 | 9 (loss) | 0.95* | None | Sex, source of infection, peak |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | hospital-based | ENCHB) | | | | | | | | | ALT before seroconversion, duration of HBeAg(+) phase |
| Iorio R, 2007[82] | Italy (Naples), Hospital-based | IT & EPCHB | Median 6.7 (1-13) | 1981-2005 | ≤6 mo | 62 | Median 10.0 (5-23) | 6 (seroconversion) | 0.97* | N/R | N/R |
| Liu J, 2010[89] | Taiwan (REVEAL), population-based | Mixed (HBeAg(+) 16%, ALT>ULN 6%) | 30-65 | 1991-1992 | 6-12 mo | 3087 | 8.0 | Overall: 562 (loss) | 2.3 | Older age, low VL at baseline, reduction in VL over time, high ALT when VL became undetectable, obesity, ethnicity | Sex, ALT at baseline, cigarettes |
| | | | | | | 1994 | 9.2 | Men: 399 | 2.2 | | |
| | | | | | | 1093 | 5.9 | Women: 163 | 2.5 | | |
| Tseng TC, 2012[90] | Taiwan (NTUH), hospital-based | IC (includes some with VL <2000 IU/ml with ALT >ULN) | ≥28 | 1985-1995 | ≤6 mo | 688 | 11.6 | 130 (loss) | 1.6 | Female, low serum HBsAg levels | Age, ALT, VL |

Table 1.3-c Annual rate of HBV-related HCC

| Author, year | Country, settings | Baseline stage of cohort | Mean age at baseline (years, range) | Recruitment period | Frequency of F/U | N | Mean length of F/U (years, range) | No. with outcome | Annual rate (%) | Factors associated with outcome | Other risk factors examined in the study |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Alward WLM, 1985[77] | Alaska, population-based | Mixed (HBeAg(+) 69%) | 20.5 (0-68) | 1971-73 | ≥6 mo | 150 | 6.1 ± 2.3 | Overall:3 | 327.9 | N/R | N/R |
| | | | | | | 90 | 6.1 | Men: 2 | 364.3* | | |
| | | | | | | 60 | 6.1 | Women:1 | 273.2* | | |
| McMahon BJ, 1990[96] | Alaska, population-based | Mixed | 0-70+ | 1971-87 | ≥6 mo | 1400 | 5.6 | Overall: 20 | 255.9 | Male, older age | N/R |
| | | | | | | 824 | N/R | Men: 18 | 386.8 | | |
| | | | | | | 576 | N/R | Women: 2 | 63.3 | | |
| Evans AA, 1998[97] | Senegal, male soldiers | N/R | 29.9 (range 20-55) | 1992-93 | N/R | 2611 | N/R | N/R | 68.3 | N/R | N/R |
| McMahon BJ, | Alaska, population- | Mixed (HBeAg(+) | Median 19.9 | N/R | 6 mo | 1536 | 12.6 | Overall: 36 | 185.3 | Older age, ethnicity, reversion to | Sex |
| | | | | | | 908 | 13.9 | Men: 29 | 229.8 | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2001[81] | based | 42%) | (1-87) | | | 628 | 9.3 | Women: 7 | 119.9 | HBeAg(+), multiple switches in HBeAg status | |
| Evans AA, 2002[98] | China, population-based | Mixed | Range: 25-64 | 1992-3 | Link with death registry | 11506 | 7.1 | Overall:704 | 863.8 | N/R | N/R |
| | | | | | | 8795 | 7.1 | Men: 643 | 1029.7 | | |
| | | | | | | 2711 | 7.0 | Women: 61 | 320.2 | | |
| Crook PD, 2003[99] | England & Wales, blood donors | N/R (HBsAg(+) once) | Median 29 (17-64) | 1970-82 | Link with death registry | 3658 | 22.4 | Overall: 21 | 25.5 | Male, older age, older calendar time | N/R |
| | | | | | | 2681 | N/R | Men: 20 | 33.5 | | |
| | | | | | | 977 | N/R | Women: 1 | 4.4 | | |
| Manno M, 2004[87] | Italy (Modena), blood donors | IC | 36 ± 11 | 1972-77 | 12-24 mo | 263 | 29.3 ± 2.1 | 2 (HCC death) | 26.0 | N/R | N/R |
| Ribes J, 2006[100] | Spain, blood donors | N/R (HBsAg(+) once) | 33 ± 11 (men), 34 ± 12 (women | 1972-85 | Link with death registry | 2352 | N/R | Oerall:12 | 26.5 | N/R | N/R |
| | | | | | | 1575 | 20.5 ± 4.6 | Men: 11 | 34.1 | HCV, HDV, alcohol, diabetes, HBeAg(+), anti-HBc IgM | Cigarettes, HBV DNA |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ) | | | 631 | 20.7 ± 3.6 | Women: 1 | 7.6 | N/R | N/R |
| Chen CJ, 2006[101] | Taiwan (REVEAL), population-based | Mixed (HBeAg(+) 15%, ALT>ULN 6%) | 30-65 | 1991-92 | N/R | 3653 | 11.4 | Overall: 164 | 393 | Male, older age, alcohol, HBeAg, LC, VL at baseline, persistence of high VL | Cigarettes, ALT |
| | | | | | | 2260 | 11.3 | Men: 135 | 530 | | |
| | | | | | | 1393 | 11.7 | Women: 29 | 178 | | |
| Tseng TC, 2012[91] | Taiwan (NTUH), hospital-based | IC (includes some with VL >2000 IU/ml with ALT <2 ULN) | >16 | 1985-2004 | ≤6 mo | 390 | 8.5 | 28 | 240.8 | Older age | Sex, VL, genotype, ALT (lack of power) |
| Tseng TC, 2012[102] | Taiwan (NTUH), hospital-based | Mixed (HBeAg(+) 19%, ALT>ULN 29%) | >28 | 1985-2000 | <6 mo | 2688 | 14.7 ± 4.3 | 191 | 484.4 | Male, older age, HBeAg, VL, HBsAg levels, genotype C (>B) (univariable analysis) | None (univariable anlalysis) |
| | | | | | | 1634 | 14.7 | Men: 154 | 642.0 | | |
| | | | | | | 1054 | 14.6 | Women: 37 | 239.6 | | |
| | | IC (includes | >28 | 1985-2 | <6 mo | 1068 | 14.9 | 29 | 181.9 | Male, older age, | VL, persistence of |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | some with VL <2000 IU/ml with ALT >ULN) | | 000 | | 600 | 15.0 | Men: 23 | 255.9 | ALT, HBsAg levels, persistence of high ALT or high HBsAg levels | high VL |
| | | | | | | 468 | 14.9 | Women: 6 | 86.3 | | |

Unless noted, variables in the last two columns are those measured at the enrolment in the cohort.

* Denominator of the rate is duration of follow-up rather than person-years at risk.

** Rate was taken from the paper

*** Incidence of HCC and mortality were assumed to be equivalent in some studies because most of HCC patients die within one year after the diagnosis.[103]

Abbreviations: IT, immune toleratnt phase; EPCHB, HBeAg-positive chronic hepatitis B disease; ENCHB, HBeAg-negative chronic hepatitis B disease; IC, inactive HBsAg carrier; LC, liver cirrhosis; ALT, alanine aminotransferase; VL, viral load.

1.4.3. Role of transient elastography (Fibroscan) in the natural history of chronic HBV infection

Transient elastography (TE) is a non-invasive method developed in 2003 to assess the degree of liver fibrosis by measuring the stiffness of the liver.[104] The probe of TE is applied to the right intercostal spaces of patients, and a vibration from the probe towards the liver tissue induces an elastic shear wave. TE estimates the stiffness of liver tissue by measuring the speed of the shear wave propagation. The technique has been validated against liver histopathology as a gold standard in patients with CHB infection.[105–108] Meta-analysis of the validity of TE in chronic HBV carriers showed that for the diagnosis of significant liver fibrosis or above (≥F2 in Metavir scores), the sensitivity was 74.3% and the specificity was 78.3% based on a cut-off of 7.9 kPa. For the diagnosis of liver cirrhosis (≥F4 in Metavir scores), the sensitivity was 84.6% and the specificity was 81.5% using a cut-off of 11.7 kPa.[109]

To date, few studies have used the degree of liver fibrosis determined by TE (Fibroscan, Echosens, France) as an outcome of CHB infection. A hospital-based cross-sectional study in Hong Kong examined 453 treatment-naïve HBeAg-positive chronic HBV carriers (median age 37, IQR; 30-48, male 60%) using TE. Severe fibrosis (>9.0 kPa in patients with normal ALT and >12.0 kPa in those with increased ALT levels) was observed in 22%. Older age and higher ALT levels were associated with severe fibrosis whilst sex, obesity and HBV DNA were not. A subset of the patients (n=28) were followed for a median of 102 months (range 95-110) and longitudinal change in ALT levels were associated with the severe fibrosis determined at the end of the follow-up.[110] The same authors studied 1,197 treatment-naïve HBeAg-negative chronic HBV carriers (mean age 48.0 ± 11.4 years, male 65%).[111] Eleven percent of the patients were categorised as probable cirrhosis (>13.4 kPa) and older age, abnormal ALT and high HBV

DNA levels were associated with this condition. A subset of patients (n=72) were followed for a mean duration of 91.2 (± 14.6) months, and were assessed for possible cirrhosis (> 8.4 kPa) at the end of the follow-up. The study identified ALT levels and the number of reactivation episodes (defined as ALT >58 IU/L) as risk factors for cirrhosis.

## 1.5. Hepatitis B vaccines

### 1.5.1. History and current situation

In 1982 a plasma-derived hepatitis B vaccine was introduced. This vaccine has been gradually replaced by a recombinant hepatitis B vaccine (introduced in 1986) because of concerns that the plasma-derived vaccine might be contaminated with HIV and other infections.[112] During the 1980's several hepatitis B vaccine trials started in Asia and Africa including two Gambian studies (a pilot study in Keneba and Manduar, and the nationwide Gambia Hepatitis Intervention Study). These studies demonstrated that the vaccine had good immunogenicity and the efficacy against HBsAg carriage was >95%.[113,114,115] Consequently, in 1992 the World Health Organisation (WHO) urged member states to include hepatitis B vaccine in national vaccination programmes.[116]

By the end of 2013, 181/193 (94%) countries have adopted this recommendation,[117] and the vaccine has been introduced in all African countries (with the exception of Western Sahara). Coverage with 3 doses of the vaccine is 79% worldwide, 72% in Africa,[117] and 95% in The Gambia.

### 1.5.2. Birth dose of hepatitis B vaccine

#### *1.5.2.1. WHO recommendation*

Currently, the WHO recommends that all neonates receive the first dose of hepatitis B vaccine as soon as possible after birth (i.e., a birth dose), ideally within 24 hours (i.e., timely birth dose).[116] WHO emphasises the importance of the birth dose not only in countries high endemic for HBV infection where mother-to-infant transmission is common, but also in countries with low to intermediate endemicity because many people acquire the infection through child-to-child transmission during early childhood. There are different formulations for hepatitis B vaccine: monovalent or a fixed combination with other vaccines including diphtheria-tetanus-pertussis (DTP), Haemophilus influenza type b, hepatitis A and inactivated polio.[116] Only monovalent hepatitis B vaccine can be used when immunising a neonate at birth.[116]

#### *1.5.2.2. Delivery of the timely birth dose*

Despite moderate coverage of hepatitis B vaccine worldwide, the birth dose has not yet been successfully implemented. By the end of 2012, out of 181 countries implementing national hepatitis B vaccine programme, only half (52%, 94/181) recommended the first dose within 24 hours.[117] In sSA, only 6 countries are undertaking the birth dose and its coverage varies: Botswana (99%), Cape Verde (99%), Djibouti (87%), The Gambia (95%), Nigeria (12%) and Sao Tome and Principe (2%). However, these figures are highly questionable. In a recent study in urban Nigeria, the median age at which the first dose of hepatitis B vaccine was given was 14 days, and none received the vaccine within 24 hours.[118] In The Gambia, only 3% of children were given the first dose within 24 hours according to the dataset from Keneba and Manduar,

two rural Gambian villages where the vaccine efficacy has been studied (see Chapter 6). Most of African countries adopted a vaccine schedule using the fixed combination starting from 6 weeks after the birth, because of low HBeAg prevalence in African mothers and relatively infrequent perinatal transmission.[119]

*1.5.2.3. Rationale for the timely birth dose*

Although WHO strongly recommends the timely birth dose within 24 hours of birth, there is a paucity of evidence to support this. A systematic review with meta-analysis assessed the preventive effect of hepatitis B vaccines when given within one month of birth in infants with HBsAg-positive mothers.[112] The review included randomised studies where hepatitis B vaccines were given alone or together with hepatitis B immunoglobulin (HBIG). The outcome was HBV infection, which was defined as positive anti-HBc, positive HBsAg or positive HBeAg. The findings are summarised as below.

i) The efficacy of hepatitis B vaccine preventing HBV infection compared to placebo or no intervention in children born to HBsAg-positive mothers

- Overall, hepatitis B vaccine alone reduced HBV infection (RR 0.28, 95% CI; 0.20-0.40).
- There was a marginal evidence of heterogeneity (p=0.11) in vaccine efficacy according to maternal HBeAg status; risk ratio (RR) for HBV infection in vaccinees varied from 0.27 (95% CI; 0.18-0.40) in those born to HBeAg-positive mothers to 1.12 (95% CI; 0.31-3.97) in those born to HBeAg-negative mothers. The latter was examined in only one trial.
- There was also a marginal evidence of heterogeneity (p=0.07) in vaccine efficacy according to the timing of the first dose; RR for HBV infection in vaccinees differed between the first dose

within 12 hours (RR 0.23, 95% CI; 0.12-0.42, two studies), within 24 hours (0.44, 95% CI; 0.27-0.72, two studies) and 48 hours (0.14, 95% CI; 0.05-0.41, one study).

- One trial[38] compared first dose at birth (4-7 days after the birth) and at one month, and not find the difference (RR 0.70, 95% CI; 0.18-2.77). In this trial all received HBIG at birth.

ii) The preventive efficacy of hepatitis B vaccine plus HBIG on HBV infection compared to vaccine alone in children born to HBsAg-positive mothers

- Overall, a combination of vaccine and HBIG further reduced HBV infection compared to vaccine alone (RR 0.54, 95% CI; 0.41-0.73).

- There was no evidence of heterogeneity (p=0.47) in efficacy of vaccine plus HBIG versus vaccine alone according to maternal HBeAg status; RR for HBV infection in vaccinees was 0.51 (95% CI; 0.42-0.61) in those born to HBeAg-positive mothers and 0.24 (95% CI; 0.01-4.06) in those born to HBeAg-negative mothers. The latter was examined only in one trial.

- There was no evidence of heterogeneity (p=0.38) in efficacy of vaccine plus HBIG versus vaccine alone according to the timing of the first dose of HBIG; RR for HBV infection in vaccinees was 0.52 (95% CI; 0.43-0.62) for the first dose within 12 hours of birth, 0.60 (95% CI; 0.35-1.05) for the first dose within 24 hours, and 0.14 (95% CI; 0.01-2.64) for the first dose within 48 hours.

In this systematic review, 18 studies recruited neonates of HBsAg-positive HBeAg-positive mothers, while only three studies recruited both neonates of HBeAg-positive mothers and HBeAg-negative mothers. The number of neonates in the subgroup analysis of the timing of the first dose was also small. Consequently, the authors noted that the meta-analysis was not

powered to answer whether the effect of vaccine differed according to maternal HBeAg status or the timing of the vaccine.[112] To date, no randomised vaccine trials have explored the timing of the birth dose.[120]

In contrast, a few observational studies have examined the timing of the first dose. A Canadian cohort study suggested the importance of birth dose in children born to HBsAg-positive mothers. A cohort of children who had been born to HBsAg-positive mothers and vaccinated against HBV between 1984 and 1989 were followed up in 1992 for the assessment of HBV sero-markers. HBIG was given to some but not all children. The study divided children into four groups according to their age (days) at first dose of hepatitis B vaccine: 1-3, 4-7, 8-61 and ≥62. The study reported that the risk of breakthrough infection (positive anti-HBc) increased with increasing delay in the first dose (OR 4.3, 95% CI; 2.2-8.4, for each unit increase in the age group of the first dose). Similarly, the risk of HBsAg carriage (positive HBsAg) increased with increasing delay in the first dose (OR 3.3, 95% CI; 1.3-8.2, for each unit increase in the age group of the first dose).[121] A study in China assessed the prevalence of HBsAg amongst children born between 1992 and 2005 and who were vaccinated with three doses of hepatitis B vaccine without HBIG. After adjusting for confounding factors, the ORs of HBsAg positivity in relation to those who received first dose of hepatitis B vaccine within 24 hours were; 0.54 (95% CI; 0.27-1.11), 1.53 (0.80-2.92), 1.26 (0.69-2.30), 1.31 (0.95-1.81) and 1.54 (1.10-2.20) for those received the first dose at 2-7, 8-14, 15-27, 28-180, and >180 days, respectively.[122] The study showed that a timely birth dose (<24 hours) was associated with lower HBsAg when compared to a dose given >24 hours, but the study did not find a difference between a dose given <24

hours and at 2-7 days of birth. The study was limited as maternal HBV sero-status was not taken account.

In a non-randomised controlled trial in Côte d'Ivoire, West Africa, two health centres provided HBV vaccines with routine schedules (starting at 6 weeks followed by 10 and 14 weeks) whilst two other centres provided the timely birth dose regimen (<24 hours, 6 weeks and 14 weeks). HBIG was not available in the study. The prevalence of maternal HBsAg was similar in both groups (7.7%) and 14.5% of such mothers also carried HBeAg. At 9 months of age, 0.5% (9/1896) of children in birth dose group and 0.5% (10/1900) of those in 6 weeks group were tested positive for HBsAg, and all of these children were born to HBeAg-positive mothers. Risk of HBsAg positivity at 9 months in children born to HBeAg-positive mothers was lower in birth dose group than the 6 weeks group (37.5% versus 58.8%) although the difference was not statistically significant (p=0.16).[119]

*1.5.2.4. Frequency of immunoprophylaxis failure*

Table 1.4 presents the risk of immunoprophylaxis failure (positive HBsAg and/or HBV DNA) by maternal HBV status in individuals who were vaccinated against HBV and received HBIG. The risk is almost 0% when the mother is negative for HBeAg or maternal HBV DNA is undetectable, and 2-14% when the mother is HBeAg-positive or viraemic.[23,29,35,38,123–127] The risk may be even higher when both positive-HBeAg and high HBV DNA levels are observed than when only either of these is present.[29,35,123,127]

This might be because mother-to-foetus intrauterine transmission is the major cause of immunoprophylaxis failure, and the risk of intrauterine transmission is determined by maternal HBeAg and HBV DNA levels. In infants born to HBsAg-positive mothers, the frequency of immunoprophylaxis failure despite passive and active immunisation is between 0-0.6% in children without evidence of intrauterine transmission (i.e., negative HBsAg and undetectable HBV DNA at birth) and 15-32% in children positive for HBsAg or HBV DNA at birth.[12,26,125]

Table 1.4 Frequency of HBsAg positivity after the age of 3 months in children who were born to HBV-infected mothers and who received immunoprophylaxis, by maternal HBeAg status or maternal HBV DNA levels (case-control study and study providing anti-viral treatment to mothers were excluded)

Table 1.4-a According to maternal HBeAg

| Author, year | Country | Study design | HBIG | First HBV vaccine | Type of outcome | Timing of sample | Type of exposure ($log_{10}$ copies/ml) | HBsAg prevalence in children born to HBeAg(+) mothers | HBsAg prevalence in children born to HBeAg(-) mothers | Other factors associated with vaccine failure |
|---|---|---|---|---|---|---|---|---|---|---|
| Beasley RP, 1983[38] | Taiwan | RCT | <7 h | 4-7 d | HBsAg | 9 mo | HBeAg | 6.0% (3/50) | N/A | N/R |
| | | | | 1 mo | HBsAg | 9 mo | HBeAg | 8.6% (5/58) | N/A | N/R |
| | | | <7 h + 3mo | 3 mo | HBsAg | 9 mo | HBeAg | 2.0% (1/51) | N/A | N/R |
| Stevens CE, 1985[128] | Asian in USA | Co | <24 h | <24h | HBsAg | 9 mo | HBeAg | 20.0% (5/25) | N/A | N/R |
| | | | | 1 mo | HBsAg | 9 mo | HBeAg | 12.5% (11/88) | N/A | N/R |
| Ip HMH, 1989[35] | Hong Kong | RCT | <1 h | <1 h | HBsAg | 3 yo | HBeAg | 14.1% (9/64) | N/A | N/R |
| | | | <1 h + monthly for 6mo | <1 h | HBsAg | 3 yo | HBeAg | 8.3% (5/60) | N/A | N/R |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| del Canho R, 1997[123] | Netherlands | RCT | <2 h | 0-3 mo | HBsAg | 12 mo | HBeAg | 7.0% (8/114) | 0% (0/??) | N/R |
| Wang Z, 2003[23] | China | Co | <24 h + 1mo | 1 mo | HBsAg | 12 mo | HBeAg | 12.1% (4/33) | N/A | N/R |
| Wiseman E, 2009[124] | Australia | Co | <12 h | <12 h | HBsAg | 9 mo | HBeAg | 6.6% (4/61) | 0% (0/77) | N/R |
| Chakvetadze C, 2011[129] | Mayotte | Co | <12 h in 83% | <12 h in 83% | HBsAg or DNA | 5 yo (2-8) | HBeAg | 1.8% (1/56) | 5.4% (2/37) | Lower maternal age associated with the risk of failure (p=0.09) |
| Zou H, 2012[125] | Taiwan | Co | <12 h | <12 h | HBsAg | 7-12 mo | HBeAg | 5.6% (27/484) | 0% (0/385) | Lower maternal age associated with risk of failure (p=0.06), HBV DNA in cord blood associated with failure |
| Chen HL, 2012[126] | Taiwan | Co | <24 h | < 7 d | HBsAg | 6 mo – 10 yo | HBeAg | 9.3% (54/583) | 0.1% (1/723), (0.3% (3/1050) for those without HBIG) | N/R |
| Wen WH, | Taiwan | Co | <24 h | <7 d | HBsAg | 4 mo – | HBeAg | 12.3% (10/81) | 0% (0/189), | Higher maternal age |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 2013[127] | | | | | | 3 yo | | | (0% (0/33) for those without HBIG) | associated with risk of failure (p=0.07) |

Table 1.4-b According to maternal HBV DNA levels

| Author, year | Country | Study design | HBIG | First HBV vaccine | Type of outcome | Timing of sample | Type of exposure ($log_{10}$ copies/ml) | HBsAg prevalence in children born to HBV DNA(+) mothers | HBsAg prevalence in children born to HBV DNA(-) mothers | Other factors associated with vaccine failure |
|---|---|---|---|---|---|---|---|---|---|---|
| Wiseman E, 2009[124] | Australia | Co | <12 h | <12 h | HBsAg | 9 mo | Detection | 2.9% (4/138) | 0% (0/??) | N/R |
| | | | | | | | ≥8 | 8.5% (4/47) | 0% (0/91) | |
| Chakvetadze C, 2011[129] | Mayotte | Co | <12 h in 83% | <12 h in 83% | HBsAg or DNA | 5 yo (2-8) | <5 (IU/ml) | 0% (0/23) | N/A | Lower maternal age associated with the risk of failure (p=0.09) |
| | | | | | | | 5-7 | 16.7% (2/12) | | |
| | | | | | | | ≥7 | 0% (0/22) | | |
| Zou H, 2012[125] | Taiwan | Co | <12 h | <12 h | HBsAg | 7-12 mo | Detection | 4.8% (27/562) | 0% (0/307) | Lower maternal age associated with risk of |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | <6 | 0% (0/426) | N/A | failure (p=0.06), HBV DNA in cord blood associated with failure |
| | | | | | | | 6-7 | 3.2% (3/95) | | |
| | | | | | | | 7-8 | 6.7% (19/282) | | |
| | | | | | | | ≥8 | 7.6% (5/66) | | |
| | | | | | | | 6-8 | 0% (0/1) | | |

Table 1.4-c According to maternal HBV DNA levels in children born to HBeAg-positive mothers

| Author, year | Country | Study design | HBIG | First HBV vaccine | Type of outcome | Timing of sample | Type of exposure ($log_{10}$ copies/ml) | HBsAg prevalence in children born to HBV DNA(+) mothers | HBsAg prevalence in children born to HBV DNA(-) mothers | Other factors associated with vaccine failure |
|---|---|---|---|---|---|---|---|---|---|---|
| Ip HMH, 1989[35] | Hong Kong | RCT | <1 h | <1 h | HBsAg | 3 yo | >5 pg/ml | 17.6% (9/51) | 0% (0/13) | N/R |
| | | | <1 h + monthly for 6mo | <1 h | HBsAg | 3 yo | >5 pg/ml | 9.1% (4/44) | 6.3% (1/16) | N/R |
| del Canho R, | Netherlands | RCT | <2 h | 0-3 mo | HBsAg | 12 mo | ≥7.5 | 29.2% (7/24) | 0% (0/48) | N/R |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1997[123] | | | | | | | | | | |
| Xu WM, 2009[29] | China | RCT | <24 h | <24 h | HBsAg | 12 mo | ≥9 | 12.2% (5/41) | N/A | N/R |
| Wen WH, 2013[127] | Taiwan | Co | <24 h | <7 d | HBsAg | 4 mo – 3 yo | <6 | 0% (0/16) | N/A | Higher maternal age associated with risk of failure (p=0.07) |
| | | | | | | | 6-8 | 5.6% (1/18) | | |
| | | | | | | | 8-9 | 17.1% (6/35) | | |
| | | | | | | | ≥9 | 25.0% (3/12) | | |

Table 1.4-d According to maternal HBV DNA levels in children born to HBeAg-negative HBsAg-positive mothers

| Author, year | Country | Study design | HBIG | First HBV vaccine | Type of outcome | Timing of sample | Type of exposure ($log_{10}$ copies/ml) | HBsAg prevalence in children born to HBV DNA(+) mothers | HBsAg prevalence in children born to HBV DNA(-) mothers | Other factors associated with vaccine failure |
|---|---|---|---|---|---|---|---|---|---|---|
| Wen WH, 2013[127] | Taiwan | Co | Some not given | <7 d | HBsAg | 4 mo – 3 yo | <6 | 0% (0/221) | N/A | Higher maternal age associated with risk of failure (p=0.07) |

Abbreviations: RCT, randomised-controlled trial; Co, cohort study; N/A, not applicable; N/R, not reported.

*1.5.2.5. Logistical challenges for implementation of birth dose vaccination*

The WHO summarised evidence on facilitators and barriers to improve coverage of the hepatitis B timely birth dose through a systematic review.[130] The review found that births at health facilities were associated with higher coverage of the birth dose than home births. For children born at health facilities, the integration of birth dose with maternal and newborn care is a key to improve its coverage, and this can be achieved through a local health policy specifying birth dose vaccination, ensuring the availability of the vaccine in the delivery room, giving a responsibility for vaccination to birth attendants, and coordination between immunisation staff and maternal health staff.

In contrast, for children born at home, there is some evidence mainly from China that home visits to provide timely birth dose by village health workers are more effective in increasing birth dose uptake than asking families to bring neonates to health facilities. Factors associated with higher birth dose coverage at home delivery include vaccine storage out of the cold chain, birth notification and pregnancy tracking, and the use of Uniject™ (a compact pre-filled auto-disable injection device) instead of standard needles and syringes.[130] Of note, storing hepatitis B vaccine outside the cold chain was shown to have similar immunogenicity and protective efficacy when compared to storage at a temperature of 2-8 $^{o}$C as recommended by manufacturers.[131]

In SSA including The Gambia, there has been no study investigating practices to improve the coverage of hepatitis B vaccine birth dose.[130] According to the statistics of UNICEF (2008-2012), although 98.1% of pregnant women visit antenatal care at least once in The

Gambia, only 55.7% women deliver their child at health facilities.[132] These imply that in order to improve the access to the birth dose in The Gambia, home delivery of timely birth dose needs to be considered together with the integration of birth dose with maternity care at health facilities. Otherwise, universal screening of maternal HBV infection at antenatal care and subsequent provision of selective birth dose to neonates born to infected mother can be an alternative strategy. However, no study has examined its efficacy and feasibility in SSA.

## 1.6. Factors modifying the progression of liver disease in individuals with chronic hepatitis B infection

### 1.6.1. Viral replication

HBe antigenaemia (as a proxy for high viral replication) and a high HBV DNA levels are associated with an increased risk of cirrhosis and HCC. Several population-based longitudinal cohort studies in Taiwan and China have examined the risk of cirrhosis and HCC in relation to HBV DNA levels at baseline. These studies consistently revealed that the risk of cirrhosis[133] or HCC[134,101,135] starts to increase when HBV DNA levels exceed 2,000 IU/mL. Similar findings were reported in case-control studies in Senegal,[136] South Africa,[137] and The Gambia.[138] Furthermore, several studies have investigated the trajectory of HBV DNA levels over time and its effect on HCC.[101,139,102] These studies found that the maintenance of high viral DNA levels plays a crucial role in determining the risk of HCC.

A number of studies have examined the association between viral replication and predictors of HCC. Tseng *et al*. prospectively followed a hospital-based cohort of people with CHB infection in Taiwan who experienced spontaneous HBeAg serovconversion and found that viral load after the HBeAg seroconversion was positively associated with the risk of HBeAg-negative CHB disease (ALT ≥80 IU/L and viral load ≥2,000 IU/ml) and hepatitis flare (ALT ≥200 IU/L and viral load ≥2,000 IU/ml).[91] They also demonstrated that the occurrence of HBeAg-negative CHB disease was associated with cirrhosis and HCC.

### 1.6.2. Serum HBsAg levels

The risk of HCC among chronic HBV carriers is positively associated with HBV DNA levels. However, the rates of HCC remain reasonably high even among carriers with low HBV DNA levels.[101,102] Several studies have therefore identified predictors of HCC in people with low viral replication. Tseng *et al*. prospectively assessed the effect of HBsAg levels on HCC incidence in a hospital-based cohort of people with CHB infection. Overall analysis showed that viral load at baseline predicts HCC risk better than HBsAg levels. However in a subgroup of people with negative-HBeAg and low viral load (<2,000 IU/ml), the positive association between HBsAg levels (≥1,000 vs. <1,000 IU/ml) and HCC risk was observed while viral load (<200 vs. 200-2,000 IU/ml) was not associated with HCC, suggesting that the measurement of HBsAg levels may complement viral load in predicting the HCC risk in people with low HBV DNA levels.[102]

Higher serum HBsAg levels were associated with other outcomes such as lower rate of spontaneous HBsAg loss[90] and a higher risk of HBeAg-negative CHB disease in a cohort with negative HBeAg and low viral load (<2,000 IU/ml) in Taiwan.[92]

1.6.3. Viral genotype

There are at least eight genotypes (A-H) of HBV, based on a nucleotide diversity of more than 8% in the complete genome sequence.[6] The viral genotype appears to modify the natural history of infection and risk of HCC. In Taiwan where genotype B (80%) and C (20%) predominate, genotype C was associated with delayed HBeAg seroconversion[83,140] and increased HCC incidence.[134] Furthermore, a Taiwanese study comparing 107 paediatric cases who became chronic carriers despite HBV vaccine and HBIG with 214 age-matched controls who became

carriers without being given the vaccine found that genotype C was associated with vaccine failure.[141] A subsequent study of children with CHB infection by the same authors showed that children with HBeAg-positive mothers have higher prevalence of genotype C than those with HBsAg-negative mothers, suggesting a link between genotype C and mother-to-infant transmission.[83] A similar finding has been reported in a study conducted in Japan.[142]

Although most HBV endemic areas have only two HBV genotypes, five genotypes (A, B, C, D and F) are found in Alaska. Livingston *et al*. found an association between genotype C and the persistence of HBeAg,[143] and between genotype F and HCC.[144]

In Africa, genotype A, D and E are common, and the predominant genotype varies with the geographical area: genotype A in South, East and Central Africa; genotype D in North Africa; and genotype E in West Africa.[145] Few African studies have related viral genotypes to clinical outcomes.[145] In Bantu-speaking South Africans, a case-control study of HBV-related HCC with asymptomatic HBV-positive controls matched for age and sex found that the prevalence of genotype A was significantly higher in cases (86.5%, 96/111) than in controls (68.5%, 76/111).[146] The majority of non-A genotypes in this study was D. In The Gambia, the vast majority is genotype E (75-95%) followed by genotype A.[147,148149] The Gambia Liver Cancer Study (GLCS) did not find an association between HBV genotype and HCC or cirrhosis, but the sample size was small. The prevalence of genotype E was 77% (33/43) in HBV-related HCC, 79% (15/19) in HBV-related cirrhosis and 50% (2/4) in HBsAg-positive controls.[149] Preliminary data from other West African countries showed that HBeAg prevalence was higher in subjects infected with genotype E (89.8%, 35/39) than in those with genotype A (33.3%, 2/6).[150]

### 1.6.4. Aflatoxin

Aflatoxin is a mycotoxin produced by fungal species of the genus *Aspergillus*. The contamination of agricultural products such as groundnuts and maize with aflatoxin is facilitated by hot and humid climates.[151] Aflatoxin exposure alone is associated with an increased risk of HCC, and the combined effect of aflatoxin and CHB infection is greater than multiplicative.[152]

In The Gambia, where groundnuts are dietary staples, people are highly exposed to this toxin.[153,154] Studies have demonstrated seasonal variation in the serum aflatoxin-albumin adduct levels, with a peak in the dry season, related to the availability of groundnuts. Cross-sectional studies have examined the association between aflatoxin-albumin adduct levels and HBV seromarkers in adults and children. In adults, aflatoxin-albumin adduct levels were not associated with HBsAg, HBeAg, or HBV-DNA positivity[153] whilst in children acute hepatitis B was associated with higher levels of aflatoxin-albumin adduct.[154]

Aflatoxin-albumin adducts only reflect recent exposure (over the past 2-3 months).[155] Recent studies have used biomarkers of historic aflatoxin exposure. Kirk *et al*. examined the prevalence of a specific mutation at codon 249 of TP53 tumor suppression gene ($249^{ser}$; AGG to AGT, Arg to Ser), a mutation induced by aflatoxin exposure, in a case-control study of HCC in The Gambia. The HCC risk was increased in HBsAg-positive subjects without the $249^{ser}$ mutation (OR, 10.0; 95% CI: 5.2-19.6), in those with positive $249^{ser}$ mutation without HBsAg (OR, 13.2; 95% CI: 5.0-35.0) and in those with both positive HBsAg and the mutation (OR, 399; 95% CI: 48.6-3270), suggesting that the combined effect is greater than multiplicative.[156] However the

association was not found between the 249$^{ser}$ mutation and hepatitis B e antigenaemia[156] or serum HBV viral load in The Gambia.[148]

### 1.6.5. Alcohol

Alcohol is known to cause liver disease including HCC.[155] However, the interaction between alcohol and CHB infection is controversial. Case-control studies mainly from Europe suggest that the combined effect of CHB infection and alcohol intake on HCC is more than additive (and sometimes even more than multiplicative).[157] In contrast, many Asian cohort studies found the effects of CHB infection and alcohol combine additively.[98,158,159] Because alcohol consumption is usually influenced by disease status, case-control studies are less informative than cohort studies.[8] In The Gambia, more than 95% of the population are Muslim[160] and regular alcohol use was observed only in 8% of controls in a case-control study of HCC.[161]

### 1.6.6. Metabolic factors

Three systematic reviews of cohort studies examining the effect of diabetes mellitus on HCC support a positive association between these diseases.[162–164] The excess risk of HCC attributable to obesity or diabetes also occurs in people with CHB infection. A cohort study in Taiwan, the relative risk (RR) for joint exposures of obesity (body mass index: BMI ≥30 kg/m$^2$) and CHB infection on HCC (22.0, 95% CI; 10.3-46.9) was almost equal to the sum of the RRs for CHB infection (19.9, 95% CI; 14.3-27.6) and for obesity alone (2.50, 95% CI; 0.99-6.32), whilst the RR for combined effect of diabetes and CHB infection on HCC (43.5, 95% CI; 20.5-92.3) was more than the sum of the RRs for CHB infection (18.7, 95% CI; 13.6-25.9) and for diabetes alone (3.49, 95% CI; 1.08-11.3).[165] In The Gambia, very low prevalence of obesity (BMI ≥30

kg/m$^2$, 2.3%, 140/6024) and diabetes (fasting glucose ≥6.7 mMol/L, 0.2%, 14/5898) were reported in the general population aged over 15 years.[166] However, these estimates are from a nationwide survey conducted in 1995 and the figures might have changed since then.

### 1.6.7. Co-infection with hepatitis C virus

Two meta-analyses have examined the effect of dual infection with HBV and hepatitis C virus (HCV) on HCC. Both have shown that the combined effect on HCC are between additive and multiplicative. The first meta-analysis of 32 case-controls studies reported that the summary OR for HBsAg positivity and anti-HCV/HCV RNA negativity was 20.4 (95% CI; 18.0-23.2), the OR for HBsAg negativity and anti-HCV/HCV RNA positivity was 23.6 (95% CI; 20.0-28.1) and the OR for both markers of positivity was 135 (95% CI; 79.7-242).[167] The second meta-analysis was restricted to studies in China and reported that the summary OR for HBsAg positivity and anti-HCV/HCV RNA negativity was 15.6 (95% CI; 11.5-21.3), the OR for HBsAg negativity and anti-HCV/HCV RNA positivity was 8.1 (95% CI; 5.0-13.0) and the OR for both markers positivity was 35.7 (95% CI; 26.2-48.5).[168]

In The Gambia, the Gambia Liver Cancer Study (GLCS), a case-control study of HCC between 1997 and 2001, reported that the OR for joint exposures of dual infection on HCC (35.3, 95% CI; 3.9-323) was slightly greater than the sum of the ORs for HBsAg positivity (16.7, 95% CI; 9.7-28.7), and for anti-HCV/HCV RNA positivity alone (16.7, 95% CI; 6.9-40.1).[169] In this study, the prevalence of HCV infection differed between generations: the prevalence was <1% in controls younger than 50 years of age and 5.7% in controls aged over 50 years. An earlier case-control study of HCC conducted in The Gambia in 1988 found that the prevalence of HCV

infection in controls was 3.3% in those less than 50 and 6.3% in those over 50 years old.[169] The decrease in HCV prevalence after 10 years in the younger controls (from 3.3% to <1%) was thought to be related to a cohort effect of HCV infection in The Gambia due to historical unsafe injections,[169] as has been observed in other African countries.[170,171] Subsequent studies supported this hypothesis. In a sero-survey of blood donors (mean age 30.6 years) in The Gambia in 2002, the prevalence of anti-HCV was 1.1% (5/460).[172] In a population-based sero-survey in 2008 recruiting young adults (18 to 22 years old) estimated the prevalence of anti-HCV (3rd generation ELISA) to be 0.5% (13/2598).[173]

### 1.6.8. Co-infection with hepatitis delta virus

Hepatitis delta virus (HDV) is a defective RNA virus which needs the presence of HBV for its own life cycle, and therefore can only infect the people infected with HBV.[174] Routes of transmission include parenteral or sexual through exposure to contaminated blood or body fluid.[175] There are two patterns of HDV infection: acute co-infection where uninfected individuals are simultaneously infected with HDV and HBV, and superinfection with HDV in people with established CHB infection. In the concomitant infection, the clinical course of acute hepatitis is more severe but the risk of progression to chronic HBV infection seems to be similar to acute HBV mono-infection.[174] The risk of liver cirrhosis and HCC are higher after superinfection with HDV than in HBV mono-infected individuals.[72] In The Gambia, HDV is rare. In the villages of Keneba and Manduar no one under 20 years was positive for anti-HDV.[60]

### 1.6.9. Co-infection with HIV

The epidemiology of HBV and HIV co-infection differs considerably between high-income countries (Europe and North America) where the HBV prevalence is low and low-income countries (Asia and sSA) where the HBV prevalence is high. In high-income countries, the prevalence of CHB infection is higher in HIV-infected than non-infected people, because HBV and HIV share common routes of transmission (i.e., sexual or contaminated needles and syringes). In low-income countries, the prevalence of CHB infection is usually similar in HIV-infected and non-infected people because HBV infection mainly occurred before adolescence in the pre-hepatitis B vaccination era.[176] In The Gambia, where HIV prevalence is 1.5%,[177] 12.2% of individuals infected with HIV are HBsAg-positive (95% CI; 9.0-15.0%),[178] which is similar to the prevalence in Gambian adults in the general population (14-16%).[55,169]

HIV co-infection modifies the natural history of CHB infection and *vice versa*. Compared to people without HIV infection, HIV-infected people are more likely to progress to chronic infection after exposure to HBV.[179] Once HIV-infected people become chronic HBV carriers, they have a slower rate of HBeAg seroconversion,[180] higher HBV DNA levels,[181] and higher risk of liver cirrhosis[181] than HBV mono-infected people. In a cohort of 5,293 men who have sex with men in the USA the combined effect of HBV and HIV on liver diseases was more than additive. The rate of liver-related mortality (per 1,000 person-years) was 0.0 in both HBsAg and HIV negative men, 0.8 in HBsAg-positive and HIV-negative men, 1.7 in HBsAg-negative and HIV-positive men and 14.2 in dually infected men.[182]

# 1.7. The effect of early age at hepatitis B virus infection on the risk of hepatocellular carcinoma and its predictors

1.7.1. Evans's hypothesis

As presented earlier (section 1.1.), the prevalence of CHB infection varies considerably by geographical region, as does the incidence of HCC. And highly endemic areas of HBV infection have a high HCC incidence.[1] However, the risk of HCC amongst people with CHB infection also varies markedly by geographical areas. Population-based cohort studies in HBsAg-positive men have estimated age-standardized HCC incidence rates of 34/100,000 carrier-years in Europe[99,100] to 68/100,000 in Senegal, [97] 230/100,000 in Alaska[81] and 530-1,030/100,000 in East Asia[98,101] (table 1.3-c). This variation in the incidence of HCC appears to be due to variation in the natural history of CHB infection as is discussed below.

The persistence of high HBV viral load,[101] or e antigenemia,[183] plays an important role in increasing the risk of HCC, and variations in the prevalence of e antigenaemia may explain the observed geographical difference in HCC incidence. In Taiwan, about half of HBsAg-positive children remain HBeAg-positive into their twenties,[184] while in sub-Saharan Africa, where HBV endemicity is similar, the prevalence of HBeAg among HBsAg-positive people declines to 10% in the second decade of life.[185] Evans *et al*. identified factors responsible for the persistence of high viral replication in an ecological study comparing Chinese and African populations. They suggest that earlier age at HBV infection and prolonged maintenance of viral replication in Asians with CHB infection is the cause of the higher HCC incidence in Asia.[97] In the pre-vaccination era, the average age at which persistent infection is established should have

been earlier in Asia where perinatal transmission was common than in sSA where child-to-child transmission predominated.

It is well established that early age at HBV infection increases the risk of chronic infection after exposure to HBV.[68,69] Early infection is therefore associated with a higher risk of HCC through the increased risk of chronic infection.[186] The hypothesis proposed by Evans *et al*. implies that early HBV infection should further increase the risk of HCC through the persistence of high viral replication, beyond its effect of increasing the chance of becoming a chronic carrier.[83] This thesis examines whether early age at HBV infection is associated with higher risk of HCC and its predictors amongst chronic carriers of HBV in The Gambia, West Africa.

### 1.7.2. Outline of the thesis

In Chapter 2, the use of birth order as a proxy indicator for the age at HBV infection is discussed. In Chapter 3, a systematic review of observational studies examining the association between early age at HBV infection and HCC and its predictors is presented. In Chapter 4, a historical dataset collected in The Gambia is analysed to determine the association between familial HBV sero-marker and the risk of hepatitis B e antigenaemia. An historical case-control study of HCC in The Gambia is analysed in Chapter 5 to determine the effect of birth order on HCC. Results from the long-term follow-up of chronic HBV carriers in three rural villages in The Gambia are described in Chapter 6. In particular this chapter examines the effect of having an HBsAg-positive mother on the predictors of HCC (HBeAg/HBsAg seroclearance, mean HBV DNA levels over time, and significant liver fibrosis). In Chapter 7, the association

between birth order and HCC is again examined in a new HCC case-control study within the PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa) project in The Gambia.

# Chapter 2. The use of birth order in the epidemiology

## 2.1. Introduction

This thesis examines the relationship between age at hepatitis B virus (HBV) infection and risk of hepatocellular carcinoma (HCC) in individuals with chronic HBV infection. Ideally the age at the time of HBV infection should be estimated by repeatedly testing a cohort of uninfected people for hepatitis B surface antigen (HBsAg). However, this study design requires a long period of follow-up and is therefore difficult to conduct.[187] Alternatively, proxy markers for different modes of HBV transmission can be used to estimate the age at infection. Maternal HBV sero-status is an indicator for perinatal maternal transmission: a chronic HBV carrier whose mother is HBsAg-positive or hepatitis B e antigen (HBeAg)-positive is more likely to have been perinatally infected by their mother than a chronically infected individual with a seronegative mother. The serological profile of older siblings may also serve as a proxy for early childhood transmission, since a chronic HBV carrier who has older siblings with positive HBV sero-marker is more likely to have been infected by their siblings during early childhood than a chronic HBV carrier without any seropositive older siblings. Finally, some studies have used birth order as a proxy for age at infection by assuming that in the absence of immunisation, children with low birth order are exposed to HBV after they start schooling, whilst children with high birth order are infected much earlier by their older siblings who got the infection outside the household.[187]

Birth order has been used as surrogate for biological and social risk factors in a number of studies, including diabetes,[188] cancer,[189] allergic conditions,[190] and all-cause mortality.[191] In this chapter, the definition of birth order, methods to examine the effect of birth order, potential confounding factors for the effect of birth order on diseases, and previous epidemiological studies applying this are discussed.

## 2.2. Definition of birth order

The definition must specify how miscarriages, multiple pregnancies and half-siblings are handled, and should reflect the period when the postulated aetiological factor such as infection plays its role.[192] When the risk factor is thought to affect an individual before their birth (e.g. intrauterine environment), miscarriages and stillbirths should be included in birth, whereas twins or triplets should be counted as one and half-siblings should not be included (table 2.1). In contrast, when the exposure to the risk agent is postulated to occur postnatally (e.g. childhood environment), birth order should be obtained by enumerating the number of older siblings who were alive during the risk period.[192] Table 2.1 shows wide variation in what the birth order indicates in birth order studies of different cancer sites.

Table 2.1 Definition of birth order

| **Hypothesised factor** | **Miscarriages / stillbirths** | **Multiple pregnancies** | **Half-siblings living with an affected individual** |
|---|---|---|---|
| Prenatal environmental factor | Counted | Counted as one | Not counted |
| Postnatal environmental factor | Not counted | Counted as independent births | Counted |

Table 2.2. Sources and definitions of birth order in previous epidemiological studies assessing the effect of birth order on the risk of cancer

| Author, year | Country | Study design | Population studied | Source of birth order | Definition of birth order | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Basis for definition | Miscarriages and stillbirths | Twins | Half siblings |
| **HCC** | | | | | | | | |
| Ryder, 1992[193] | Gambia | CC | Adults | Self-reported | N/R | N/R | N/R | N/R |
| Hsieh, 1992[187] | Greece | CC | Adults | Self-reported | Children in the family | Not counted | Counted as independent birth | N/R |
| Kuper, 2000[194] | Greece | CC | Adults | Self-reported | Children in the family | Not counted | Counted as independent birth | N/R |
| **Swedish Family-Cancer Database** | | | | | | | | |
| Hemminki, 2001[195]; Altieri, 2007[196]; Bevier, 2011[189] | Sweden | Co | Children & adults | Second-Generation Register | Maternal parity | N/R | N/R | In the case of divorce, assumption that all children lived with the mother |
| **Breast cancer** | | | | | | | | |
| Hsieh, 1991[197] | USA, Wales, Japan | CC | Adults | Self-reported | Children in the parental family who lived to 40 years | N/R | Counted as single pregnancy | N/R |
| Hodgson, 2004[198] | USA | CC | Adults | Self-reported | N/R | N/R | N/R | N/R |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Sorensen, 2005[199] | Denmark | CC | Adults | Midwife record | N/R | N/R | N/R | N/R |
| **Testicular cancer** | | | | | | | | |
| Prener, 1992[200] | Denmark | Nested CC | Children | Parental information | Children in the family | N/R | N/R | N/R |
| | | | | Midwife record | Maternal parity | Counted (miscarriages) | N/R | N/R |
| Westergaard, 1998[201] | Denmark | Co | Children | Danish Civil Registration System | Maternal parity | Not counted | N/R | N/R |
| Richiardi, 2004[202] | Sweden | Nested CC | Adults | Swedish Multi-Generation Register | N/R | N/R | Counted as singleton birth | N/R |
| **Gastric cancer** | | | | | | | | |
| Blaser, 1995[203], 2007[204] | USA | Co | Adults | Self-reported | Children in the family | Not counted | Counted as independent birth | N/R |
| **Acute leukaemia** | | | | | | | | |
| MacMahon, 1962[205] | USA | CC | Children | National Office of Vital Statistics | Maternal parity | Not counted | N/R | N/R |
| Dockerty, 2001[206] | UK | CC | Children | Birth record | Maternal parity | Counted (stillborns) | N/R | Excluding children born outside marriage |
| **Non-Hodgkin lymphoma** | | | | | | | | |
| Mensah, 2007[207] | UK | CC, | Adults | Self-reported | N/R | N/R | N/R | N/R |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | GY | | | | | | |
| Grulich, 2010[208] | Multi-national | CC | Adults | Self-reported | N/R | N/R | N/R | N/R |
| Crump, 2012[209] | Sweden | Co | Children & adults | Birth registry & national census | N/R | N/R | N/R | N/R |
| **Hodgkin lymphoma** | | | | | | | | |
| Gutensohn, 1981[210] | USA | CC | Adults | Self-reported | N/R | N/R | N/R | N/R |
| Westergaard, 1997[211] | Denmark | Co | Children & adults | Danish Civil Registration System | Maternal parity | Not counted | N/R | N/R |

Abbreviations: N/R, not reported; Co, cohort study; CC, case-control study; GY, Greenwood-Yule methods.

## 2.3. Design of birth order study

There are two methods for investigating birth order associations. One is the case-control approach, the other is the Greenwood-Yule method.[192] The latter assumes a random distribution of individuals by birth order in the population and studies whether the observed distribution of birth rank among affected individuals is different from what would be expected by chance.[212] The expected distribution of birth order is derived from a total sibship size of affected individuals, and the ratios of observed/expected in each birth order are examined. Haldane and Smith developed a statistical test which compares the sum of the birth orders of all affected cases with the expected value. If the observed sum is significantly higher than the expected sum, it can be concluded that higher birth order is associated with the disease.[213]

These classical methods have advantages over the case-control method since it can be applied to a study without having a control group; the analysis of birth order is made within sibships and is therefore less prone to confounding by socioeconomic status and demographic factors.[214] However, the method is susceptible to bias when there are incomplete sibships or changes in population dynamics.[215–217] Sibships are often incomplete in studies of children or younger adults because mothers of cases may have further children after the data were collected. Incomplete sibships violate the assumption that individuals are randomly distributed by birth order because there is an excess of later-born children.[216] Changes in population dynamics may also result in bias in the Greenwood-Yule method. First, a change in the annual birth rate will influence the distribution of birth order in the population;[216,217] increased birth rate will lead to an excess of early-born children. Second, migration causes the birth order distribution to depart from being random, because population movement generally is related with birth order.[216] Because of these potential biases in the Greenwood-Yule method, modern cancer epidemiology generally employs either case-control or cohort study to examine for birth order associations (table 2.2).

## 2.4. Potential confounding factors for the association of birth order with a disease

Studies of the effect of birth order on cancer are inconsistent in whether they adjust for total sibship size or number of younger siblings (Table 2.3). Out of 21 studies examined, 4 adjusted for sibship size, 2 adjusted for number of younger siblings, while 15 did not adjust for either

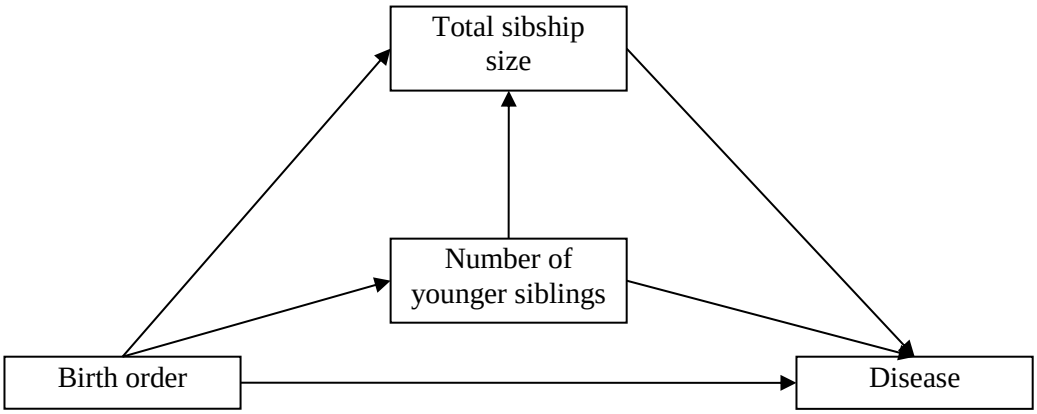
factor. The arguments for and against adjusting for total sibship size and the number of younger siblings are presented below.

### 2.4.1. Total sibship size

The importance of adjusting for total sibship size in birth order studies has been advocated by several investigators to obtain an accurate estimate of the effect of the birth order.[187,218] This is because a disease which tends to occur in large sibships may provide a false impression that the disease is more common in higher birth orders,[214] and that large sibship size is often related to lower socioeconomic status,[207] which is a known risk factor for many of the diseases. In contrast, others claim that total sibship size should not be introduced in the same model with birth order because the two variables are substantially correlated. Spearman's correlation coefficient for birth order and total sibship size was 0.41 in a study of gastric cancer,[203] 0.69 in a study of testicular cancer,[202] and 0.50 in the study of HCC;[194] and thus, collinearity was suspected.[202] In addition, Richiardi *et al*.[202] argued that empty cells in the regression models created by impossible combinations (e.g., a fifth-born child in a family with sibship of three) hamper inclusion of total sibship size in the model. Instead of total sibship size, several investigators controlled for the number of younger siblings.[202,211]

It is important to consider the direction of causation between birth order and total sibship size in order to judge whether total sibship size confounds the association of birth order with disease incidence. Birth order is always determined at birth and temporally precedes total sibship size, which depends on the number of children subsequently born into the family. Consequently, total sibship size is affected by birth order and the number of younger siblings (figure 2.1), and thus should not be regarded as a confounding factor in birth order studies, unless the objective is to examine the effect of birth order which is not mediated through total sibship size.

Figure 2.1 A causal diagram of birth order, number of younger siblings and total sibship size




2.4.2. Number of younger siblings

Birth order is equal to the number of elder siblings plus one, in the absence of miscarriage, stillbirth or child death. The number of younger siblings is determined after birth order has been established, and the birth order might affect the number of younger siblings because parental behavior pertaining to conception is at least partly influenced by the number of children they already have. Consequently, the number of younger siblings cannot be considered as a confounding variable in studies examining the birth order effect, unless the objective is to estimate the effect of birth order which is not mediated by the number of younger siblings (figure 2.1).

Due to the controversy, two distinct models are used throughout this thesis to assess the effect of birth order on liver disease. First model includes parental education levels or index cases' education levels as proxy measures for socioeconomic status. The second model includes total sibship size as a surrogate for the socioeconomic status.

Table 2.3 Confounding factors in previous observational studies assessing the effect of birth order on the risk of cancer

| Author, year | Birth order as a proxy for | Confounding factors | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sex | Age | Birth year | Race | Region | SES | Family history of cancer | Maternal age at birth | Paternal age at birth | Total sibship size | No. younger siblings | Others |
| **HCC** | | | | | | | | | | | | | |
| Ryder, 1992[193] | Age at HBV infection | M | M | M | - | M | - | - | - | - | - | - | - |
| Hsieh, 1992[187] | | A | A | A | R | - | - | - | - | - | A | - | Cigarettes, HCV |
| Kuper, 2000[194] | | M/A | M/A | M/A | - | - | A | - | - | - | A | - | Cigarette, alcohol |
| **Swedish Family-Cancer Database** | | | | | | | | | | | | | |
| Hemminki, 2001[195] | N/A | Str | A | A | - | A | A | R | - | - | - | - | Participant's parity and age at first childbirth (only for breast cancer) |
| Altieri, 2007[196] | N/A | A | A | A | - | A | A | A | - | - | - | - | - |
| Bevier, 2011[189] | N/A | A | A | A | - | A | A | R | - | - | - | - | - |
| **Breast cancer** | | | | | | | | | | | | | |
| Hsieh, 1991[197] | Intrauterine oestrogen levels | R | A | - | - | A | - | - | A | - | - | - | Participant's own parity, menopause, age at first |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | childbirth, age at menarche, height, and Quetelet's index |
| Hodgson, 2004[198] | | R | A | R | A | - | A | - | A | - | - | - | Current BMI, sampling fraction |
| Sorensen, 2005[199] | | R | A | M | - | - | - | - | - | - | - | - | Birth weight, marital status of the mother |
| **Testicular cancer** | | | | | | | | | | | | | |
| Prener, 1992[200] | Intrauterine oestrogen levels | R | M | A | - | - | A | - | - | - | - | - | Cryptorchidism, birth weight |
| Westergaard, 1998[201] | | R | A/Str | A/Str | - | - | - | - | A/Str | - | - | - | Maternal age at first birth, interval to previous delivery, sex of the first-born child |
| Richiardi, 2004[202] | | R | A | A | - | A | A | R | A | A | - | A | - |
| **Gastric cancer** | | | | | | | | | | | | | |
| Blaser, 1995[203], 2007[204] | Age at *H. pylori* infection | R | M/A | M/A | R | R | - | - | - | - | - | - | Cigarettes (2007 study only) |
| **Acute leukaemia** | | | | | | | | | | | | | |
| MacMahon, | Age at | - | M/St | M/St | Str | - | - | - | Sta | - | - | - | - |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1962[205] | common infection | | a | a | | | | | | | | | |
| Dockerty, 2001[206] | | M | Str | M | - | M | A | - | A | - | - | - | - |
| **Non-Hodgkin lymphoma** | | | | | | | | | | | | | |
| Mensah, 2007[207] | Age at common infection, EB virus | M/A | M/A | M | - | A | - | - | - | - | - | - | - |
| Grulich, 2010[208] | | M/A | M/A | - | R | M/A | Str | - | - | - | Str | - | - |
| Crump, 2012[209] | | A | - | A | - | - | A | A | A | - | - | - | Fetal growth, gestational age, multiple birth, |
| **Hodgkin lymphoma** | | | | | | | | | | | | | |
| Gutensohn, 1981[210] | Age at common infection | M | M | - | - | - | A | - | - | - | A | - | Religion, playmates, infectious mononucleosis |
| Westergaard, 1997[211] | | A | A | A | - | - | - | - | A | - | - | A | - |

Abbreviations: M, variable matched on; A, variable adjusted for; Str, variable stratified by; R, variable restricted for; Sta, variable standardised by; SES, socioeconomic status.

## 2.5. Association between higher birth order and lower age at infection with common pathogens

Age of infection is often assumed to be negatively correlated with birth order. For example, Stallybrass states that "*the child attending the day school is much more likely to introduce the disease to the younger members of his own family than is the boarder. And so the attendance of the first-born at a day school often involves the repeated introduction of infection into his family*".[219] Similarly, Fox, Hall and Elveback suggest that "*the aspect of birth order is the influence it has upon the age and the overall probability of acquiring infections that are transmitted by contact. Contact between family members is particularly close so that, when one member becomes infected, he typically shares it with those not already immune. As a corollary, the larger the family, the greater is the probability that one member will bring an infection home. Also, the higher the birth order (i.e., the younger the child), the greater is the probability of contracting an infection at an early age from an older sibling*".[35] The evidence for the association between birth order and age at infection is outlined below.

### 2.5.1. Acute childhood infection

Badger *et al*.[220] identified the characteristics of family members at risk of introducing respiratory infection into the home in a cohort study in Cleveland. An index case was defined as a person who developed acute respiratory symptoms at least 10 days after the onset of any respiratory illness within a family. School children were more likely to become index cases than preschool children. In addition, amongst preschool children with one sibling, the incidence of

respiratory diseases was higher when the sibling was a school child than when the sibling was a preschool child. These findings suggest that school children serve as the most frequent source of respiratory infections within the family.

In Houston a cohort of children was followed for influenza infection over the first year of life.[221] The incidence increased with increasing birth order from 18 per 100 infant-years in first-born children to 44 in third-born children.

A UK birth cohort was used to determine the effect of birth order on the risk of childhood infections by age seven.[222] After controlling for socioeconomic status, higher birth order was associated with an increased risk of pertussis. However, birth order had no effect on the risk of measles, rubella, varicella and mumps. The lack of association was explained by: 1) birth order having a limited effect after children start school, and 2) episodes of infection were retrospectively elicited from parents leading to potential recall bias.[223]

In Guinea-Bissau, West Africa, the attack rate of measles in children aged 6-35 months was greater with increasing number of children aged <6 years in the same house (26.4%, 32.0%, and 40.5% in households with 1-2, 3-4 and ≥5 children, respectively).[224] A positive association between measles and the number of children <6 years old in the house was also observed in infants aged 0-5 months. Unfortunately the study did not examine the relationship between birth order and measles.

### 2.5.2. *Helicobacter pylori* infection

About half the global population is infected with *Helicobacter pylori*, and in some areas the majority of adults show evidence of the infection. Having an infected sibling is a risk factor for *H. pylori* infection in children, illustrating the importance of sibling-to-sibling transmission during childhood.[225] Other studies have found a positive association between birth order and prevalence of *H. pylori* infection.[226,227] <sup>226</sup>For example in Columbia, the odds of infection in children increased with increasing birth order; adjusted ORs compared with first-born children were 1.8 (95% CI: 1.0-3.3), 2.1 (1.0-4.4) and 2.2 (0.9-5.2) in children born 2$^{nd}$, 3$^{rd}$ and ≥4$^{th}$, respectively.[228] These findings support the role of birth order as a marker of early exposure to *H. pylori*.

## 2.6. Use of birth order in cancer epidemiology

Birth order has been used in cancer epidemiology as a surrogate for the intrauterine hormonal environment (breast and testicular cancer) or age at infection (liver, stomach and haematologic malignancy including childhood leukaemia, non-Hogdkin's lymphoma, and Hodgkin's disease).

### 2.6.1. Breast cancer

Trichopoulos[229] proposed that an intrauterine environment with high oestrogen levels would lead to increased risk of breast cancer decades later. Birth order can be used as a proxy for oestrogen levels since the first pregnancy has higher oestrogen levels than subsequent

pregnancies.[198,230,231] The hypothesis has been confirmed in some studies[197,199] but other studies have not found an association.[189,195,196,198]

### 2.6.2. Testicular cancer

A high level of maternal oestrogen is thought to increase the risk of cryptorchidism and testicular cancer.[200,201] Thus an inverse association between birth order and risk of testicular cancer is predicted, and this has generally been observed.[189,200–202] However, a cohort study in Sweden found no association.[195]

### 2.6.3. Gastric cancer

A positive association between birth order and risk of *Helicobacter pylori*-associated stomach cancer has been reported in Japanese-American men infected with *H. pylori*.[203,204] The hypothesis is that children with a high birth order (i.e., a birth order greater than two) acquire *H. pylori* infection at a younger age than first- or second-born children, and early exposure to *H. pylori* infection is associated with: 1) a longer duration of the infection, 2) immaturity of host immune system, and 3) interaction with other childhood infections, which may further enhance the gastric cancer risk associated with *H. pylori* infection.[203,204]

### 2.6.4. Haematologic malignancy

Birth order has been used as a surrogate for the timing of exposure to infections in the epidemiology of haematologic malignancy. An increased risk of childhood leukaemia for first-born children has been observed in many studies.[205,206,232–235] Greaves has proposed that

delayed or diminished exposure to common infections in infancy is associated with an increased risk of the precursor B-cell subtype of acute lymphoblastic leukaemia.[236]

There is inconsistent evidence regarding the association between birth order and risk of non-Hodgkin lymphoma (NHL). Some case-control studies have demonstrated a positive association, however, these could be attributable to a selection bias due to differential study participation between cases and controls according to socioeconomic status and birth order (see further discussion in Chapter 5 & 7).[207,208] A recent large-scale Swedish national cohort study found an inverse association between birth order and NHL in children and young adults. The study supports the hypothesis that delayed exposure to Epstein-Barr virus and other infectious agents impedes the maturation of the immune system, and this may predispose individuals to NHL.[209] Studies have yielded conflicting results for the association of birth order with Hodgkin lymphoma.[210,211,237]

## 2.7. Birth order and the epidemiology of hepatitis B virus infection in The Gambia

The definition of birth order used to estimate the timing of HBV infection in chronic HBV carriers should differ according to local epidemiology of the infection. Where perinatal mother-to-infant transmission of HBV is common, low birth order is a proxy for perinatal transmission; this is because low birth order children have younger mothers who were more

viraemic than older mothers at the birth of the children (viraemia decreases with age). Indeed, a study in Taiwan demonstrated that low maternal age was associated with higher risk of perinatal transmission.[125] To reflect maternal age, birth order should count miscarriages and stillbirths but not twins and half-siblings.

In contrast, where horizontal transmission during early childhood is common, high birth order may indicate earlier age at HBV infection through sibling-to-sibling transmission.[187] Consequently, birth order should reflect the number of older children that lived in the same household during the childhood.[187,194] Therefore when birth order is a proxy for horizontal transmission in early childhood it should include half-siblings and count twins independently.

In the pre-HBV vaccine era in The Gambia, most HBV infection occurred during early childhood.[42,60] Therefore, a positive association between birth order and risk of liver diseases is expected, and birth order should exclude miscarriages and stillbirths, whilst twins or triplets need to be enumerated independently. Half-siblings who grew up together with the participants also need to be included. It is particularly important to include half-siblings because The Gambia is a polygamous society. Perinatal maternal transmission is less common in The Gambia than in Asia,[63] but it certainly exists in The Gambia.[238] Therefore, the positive association of birth order with liver diseases might be J- or U-shape rather than linear (i.e., a moderate risk for low birth order, the lowest risk for intermediate birth orders and the highest risk in high birth orders).

## 2.8. Summary

It is difficult to estimate the age at HBV infection in epidemiological studies. Birth order may be a useful proxy for the age at infection. Definition of birth order depends on the study hypothesis. Where postnatal child-to-child transmission of HBV is common, higher birth order predicts earlier age at HBV infection during childhood, and birth order should count twins independently and include half-siblings. There is evidence for an inverse association between birth order and age at infection in common acute childhood infection and *H. pylori*. In contrast, where mother-to-infant transmission of HBV is common, lower birth order predicts earlier age at HBV infection because high viral replication that determines the risk of perinatal transmission is more frequent in younger mothers. To reflect maternal age, birth order should be defined to include miscarriages and still births. In this thesis, confounding due to socio-economic status is accounted for using education or total sibship size as a proxy measure.

# Chapter 3. Association of early age at establishment of chronic hepatitis B infection with persistent viral replication, liver cirrhosis and hepatocellular carcinoma: a systematic review

## 3.1. Abstract

**Background**

Age at infection with hepatitis B virus (HBV) is a known risk factor for chronic HBV infection. In addition, there is evidence that early age at infection further increases the risk of primary liver cancer beyond its association with increased risk of chronic infection. This systematic review of observational studies assesses the association between age at initiation of chronic HBV infection and liver cirrhosis, hepatocellular carcinoma, and their predictors including indicators of ongoing viral replication and hepatic damage.

**Methods**

The review includes birth order and maternal HBV serology as proxies for age at infection. Electronic searches in two English-language (Medline and Embase, until Jan 2012) and two Chinese-language (CNKI and SinoMed, until Sep 2012) databases without language restriction and manual search through reference lists were conducted.

**Results**

Of 7,077 papers identified, 19 studies of 21 outcomes (8 primary liver cancer, 1 liver cirrhosis, 10 viral replication and 2 liver inflammation) are included. No African study was identified. One study directly examined the age at infection in a longitudinal cohort, 12 assessed maternal sero-status and 6 investigated birth order. The direction of associations in all studies was in accordance with the hypothesis that earlier age at infection is associated with worse outcomes in addition to its effect of increasing the probability of chronic HBV infection, although there was evidence of publication bias.

**Conclusion**

Early age at HBV infection increases the risk of liver cancer and its predictors in chronic carriers of HBV. This has implications for the control of hepatitis B.

## 3.2. Introduction

Age at infection is known to influence the establishment of HBV infection. Infection persists in 80-90%, 20-30%, <10%, and <5% of people infected perinatally, in early childhood, adolescence, and adulthood, respectively.[68] However, it is unclear whether early HBV infection also increases the risk of liver cirrhosis and/or HCC through the persistence of high viral replication, in addition to increasing the risk of chronic infection.[239] I therefore undertook a systematic review of observational studies where the association between age at establishment of chronic HBV infection and risk of cirrhosis and/or HCC has been investigated among people chronically infected with HBV.

In addition to liver cirrhosis and HCC as endpoints, two indicators of ongoing viral replication (serum HBV DNA level and presence or persistence of hepatitis B e antigen (HBeAg)) and two of hepatic damage (elevated serum alanine transaminase (ALT) and degree of hepatic fibrosis) were included. These are important predictive factors for cirrhosis and HCC, and often used as indicators for antiviral treatment of CHB infection.[6] Our hypothesis is that earlier age at infection is associated with worse outcomes in addition to its effect of increasing the probability of chronic HBV infection.

## 3.3. Methods

3.3.1. Inclusion criteria

*3.3.1.1. Types of participants*

CHB infection was defined as serum HBsAg positivity on two occasions at least 6 months apart. However, because new HBV infections in adults are uncommon in highly endemic areas where the vast majority of HBsAg-positive people acquire the infection perinatally or during childhood, HBsAg positivity on only one occasion in adults living in highly prevalent communities was assumed to reflect chronic carriage of HBsAg.[97]

*3.3.1.2. Exposures of interest*

The age at the time of infection with HBV was estimated by one of the following: 1) direct measurement through frequent serological examination of an uninfected cohort to determine the time point at which a person sero-converted from negative- to positive-HBsAg; 2) the HBV

serological profile (HBsAg and/or HBeAg) of the mother of the participant; or 3) the person's birth order. Maternal history of HBV infection was not considered in the review because it is frequently unknown, and absence of a history does not necessarily indicate that the mother is negative for HBV marker.

*3.3.1.3. Outcomes of interest*

Quantitative/qualitative serum HBV DNA, presence of serum HBeAg, levels of serum ALT, degree of liver fibrosis, cirrhosis, and HCC.

*3.3.1.4. Types of studies*

Any observational studies (i.e., cross-sectional, case-control or cohort) published in any language which met all of the following criteria were included: the study examined the association between any of the exposures of interest and any of the outcomes of interest described above; individuals without CHB infection were excluded from the analyses (because the primary focus of this review is the link between age at infection and the risk of HCC beyond its effect of increasing risk of CHB infection); in studies of maternal HBV sero-status, the subjects with unknown maternal sero-status were excluded from the analyses to avoid misclassification of exposure status; case series, i.e. studies without a control group, were included if the Greenwood-Yule method, or a related approach, was used to examine the birth order distribution.[212]

3.3.2. Search strategy

We conducted a systematic search using two English-language databases (Medline, 1946 to Jan 2012 and Embase, 1974 to Jan 2012) and two Chinese-language databases (CNKI, 1979 to Sep 2012 and SinoMed, 1979 to Sep 2012). Subject headings used in Medline search included "hepatitis B", "alanine transaminase", "hepatitis B e antigens", "DNA, viral", "virus replication", "liver cirrhosis", "hepatocellular carcinoma", "age factors", "infectious disease transmission, vertical", and "birth order". Manual search through reference lists was also conducted. Gray literature was not searched. The full search strategy is reported in Appendix 1.

3.3.3. Study selection

Eligibility criteria were specified in advance and documented in a protocol (Appendix 2). The title and abstract of all papers identified by the electronic searches in English databases were screened by two independent reviewers (Yusuke Shimakawa and Naho Tsuchiya). Those identified in Chinese databases were screened by another reviewer (Hong-Jing Yan). Papers detected through the screening process were retrieved and reviewed to assess their eligibility. Disagreements were resolved by discussion with a fourth author (Andrew Hall). Twenty-seven authors of the articles were contacted for clarification of the study design or results. Seventeen responded and nine provided numerical data that were not presented in the published papers. Data extraction was independently carried out by two reviewers for articles from the English databases and by one reviewer for those from the Chinese databases. A standardized pre-piloted data extraction sheet was used (Appendix 3). The included studies were evaluated for the risk of bias (Appendix 4). The aim of assessing the risk of bias was to summarize limitations identified in each study, rather than to exclude additional studies on the basis of low methodological quality.

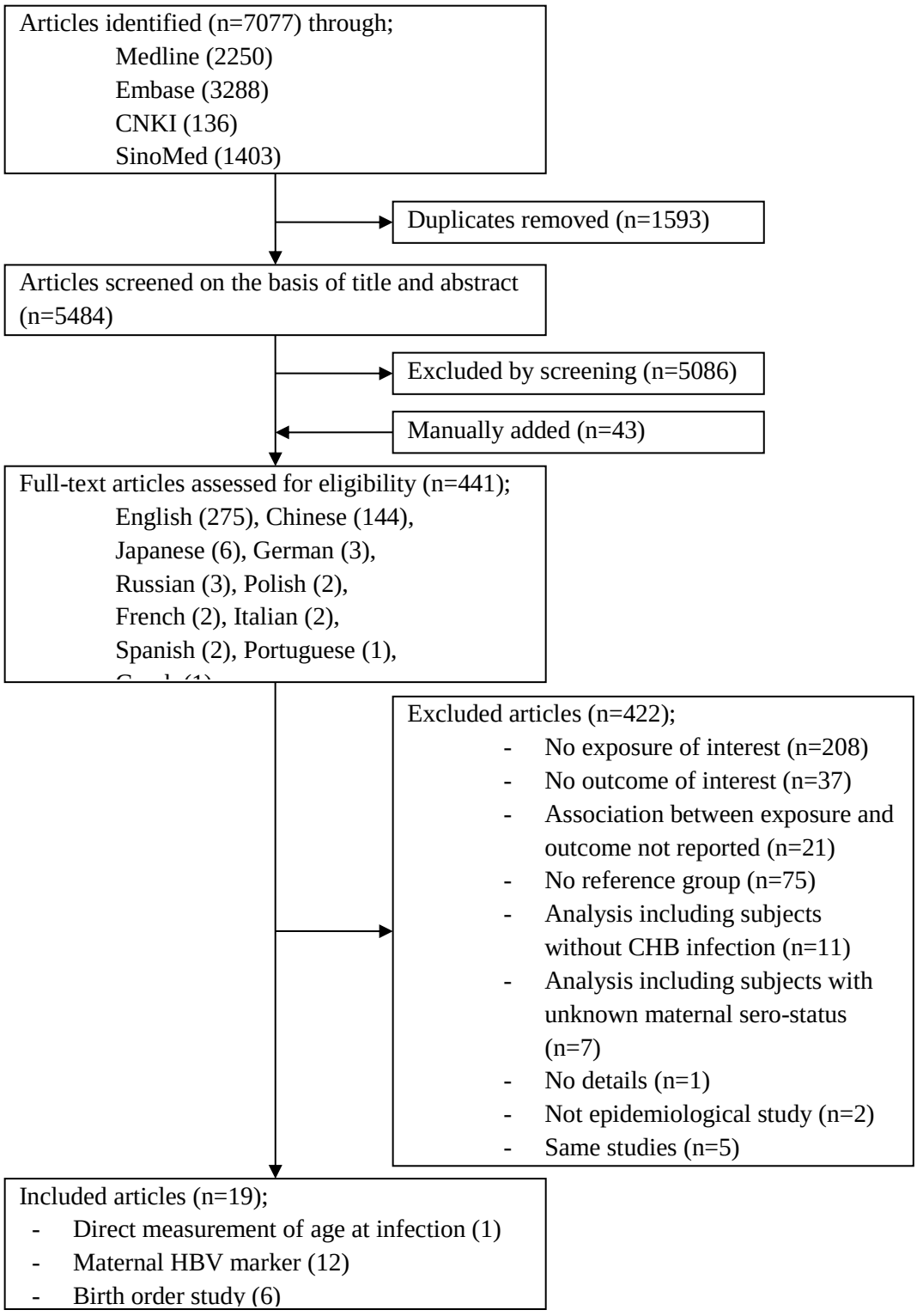
3.3.4. Statistical analysis

For the cross-sectional and case-control studies, odds ratios (OR) were estimated. In longitudinal studies, the risk ratio, rather than the rate ratio, was computed because person years at risk were not available for many of the published papers. The chi-squared test (or Fisher's exact test for small samples) was used to test the statistical significance of the crude associations. The chi-squared test for trend was presented for the association between birth order and liver disease. For studies where no events were reported in one of the comparison groups, odds ratios were estimated by adding 0.5 to each cell of the contingency table. The Greenwood-Yule method was used to estimate the expected distribution of birth order among cases in studies without a control group, and a statistical test introduced by Haldane and Smith that compares the sum of the observed birth ranks with the sum of expected birth ranks was applied.[213] We present adjusted effect estimates for studies where these were reported in the original analysis. A meta-analysis was not performed because of differences in outcomes and exposures between studies. Potential for publication bias was visually assessed using funnel plots and statistically with Egger's test.[240] This review was reported in accordance with checklists presented in the PRISMA guidelines.[241] All analyses were conducted using STATA 11.0 (Stata Corporation, College Station, Texas).

## 3.4. Results

The search of databases identified 7,077 potential articles (2,250 in Medline, 3,288 in Embase, 136 in CNKI and 1,403 in SinoMed) of which 1,593 were excluded due to duplication. Review of the titles and abstracts excluded 5,086. Forty-three papers were manually identified from reference lists. The full text of the 441 articles was examined in detail, and of these 422 was discarded leaving 19 papers eligible for the systematic review. No African study was identified. Criteria of exclusion are described in figure 3.1.

Figure 3.1 Flow diagram of study selection



Abbreviations: CHB, chronic hepatitis B; HBV, hepatitis B virus.

There was only one longitudinal study that examined the association between age at first HBsAg-positive result and persistence of HBeAg.[81] This study used an historical cohort of HBsAg-negative children in Alaska who were later identified to have a CHB infection through semi-annual serological follow-up. The cohort was followed up for greater than ten years to assess the timing of HBeAg loss (table 3.1).

A total of 12 studies assessed the association of maternal HBV sero-status with various outcomes (table 3.2 and 3.3); HCC (2 case-control studies),[242,243] cirrhosis by liver histopathology (1 cross-sectional study),[244] persistence of HBeAg (4 cohort studies),[78,83,245,246] and presence of HBeAg at one time point (5 cross-sectional studies).[247–251] The two cohort studies that evaluated HBeAg loss also examined ALT levels.[83,245] Except for four studies,[246,248–250] the studies exclusively enrolled children. In all the cohort studies, treatment was not given during follow-up except one study of steroid withdrawal therapy.[246] All studies examined HBsAg as the only maternal HBV marker, and three also considered maternal HBeAg.[83,247,251] The proportion of participants with available maternal sero-status varied markedly between studies. In two studies having a mother alive[248] or maternal serology available[245] was one of the eligibility criteria while among the remaining studies the percentage of subjects with known maternal sero-status varied from 49%[250] to 95%.[78,83] The timing of when maternal sero-status was defined in relation to the child's age also varied. One study in England[247] assessed prenatal maternal HBeAg whereas 6 studies examined mothers' HBV markers when their children were enrolled in the study (table 3.2 and 3.3). In the remaining 5 studies, the timing was unclear.

For birth order, one cross-sectional study[252] and two case-control studies[187,194] compared the frequency distribution of birth order by the presence of HBeAg-positivity and HCC, respectively (table 3.4). Three Chinese case series of HBV-related HCC used the Greenwood-Yule method (table 3.5).[253–255] All birth order studies were conducted in adults.
Except for one study,[83] all longitudinal studies compared proportions rather than incidence rates. Results of multivariable analyses were only reported in three studies[83,187,194] although some

studies presented results stratified by age group.[78,248] The author of a New Zealand study[250] provided additional data which enabled me to control for age. The risk of bias in the included studies is summarized in Appendix 5.

3.4.1. Time of HBV infection study

In the Alaskan study children who remained HBeAg-positive were infected younger (median 4.6 years) than children who lost HBeAg with subsequent reversion to HBeAg positive (median 12.5 years) or without reversion (median 7.8 years, table 3.1).[81]

3.4.2. Maternal HBV serology studies

Three paediatric studies from Taiwan, two with HCC[242,243] and one with cirrhotic changes in liver histopathology as outcome,[244] revealed a similar magnitude of association with having an infected mother (ORs range from 11.3 to 16.0, table 3.2 and figure 3.2). One longitudinal study of treatment-induced HBeAg loss in adults[246] and three studies of spontaneous HBeAg loss in children[78,83,245] found good evidence for an association of HBeAg persistence with having an HBsAg-positive mother (table 3.3 and figure 3.2). However, in one of these[83] the association was no longer statistically significant after adjusting for maternal HBeAg, peak ALT and HBV genotype (hazard ratio 1.2, 95% CI: 0.8-1.8). Another study[78] stratified the analysis by age at study entry and observed heterogeneity (test of homogeneity P<0.0001) in the risk ratios; 0.87 (95% CI: 0.79-0.95), 1.1 (0.8-1.4) and 1.3 (1.0-1.7) in age group of <1, 1-6 and >6 years old, respectively. Tseng *et al.*[83] evaluated maternal HBeAg in addition to maternal HBsAg and found a good evidence for the association with children's HBeAg persistence in univariable analysis (risk ratio 2.3, 95% CI: 1.5-3.6) and after controlling for maternal HBsAg, peak ALT and HBV genotype (hazard ratio 1.8, 95% CI: 1.1-2.8).

Of five cross-sectional studies of HBeAg prevalence (table 3.3 and figure 3.2), one from New Zealand[250] showed strong evidence for an association with positive maternal HBsAg and this remained after controlling for age (OR 1.8, 95% CI: 1.3-2.4). The paediatric study of immigrants in Sweden found weak evidence (OR 8.2, 95% CI: 0.8-400.8, P=0.09) for an association with having an HBsAg-positive mother.[251] Although the other studies did not demonstrate a statistically significant difference in HBeAg prevalence between those born to

seropositive and seronegative mothers, the direction of the association was positive. Habu *et al*.[248] found no evidence of an association after adjusting for age (OR 1.6, 95% CI: 0.9-2.9).

Two longitudinal cohort studies of children positive for HBeAg assessed peak ALT levels during follow-up (table 3.2 and figure 3.2).[83,245] In both studies, there was good evidence that the proportion that experienced abnormally high ALT levels was smaller in children born to HBsAg-positive mothers than in those born to negative mothers.

### 3.4.3. Birth order studies with a control group

Both Greek studies found that later birth order is associated with a higher risk of HCC (table 3.4). This association did not change after adjusting for other prognostic factors.[187,194] A Taiwanese cross-sectional study of HBeAg prevalence showed weak evidence for the association between earlier birth order and higher risk of positive HBeAg.[252]

### 3.4.4. Birth order studies without control group

A study from Haimen, China demonstrated that there was a higher than expected frequency of HCC patients in birth orders 1-3, and a lower than expected frequency in birth orders higher than 4 (table 3.5). The Haldane-Smith method confirmed statistical evidence (P=0.03) of this.[253] A study from Shunde district was consistent with these findings.[255] And the association was in the same direction but not statistically significant in a study from Luoyang (P=0.4).[254]

### 3.4.5. Publication bias

A funnel plot is presented for the studies examining the association between the maternal HBV marker and the persistence/prevalence of HBeAg (figure 3.3). The plot appears asymmetric (which is indicative of publication bias) and this is confirmed by Egger's test (P=0.04). There were too few studies to assess publication bias for other associations.

Table 3.1 Study of the association between time of HBV infection and the risk of HBeAg persistence in a systematic review, up to 2012

| First Author, Year (Reference No.) | Region | Study Design | Serological Course | No. of Subjects | Median Age at 1st HBsAg-positive Result | Median Duration of Follow-up | Median No. of Serological Tests |
|---|---|---|---|---|---|---|---|
| McMahon, 2001[81] | Alaska, USA | Cohort study | Remained HBeAg-positive throughout the follow-up period | 9 | 4.6 years old | 11.1 years | 12 |
| | | | Lost HBeAg during the course without reversion | 47 | 7.8 years old | 16.6 years | 17 |
| | | | Lost HBeAg during the course with reversion to positive HBeAg | 11 | 12.5 years old | 14.8 years | 20 |

Table 3.2 Studies Assessing the Effect of Maternal HBV Sero-status on HCC, Liver Cirrhosis and Peak ALT Levels in a Systematic Review, up to 2012

| First Author, Year, Region (Reference No.) | Study Design | Age Range, Years[a] | Type of Maternal HBV Sero-marker | Frequency in Cases or Exposed Group[b] | Frequency in Controls or Non-exposed Group[c] | Crude | | | Adjusted | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | OR or RR[d] | 95% CI | *P* value | OR or RR | 95% CI | *P* value |
| **1. HCC** | | | | | | | | | | | |
| Beasley, 1982, Taiwan[242e] | CC | Children | HBsAg[f] | 12/14 (86%) | 17/49 (35%) | 11.3 | 1.9, 67.8 | 0.0008 | N/R | | |
| Chang, 1989, Taiwan[243] | CC | 3-16 | HBsAg at entry | 29/31 (94%) | 22/44 (50%) | 14.5[g] | 2.5, 84.0 | 0.0001 | N/R | | |
| **2. Cirrhosis** | | | | | | | | | | | |
| Hsu, 1988, Taiwan[244] | CS | Children | HBsAg[f] | 6/23 (23%) | 0/21 (0%) | 16.0[h] | 0.8, 303.6 | 0.02[i] | N/R | | |
| **3. Peak ALT level** | | | | | | | | | | | |
| Kojima, 1985, Japan[245] | Co | 2-12 | HBsAg[f] | 3/9 (33%) | 22/28 (79%) | 0.42 | 0.17, 1.09 | 0.04 | N/R | | |
| Tseng, 2011, Taiwan[83] | Co | 0-16 | HBsAg at | 63/137 (46%) | 29/48 (60%) | 0.76 | 0.57, 1.02 | 0.09 | N/R | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | entry | | | | | | | | |
| | | | HBeAg at entry | 33/80 (41%) | 59/105 (56%) | 0.73 | 0.54, 1.00 | 0.04 | N/R | | |

Abbreviations: ALT, alanine transaminase; CC, case-control study; CI, confidence interval; Co, cohort study; CS, cross-sectional study; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HR, hazard ratio; N/R, not reported; OR, odds ratio; RR, risk ratio.

[a] When age range was not available, study was categorized as children (<20 years old), adults (≥20 years old) or both children and adults.

[b] Prevalence of exposure in cases presented in case-control design and prevalence of outcome in exposed group presented in cross-sectional and cohort study

[c] Prevalence of exposure in controls presented in case-control design and prevalence of outcome in non-exposed group presented in cross-sectional and cohort study

[d] Odds ratios for case-control or cross-sectional studies and risk ratios for cohort studies are presented.

[e] Communication with the author confirmed that all the HCC cases were positive for HBsAg.

[f] When the measurement of maternal sero-status was performed is not known.

[g] Matched design without matched analysis

[h] As a contingency table contains a zero cell, 0.5 was added to each cell.

[i] Although the 95% CI does not exceed the unity, *P* value is <0.05 due to the small sample size.

Table 3.3 Studies Assessing the Effect of Maternal HBV Sero-status on Persistence/Prevalence of HBeAg in a Systematic Review, up to 2012

| First Author, Year, Region (Reference No.) | Study Design | Age Range, Years[a] | Type of Maternal HBV Sero-marker | Frequency in Exposed Group | Frequency in Non-exposed Group | Crude | | | Adjusted | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | OR or RR[b] | 95% CI | *P* value | OR or HR[c] | 95% CI | *P* value |
| **1. Persistence of HBeAg** | | | | | | | | | | | |
| Kojima, 1985, Japan[245] | Co | 2-12 | HBsAg at entry | 7/9 (78%) | 9/28 (32%) | 2.4 | 1.3, 4.6 | 0.02 | N/R | | |
| Kojima, 1985, Japan[246] | Co | 19-48 | HBsAg at entry | 4/4 (100%) | 2/9 (22%) | 4.5 | 1.3, 15.3 | 0.02 | N/R | | |
| Chang, 1989, Taiwan[78] | Co | 0-15 | HBsAg[d] | 121/142 (85%) | 52/75 (69%) | 1.2 | 1.0, 1.5 | 0.006 | N/R | | |
| Tseng, 2011, Taiwan[83] | Co | 0-16 | HBsAg at entry | 56/137 (41%) | 8/48 (17%) | 2.5 | 1.3, 4.8 | 0.002 | 1.2[e] | 0.8, 1.8 | 0.5 |
| | | | HBeAg at entry | 41/80 (51%) | 23/105 (22%) | 2.3 | 1.5, 3.6 | <0.0001 | 1.8[e] | 1.1, 2.8 | 0.01 |
| **2. Prevalence of HBeAg** | | | | | | | | | | | |
| Wheeley, 1989, UK[247] | CS | 0-16 | Prenatal HBeAg | 32/42 (76%) | 0/1 (0%) | 9.3[f] | 0.4, 245.6 | 0.1 | N/R | | |
| Habu, 1991, Japan[248] | CS | Children & adults | HBsAg at entry | 71/101 (70%) | 96/152 (63%) | 1.4 | 0.8, 2.4 | 0.2 | 1.6[g] | 0.9, 2.9 | 0.1 |
| Tai, 1999, Taiwan[249] | CS | >15 | HBsAg at entry | 67/221 (30%) | 35/131 (27%) | 1.2 | 0.7, 1.9 | 0.5 | N/R | | |
| Hopkirk, 2000, New Zealand[250h] | CS | Children & adults | HBsAg[d] | 160/281 (57%) | 214/530 (40%) | 2.0 | 1.5, 2.6 | <0.0001 | 1.8[g] | 1.3, 2.4 | 0.0005 |
| Soderstrom, 2002, Sweden[251] | CS | 2-18 | HBeAg[d] | 15/16 (94%) | 11/17 (65%) | 8.2 | 0.8, 400.8 | 0.09 | N/R | | |

Abbreviations: ALT, alanine transaminase; CC, case-control study; CI, confidence interval; Co, cohort study; CS, cross-sectional study; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HR, hazard ratio; N/R, not reported; OR, odds ratio; RR, risk ratio.

[a] When age range was not available, study was categorized as children (<20 years old), adults (≥20 years old) or both children and adults.

[b] Odds ratios for case-control or cross-sectional studies and risk ratios for cohort studies are presented.

[c] Except for the study of Tseng et al.[83] which presented hazard ratios, odds ratios are presented.

[d] When the measurement of maternal sero-status was performed is not known.

[e] Multivariable model included maternal HBsAg, maternal HBeAg, peak ALT and HBV genotype.

[f] As a contingency table contains a zero cell, 0.5 was added to each cell.

[g] Adjusted for age.

[h] The author provided the raw data to compute odds ratio.

Table 3.4 Studies of Birth Order With Control Group in a Systematic Review, up to 2012

| First Author, Year (Reference No.) | Region | Study Design | Age Range, Years[a] | Outcome | Birth Order | No. of Cases (%) | No. of Controls (%) | Crude | | | Adjusted | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | OR | 95% CI | *P* for trend | OR | 95% CI | *P* for trend |
| Hsieh, 1992[187] | Greece | CC | Adults | HCC | 1st | 17 (20%) | 9 (27%) | 1.0 | Reference | 0.04 | 1.0[b] | Reference | 0.02 |
| | | | | | 2nd | 11 (13%) | 11 (33%) | 0.5 | 0.2, 1.7 | | 0.9 | 0.2, 4.2 | |
| | | | | | 3rd | 17 (20%) | 3 (9%) | 3.0 | 0.7, 13.8 | | 7.8 | 1.4, 42.4 | |
| | | | | | 4th | 15 (18%) | 4 (12%) | 2.0 | 0.5, 8.0 | | 4.0 | 0.8, 20.7 | |
| | | | | | ≥5th | 25 (29%) | 6 (18%) | 2.2 | 0.6, 7.6 | | 3.8 | 0.9, 16.5 | |
| Kuper, 2000[194] | Greece | CC | Adults | HCC | 1st | 42 (20%) | 13 (43%) | 1.0 | Reference | 0.0008 | 1st 1.0[c] | Reference | N/R |
| | | | | | 2nd | 59 (28%) | 9 (30%) | 2.0 | 0.8, 5.2 | | 2nd 2.0 | 0.8, 5.3 | |
| | | | | | 3rd | 49 (24%) | 7 (23%) | 2.2 | 0.8, 6.0 | | ≥3rd 4.1 | 1.3, 12.7 | |
| | | | | | ≥4th | 58 (28%) | 1 (4%) | 18.0 | 2.0, 163.4 | | | | |
| Tai, 2002[252] | Taiwan | CS | Adults | HBeAg | 1st | 29 (29%) | 65 (23%) | 1.00 | Reference | 0.09 | N/R[d] | | |
| | | | | | 2nd | 29 (29%) | 68 (25%) | 0.96 | 0.51, 1.77 | | | | |
| | | | | | 3rd | 24 (24%) | 78 (28%) | 0.69 | 0.36, 1.30 | | | | |
| | | | | | 4th | 11 (11%) | 40 (14%) | 0.62 | 0.28, 1.38 | | | | |
| | | | | | ≥5th | 7 (7%) | 27 (10%) | 0.58 | 0.23, 1.50 | | | | |

Abbreviations: CC, case-control study; CI, confidence interval; CS, cross-sectional study; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma; N/R, not reported; OR, odds ratio.

[a] When age range was not available, study was categorized as children (<20 years old), adults (≥20 years old) or both children and adults.

[b] Adjusted for age, sex, smoking and anti-HCV.

[c] Adjusted for age, sex, smoking, alcohol, schooling, anti-HCV and sibship size.

[d] Stratification by relationship with index case (i.e., children and siblings) was reported.

Table 3.5 Studies of Birth Order Without Control Group in a Systematic Review, up to 2012

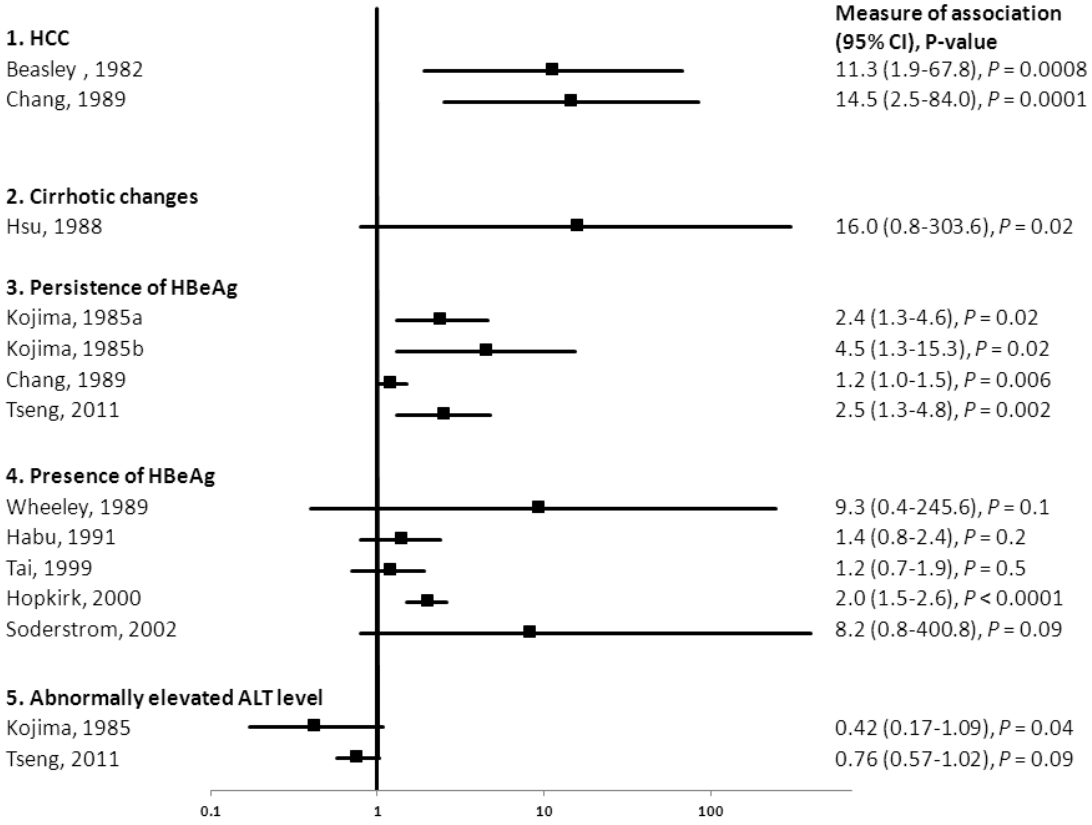
| First Author, Year (Reference No.) | Region | Study Design | Age Range, Years[a] | Outcome | Birth Order | Observed Distribution | Expected Distribution | Greenwood-Yule Method | Haldane-Smith Method |
|---|---|---|---|---|---|---|---|---|---|
| Cai, 2003[253] | Haimen, China | Case series | >36 | HCC | 1st | 29 | 26.27 | 1.10 | Observed < Expected, t = 2.17, df = 121, *P* = 0.03 |
| | | | | | 2nd | 28 | 22.27 | 1.26 | |
| | | | | | 3rd | 18 | 17.27 | 1.04 | |
| | | | | | 4th | 9 | 12.94 | 0.70 | |
| | | | | | ≥5th | 10 | 15.30 | 0.65 | |
| Cao, 2005[254] | Luoyang, China | Case series | Adults | HCC | 1st | 19 | 16.75 | 1.13 | Observed < Expected, t = 0.95, df = 62, *P* = 0.4 |
| | | | | | 2nd | 15 | 13.75 | 1.09 | |
| | | | | | 3rd | 11 | 12.25 | 0.90 | |
| | | | | | 4th | 10 | 9.25 | 1.08 | |
| | | | | | ≥5th | 8 | 11.00 | 0.73 | |
| Song, 2009[255] | Shunde, China | Case series | Adults | HCC | 1st | 17 | 13.92 | 1.22 | Observed < Expected, t = 2.20, df = 46, *P* = 0.03 |
| | | | | | 2nd | 15 | 10.92 | 1.37 | |
| | | | | | 3rd | 5 | 7.42 | 0.67 | |
| | | | | | 4th | 4 | 5.75 | 0.70 | |
| | | | | | ≥5th | 6 | 8.98 | 0.67 | |

Abbreviations: df, degree of freedom; HCC, hepatocellular carcinoma.

[a] When age range was not available, study was categorized as children (<20 years old), adults (≥20 years old) or both children and adults.

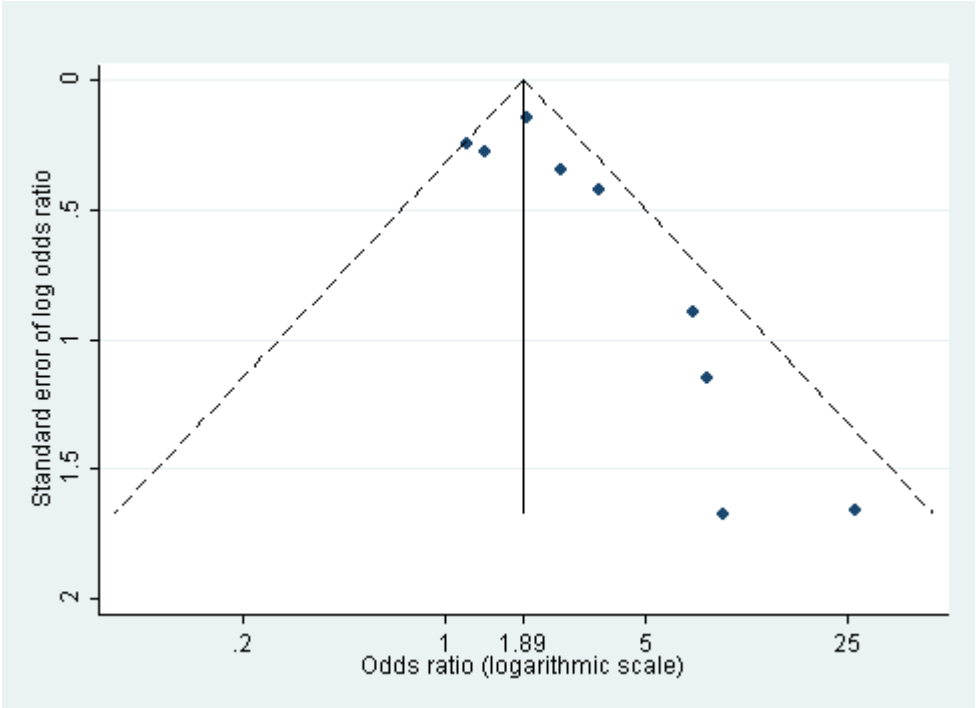
[a] Ratio of observed/expected number is presented.

Figure 3.2 Effect measures and 95% CIs for the association between maternal HBV sero-status and HBV-related outcomes




Odds ratios for case-control or cross-sectional studies[242–244,247–251] and risk ratios for cohort studies[78,83,245,246] are presented. Except the study by Wheeley[247] which assessed maternal HBeAg, all studies examined maternal HBsAg.

Figure 3.3 Funnel plot for studies investigating the relation between maternal sero-marker and the persistence/presence of HBeAg




Dashed line represents pseudo 95% confidence limits.

## 3.5. Discussion

Of 21 outcomes examined in 19 studies, univariable analysis of 14 outcomes supported our hypothesis with $P \leq 0.05$ and two with weak evidence ($0.05 < P < 0.1$). Although the other studies did not reach statistical significance, the direction of the associations was consistent with our hypothesis.

### 3.5.1. Time of HBV infection study

McMahon *et al.* directly assessed the age at HBV infection and its association with HBeAg persistence in Alaskan natives whose approximate date of infection was known because of consecutive serological tests.[81] They showed that the median age at first HBsAg-positive result was lower in those who did not clear HBeAg during the follow-up period than those who HBeAg sero-converted, suggesting that the early age at infection is associated with delayed HBeAg loss. However, the length of follow up is different for the two groups, which is a crucial determinant of the chance of HBeAg loss.

### 3.5.2. Maternal HBV serology studies

Studies of maternal HBV serology showed that having a mother with a positive HBV marker is associated with worse outcomes (HCC, cirrhotic changes, persistence of HBeAg or possession of HBeAg at one particular time) although three of these were not supported by statistical evidence. Two longitudinal studies from East Asia which evaluated both HBeAg loss and peak ALT levels during follow-up demonstrated good evidence that children with HBsAg-positive mothers experienced elevated levels of hepatic enzymes less frequently and fewer episodes of

HBeAg loss than those whose mothers are negative. It is well established that having a highly elevated serum ALT level during the immune-tolerant phase of CHB infection is a factor leading to early HBeAg seroconversion.[256]

The only maternal serological study which reported the results of a multivariable analysis[83] showed that the association with maternal HBsAg observed in the univariable analysis was no longer significant after controlling for maternal HBeAg. This reflects the fact that maternal HBeAg is a stronger predictor of perinatal transmission than maternal HBsAg. In fact, the risk of perinatal transmission ranges from 10-20% in HBsAg-positive mothers without HBeAg to 90% in mothers with positive HBeAg.[30] Nevertheless, because HBeAg sero-clearance (loss of the maker) occurs faster than HBsAg sero-clearance in chronically infected persons (3-14% *versus* 0.5-2% per year, Chapter 1), misclassification of maternal HBeAg status between when the index child was born and when he/she entered the study is greater than misclassification of maternal HBsAg status. Apart from 5 studies not reporting when mothers were bled, all but one examined maternal sero-status at the child's study entry, which might have led to non-differential misclassification of the exposure, resulting in underestimation of its association. The only study which investigated prenatal maternal HBeAg,[247] the best proxy for perinatal transmission, was inconclusive because of a small sample size.

Recent evidence suggests that viral genotypes modify the natural history of CHB infection. Certain genotypes are associated with delayed HBeAg seroconversion[143] and also HCC risk.[146] Moreover, associations between certain viral genotypes and mother-to-infant transmission have been reported.[141,142] For example, Tseng *et al.*[83] found that genotype C was associated with

delayed HBeAg seroconversion after accounting for maternal HBsAg and HBeAg status. The study also showed that maternal HBeAg was associated with persistence of HBeAg after adjusting for viral genotype.

### 3.5.3. Birth order studies

Two Greek case-control studies of birth order demonstrated that HCC cases are concentrated in higher birth orders, suggesting that later-born children who might have been exposed to HBV at a young age through their older siblings have a higher risk of developing HCC than early-born children.[187,194] In contrast, one Taiwanese study showed that HBeAg was more prevalent in earlier birth orders although the evidence was weak.[252] Furthermore, three subsequent Chinese birth order studies which applied the Greenwood-Yule methods also suggested that HCC cases are associated with lower birth order. As discussed in Chapter 2, these heterogeneities can be understood in terms of geographic differences in the main mode of transmission. In Taiwan and China, perinatal mother-to-infant transmission is relatively important compared to other parts of the world.[63] In such places, first-born children have a higher chance of having been born to a HBeAg-positive mother than later-born children, because young mothers with positive-HBeAg will clear it with older age.[6] Consequently, early-born children have a higher chance of having been infected earlier from their mother than later-born children. In contrast, in Mediterranean countries where children are infected during childhood through other children with positive-HBeAg,[71] birth order acts as an indicator for number of older siblings, and later-born children have an increased chance of being exposed to HBV at an early age through infectious older siblings than early-born children. Perinatal transmission also occurs in Greece but is much less common than in China. This could explain the results of the first Greek study where people

born as the second child have lower odds of HCC than the first birth rank (OR 0.5), with the odds increased for higher birth orders.

### 3.5.4. Limitations of the study

The funnel plot and Egger's test suggest the presence of funnel plot asymmetry in the studies assessing the association between maternal sero-marker and persistence/presence of HBeAg. Publication bias is one of a number of reasons for the asymmetry in the funnel plot, and other explanations include: poor methodological design of small studies which lead to larger effects,[257] systematic differences in the study population between smaller and larger studies, and shorter duration of follow-up in smaller cohort studies.[258] It is difficult to prove that the asymmetry was due to the publication bias in the absence of prospective registries of epidemiological studies which include the information on unpublished studies.[259] Nevertheless, observational studies are more vulnerable to publication bias than randomized trials, because epidemiological studies are often conducted retrospectively by analysing existing databases, and unless published, this kind of study is hard to trace and thus it is difficult to have the prospective registries of such studies.[240] Although we were unable to assess the publication bias in the birth order studies due to the small number of studies included, these studies are prone to selective reporting bias because birth order is easily obtained by interview without additional cost.

The study was also limited because no African study was included in the review. Sub-Saharan Africa (sSA) has high prevalence of HBV infection and high HCC incidence (Chapter 1). However, the epidemiology of HBV infection in sSA differs from East Asia, and therefore it is

questionable whether the findings observed in this review (which are mainly from East Asia) are applicable to the African context.

### 3.5.5. Public health implications

Although a robust conclusion cannot be drawn due to the potential role of publication bias and the heterogeneity of studies included in the review, this systematic review supports the hypothesis that earlier age at HBV infection is associated with an increased risk of HCC through persistence of viral replication. The implications of the effect of early age at infection on the prevention of cirrhosis and primary liver cancer are twofold. First it means that the impact of hepatitis B vaccination on raising the average age at infection will not simply be in reducing the prevalence of chronic infection but also in reducing the adverse effects of that chronic infection in those acquiring it at an older age. Second it adds emphasis to the critical importance of interrupting perinatal transmission – as reflected in the WHO recommendation for a timely birth dose within 24 hours of birth in all countries.[260]

## 3.6. Summary

A systematic review of the effect of age at HBV infection on HCC and its predictors amongst chronic HBV carriers was conducted. As the direct estimation of the age at HBV infection is difficult, the review included studies that used maternal HBV status and birth order as proxy measures for age at infection. The associations in all studies were in agreement with the hypothesis that earlier age at infection is associated with worse outcomes in chronic carriers. The review was potentially limited by publication bias and the absence of studies from Africa .

# Chapter 4. Association between perinatal mother-to-infant transmission of hepatitis B virus and the risk of hepatitis B e antigenaemia in children: a cross-sectional study in The Gambia, West Africa

## 4.1. Abstract

**Background**

Early age at infection with hepatitis B virus (HBV) increases the risk of chronic HBV infection. In addition early age at infection may further increase the risk of persistent viral replication beyond its effect on chronicity. The effects of perinatal and early postnatal transmission on the risk of prolonged hepatitis B e antigenaemia in children with chronic HBV infection are not well documented in Africa. We examine these associations using maternal HBV sero-status and the number of HBV-positive older siblings as proxy measures for perinatal and early postnatal transmission.

**Methods**

Hepatitis B e antigen (HBeAg)-positive mothers were identified in six population-based HBV sero-surveys conducted in The Gambia in 1986-1990. For every HBeAg-positive mother a positive (hepatitis B surface antigen (HBsAg)-positive but HBeAg-negative) and negative

(HBsAg-negative) control mother was selected. These mothers and their family members were tested for HBV sero-markers in a subsequent survey in 1991-1993.

**Results**

Thirty-eight HBeAg positive mothers and the same number of positive and negative controls participated in the study. Sixty-nine percent of their children and 67% of their mothers also participated. There was a non-significant positive association between HBeAg prevalence in children and the number of HBeAg-positive older siblings (64.1%, 69.2% and 83.3% in children with 0, 1 and ≥2 HBeAg-positive older siblings, respectively). After adjusting for confounders, having an HBeAg-positive mother was a risk factor for HBeAg positivity in children carrying HBsAg (adjusted OR 4.5, 95% CI: 1.0-19.5, p=0.04), whilst the number of HBeAg-positive older siblings was not.

**Conclusions**

Maternal HBeAg was associated with positive HBeAg in children with chronic HBV infection. This suggests that interrupting mother-to-infant transmission in sub-Saharan Africa might help reduce the burden of liver disease. A timely dose of HBV vaccine within 24 hours of birth, as recommended by WHO, needs to be implemented in sub-Saharan Africa.

## 4.2. Introduction

Chronic infection with the hepatitis B virus (HBV) is a cause of hepatocellular carcinoma (HCC).[8] The risk of chronic infection after exposure to HBV depends on the age at infection; infection becomes chronic in 80-90%, 20-30%, <10%, and <5% of individuals who are infected during perinatal period, early childhood, adolescence and adulthood, respectively .[68] Early HBV infection is therefore associated with a higher risk of HCC through the increased risk of chronic infection.[186] However, beyond its effect of increasing the chance of becoming a chronic carrier, early age at HBV infection may further increase the risk of persistent viral replication, which ultimately leads to HCC.[83] The immaturity of the host immune system in neonates and toddlers has been suggested as a mechanism of prolonged e antigenaemia.[71]

A systematic review of observational studies (Chapter 3)[261] found a positive association between having a mother positive for HBV sero-marker (a proxy for perinatal mother-to-infant transmission) and prolonged hepatitis B e antigenaemia (an indicator of high viral replication) amongst children with chronic HBV infection. There was also a positive association between maternal sero-status and paediatric cases of HCC.

However, the scope of the review was limited since: 1) most of the studies included were from East Asia where mother-to-infant transmission is frequent,[63] and 2) none of the studies assessing the effect of maternal sero-marker examined the infectious status of older siblings, who are known to be a major source of HBV infection in many parts of the world including sub-Saharan Africa (sSA).[42,60,71] Thus, the contribution of sibling-to-sibling HBV transmission during early childhood to the prolonged viral replication remains to be determined.

In this chapter the association between age at HBV infection and the presence of hepatitis B e antigen (HBeAg) is examined in The Gambia, West Africa, using maternal HBV sero-status and number of HBV-positive older siblings as a proxy for perinatal and early postnatal transmission, respectively. The analysis was restricted to study participants with positive hepatitis B surface antigen (HBsAg), because the aim of the analysis was to identify the effect of age at infection on the risk of hepatitis B e antigenaemia, beyond its effect of increasing the risk of chronic HBV infection.[261]

# 4.3. Methods

### 4.3.1. Setting

A nation-wide hepatitis B vaccination trial was initiated in The Gambia in 1986 and by 1990 countrywide coverage was achieved.[262] In parallel, six population-based sero-surveys for HBV infection were conducted in The Gambia between 1986 and 1990 to assess immunological response to the vaccine and to determine risk factors for HBV transmission.[55,60,262] These surveys obtained sera from both children and their mothers and tested for the presence of HBsAg in all the samples, and those that tested positive for HBsAg were further tested for HBeAg. In total, 53 HBeAg-positive mothers were identified from these studies (figure 4.1).

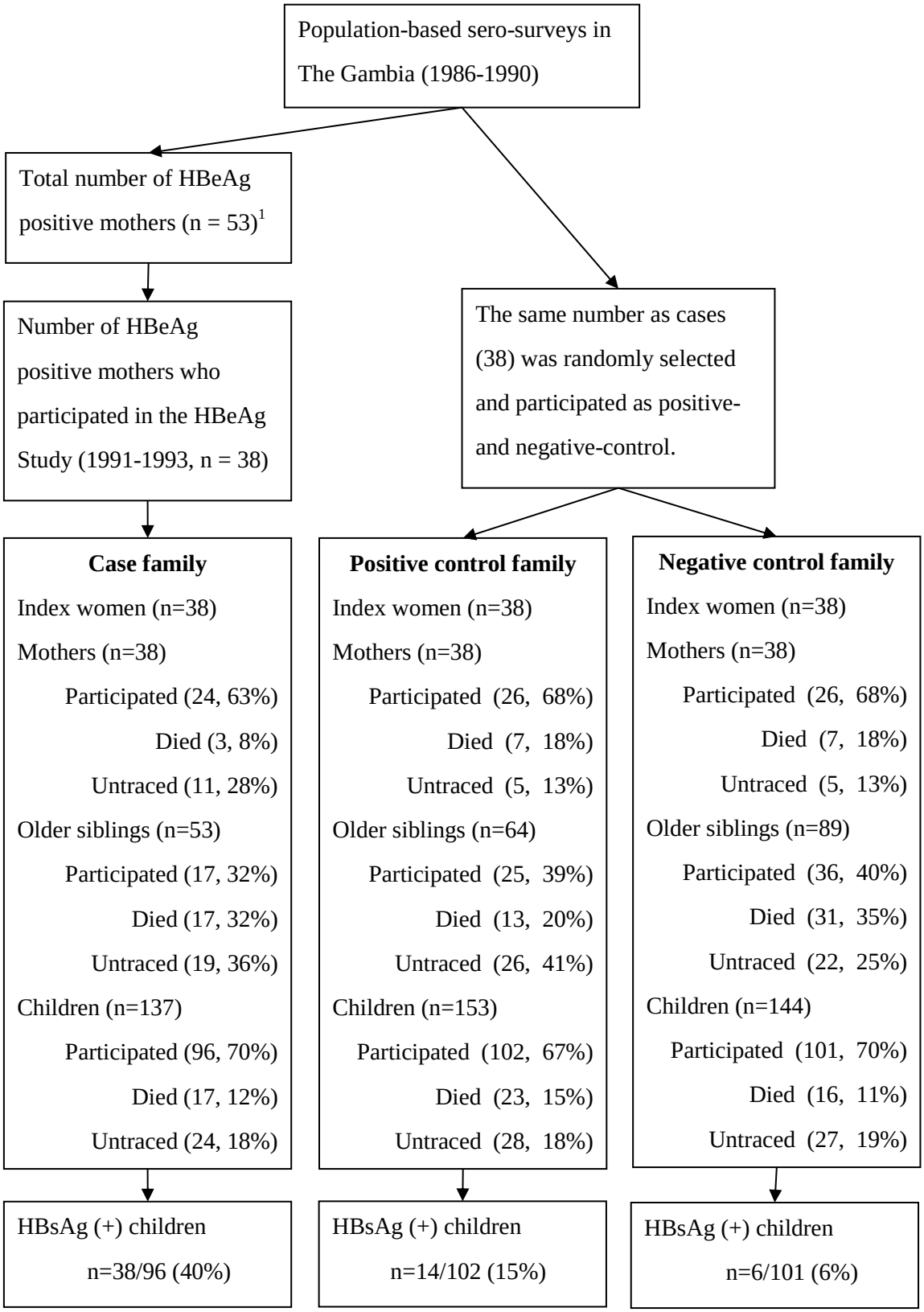
On the basis of the HBV sero-status determined at these surveys, all HBeAg-positive mothers were invited to participate in "HBeAg Study". The same numbers of positive (HBsAg-positive but HBeAg-negative) and negative (HBsAg-negative) control mothers was randomly selected

from the databases used in these surveys. Between 1991 and 1993, these mothers (i.e., index women) and their family members were bled for HBV markers to determine the effect of familial HBV sero-markers on presence of HBeAg.

After consent was obtained, the women were bled and interviewed to collect demographic information on dead and living family members. Index women were bled twice, the first bleeding occurred between 1986 and 1990 and the second at the time of the HBeAg Study (1991-1993). We used the serological status recorded in the first survey as a proxy for serological status at birth of their children.

Family members of the index woman (excluding half siblings), were contacted and if they agreed to participate in the study, were bled for HBV serology. All sera were assayed for HBsAg by reverse passive haemagglutination (Wellcotest, Murex Diagnostics, UK). HBeAg was tested by radioimmunoassay (Sorin, Biomedica, Italy) only when sera were HBsAg-positive. The study was approved by the Gambia Government/MRC (Medical Research Council) Joint Ethical Committee and the ethics committee at IARC (International Agency for Research on Cancer), France.

Figure 4.1 Flow chart of study participants in the HBeAg Study, The Gambia, 1991-93



[1] Origins of HBeAg positive mothers are the following: GHIS group 1 (n=18), GHIS group 3 (n=17), Arthropod study (n=6), Manduar sero-survey (n=4), Farafenni sero-survey (n=6), and Banjul sero-survey (n=2).

### 4.3.2. Data analysis

The association between maternal HBV markers (HBsAg, HBeAg) and e antigenaemia was examined in successive generations: mothers of index women (1st generation) and index women (2nd generation); and index women (2nd generation) and their children (3rd generation). The effect of the number of older siblings positive for HBsAg or HBeAg was evaluated in index women (2nd generation) and their children (3rd generation).

Logistic regression was used to estimate adjusted odds ratios for the associations between familial HBV sero-status (maternal sero-status and number of elder siblings positive for HBV markers) and e antigenaemia in index women. In the children of index women, adjusted odds ratios for the same associations were estimated using generalised estimating equations with an exchangeable correlation structure to account for household clustering.

#### 4.3.2.1. Potential confounding factors

In order to determine a minimally sufficient set of *a priori* confounders of the association between each of the familial sero-markers and e antigenaemia, the backdoor test was applied to a causal diagram.[263] First, using external knowledge a causal diagram was constructed (figure 4.2), in which each variable (exposures, outcome and potential confounding factors) was

assigned a fixed location and each direct causal effect of one variable on another was presented using an arrow starting from the cause with its tail to the effect with its head. Unmeasured but important variables are also presented and surrounded by dashed lines. A “path” is any unbroken route traced out along arrows connecting variables, irrespective of the direction of arrowheads. A “backdoor path from X to Y” is defined to be a path connecting X to Y with an arrowhead pointing to X. A “Collider” is a variable on the path where two arrowheads meet, and a path with one or more colliders is “blocked”; otherwise it is “unblocked” or “open”.

Based on the theory of the directed acyclic graphs (DAGs), i) the diagram only included directed arrows (in which all direct associations are causal) and thus did not include any non-directed arrow which represents a direct non-causal association; and ii) there was no feedback loop in which a directed path forms a closed loop. In this diagram (figure 4.2), the exposures of interest are familial HBV sero-status (maternal HBV sero-status at the birth of the affected child (e and f) and number of older siblings with positive sero-marker (g and h)) and the outcome is persistent viral replication (l). Year of birth (a) and parental socioeconomic status (b) determine maternal HBV sero-status when the affected child was born (e and f) and number of older siblings with positive HBV sero-markers (g and h) through intermediate variables of maternal HBV sero-status when the mother delivered older siblings (c and d). Maternal HBV sero-markers at birth of the affected child (e and f) determine the risk of perinatal HBV transmission (i) whereas the number of older siblings with positive HBV markers (g and h) determines the risk of horizontal HBV transmission during early childhood (j). Early age at HBV infection through perinatal maternal transmission (i) or early horizontal transmission (j) indirectly increases the risk of persistent HBV viral replication (l), by

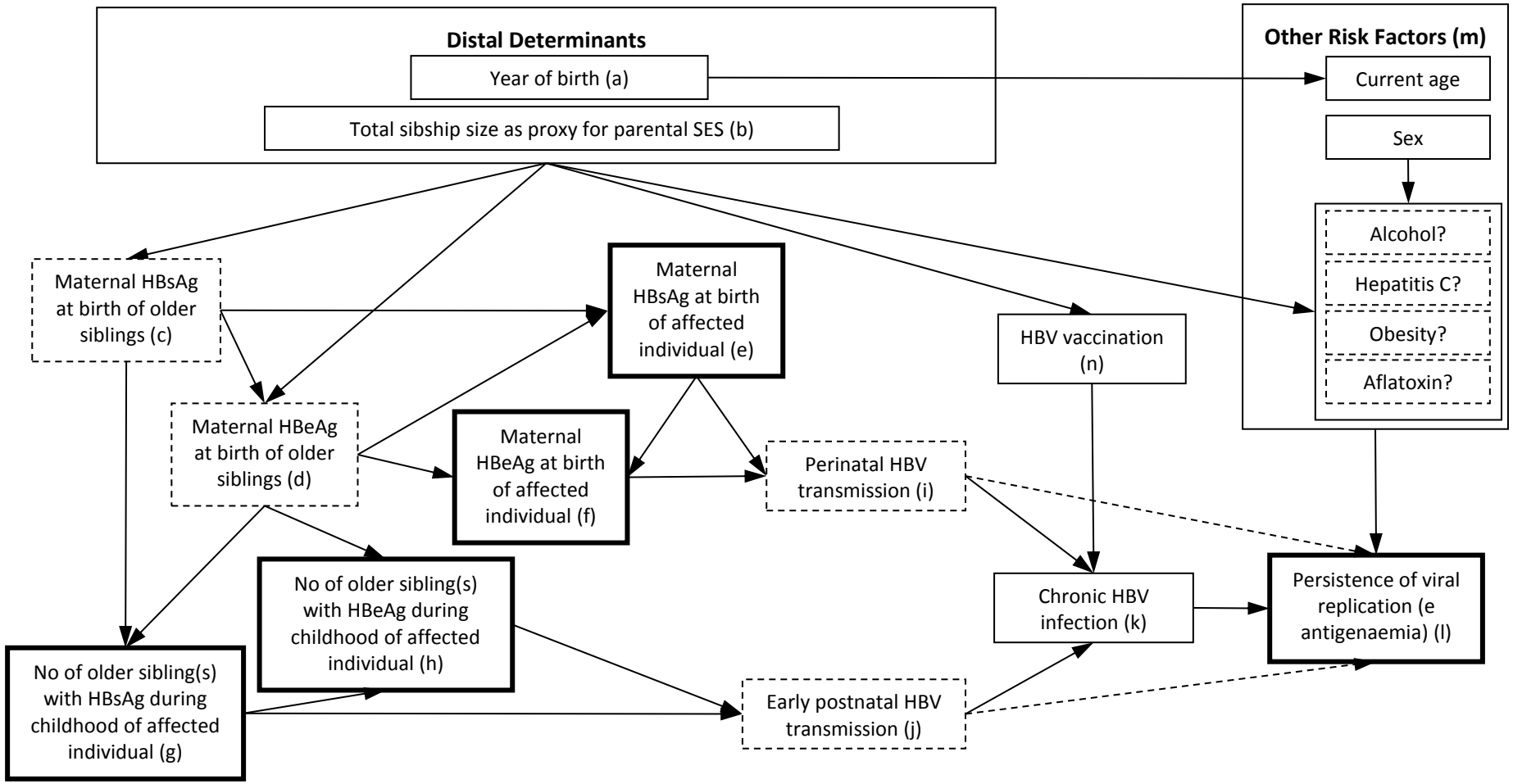
increasing the risk of chronic carriage (k). The hypothesis of this analysis is that early age at infection (i and j) increases the risk of persistent viral replication (l), beyond its effect of increasing the chronic HBV infection (k). This effect is represented by dashed arrows. Other potential risk factors for prolonged viral replication (m: current age, alcohol, hepatitis C, obesity and aflatoxin exposure) are affected by year of birth and parental socioeconomic status (a and b). Provision of hepatitis B vaccine (n) started in 1986 (a) and the likelihood of receiving vaccine was probably related to parental socioeconomic status (b). Vaccination is effective in preventing chronic HBV carriage (k).

Conditioning on a variable X can generate a non-directed arrow between the ancestors of X (i.e., variables which give directed path to the conditioned variable X). In this diagram, the analysis was restricted to study participants with positive HBsAg (i.e., conditioning on "chronic HBV infection (k)"). Consequently, additional non-directed arrows are generated between HBV vaccination (n) and each of the following variables: perinatal (i) and early horizontal transmission (j), maternal HBsAg (e) and HBeAg (f) at birth and number of older siblings with HBsAg (g) and HBeAg (h). This is not illustrated in the Figure 4.2, but has been accounted for in the selection of confounders (see below).

The causal effect of an exposure on an outcome can be estimated by eliminating all open backdoor paths between the two variables. This can be achieved by using a regression model to adjust for at least one variable from each open backdoor path. A set of variables ***S*** is minimally sufficient to identify the effect of exposure on the outcome, when removing any subset of variables from ***S*** results in an insufficient set. For the effect of maternal HBsAg at birth of

affected individual (e) on e antigenaemia (l), a minimally sufficient set of potential confounders that were measured is {year of birth (a), parental socioeconomic status (b), maternal HBeAg at birth of affected child (f), number of older siblings with positive HBsAg (g) and positive HBeAg (h)}. Similarly, for the effect of maternal HBeAg at birth of affected individual, a minimally sufficient set of potential confounders is {(a), (b), (e), (g) and (h)}. For the effect of number of older siblings with positive HBsAg (g), a minimally sufficient set is {(a), (b), (e), (f) and (h)}. Finally, for the effect of number of older siblings with positive HBeAg (h), a minimally sufficient set is {(a), (b), (e), (f) and (g)}. The mothers of index women were all negative for HBeAg and only one index woman had an HBeAg-positive older sibling. These variables were therefore omitted from multivariable models of e-antigenaemia in index women.

Figure 4.2 Causal diagram for the effect of familial HBV marker on hepatitis B e antigenaemia in the HBeAg Study, The Gambia, 1991-1993



The effect of maternal HBV sero-marker and number of older siblings with HBV sero-marker as proxies for age at establishment of chronic HBV infection on the risk for e antigenaemia was determined. The variables which were not measured are surrounded by dashed lines, and the exposure/outcome variables of interest are surrounded by lines in bold-type. SES denotes socio-economic stat

## 4.4. Results

### 4.4.1. Participation

Out of 53 HBeAg-positive mothers in the database, 38 (71.7%) were traced and agreed to participate in the study. The rate of refusal among controls was not recorded. More than 65% of the mothers and children of index women participated in the study, but participation was less than 35% among older siblings (figure 4.1).

### 4.4.2. Index women (2nd generation)

The characteristics of 38 HBeAg-positive cases and 38 HBeAg-negative controls with positive HBsAg are presented in table 4.1. The median age in index women was 25.0 years (interquartile range: 23-30) for cases and 26.5 years (interquartile range: 22-30) for controls (Wilcoxon rank-sum test: p=0.8). The associations between familial HBV sero-status and HBeAg positivity in HBsAg-positive women are presented in table 4.2. The proportion of index women with HBsAg-positive mothers was higher in cases (16.7%, 4/24) than in controls (7.7%, 2/26), although the difference was not statistically significant (p=0.3). None of the HBsAg-positive mothers were HBeAg-positive. The proportion of index women with HBsAg-positive older siblings was similar in cases (15.4%, 4/26) and controls (17.2%, 5/29). After adjusting for confounding factors, there was no evidence for an association between HBeAg positivity in index women and either maternal HBsAg (OR 1.2, 95% CI: 0.1-23.2, p=0.9) or having an HBsAg-positive older sibling (OR 0.9, 95% CI: 0.2-5.5, p=0.9).

Table 4.1 Characteristics of HBeAg-positive index women (cases) and HBsAg-positive HBeAg-negative women (controls)

| Variables | | HBsAg (+) HBeAg (+) index women (n=38) | | HBsAg (+) HBeAg (-) index women (n=38) | | p-value (test for trend) |
|---|---|---|---|---|---|---|
| | | No | % | No | % | |
| Age group | 17-20 | 6 | 15.8 | 7 | 18.4 | 0.9 |
| | 21-30 | 25 | 65.8 | 22 | 57.9 | |
| | 31-45 | 7 | 18.4 | 9 | 23.7 | |
| Year of birth | 1948-1960 | 6 | 15.8 | 8 | 21.0 | 0.8 |
| | 1961-1970 | 27 | 71.0 | 24 | 63.2 | |
| | 1971-1974 | 5 | 13.2 | 6 | 15.8 | |
| Total sibship size | 1-4 | 16 | 42.1 | 8 | 21.0 | 0.07 |
| | 5-6 | 12 | 31.6 | 15 | 39.5 | |
| | ≥7 | 10 | 26.3 | 15 | 39.5 | |

Table 4.2 Risk factors for HBeAg positivity in HBsAg-positive women

| Variables | HBsAg (+) HBeAg (+) index women (n=38) | | HBsAg (+) HBeAg (-) index women (n=38) | | Crude odds ratios | | | Adjusted odds ratios[1] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | OR | 95% CI | P | OR | 95% CI | P |
| Maternal HBsAg | | | | | | | | | | |
| Negative | 20 | 83.3 | 24 | 92.3 | 1.0 | | 0.3 | 1.0 | | 0.9 |
| Positive | 4 | 16.7 | 2 | 7.7 | 2.4 | 0.4-14.5 | | 1.2 | 0.1-23.2 | |
| Maternal HBeAg | | | | | | | | | | |
| Negative | 24 | 100 | 26 | 100 | 1.0 | | | 1.0 | | |
| Positive | 0 | 0 | 0 | 0 | N/A | | | N/A | | |
| No. of older siblings with positive HBsAg | | | | | | | | | | |
| 0 | 22 | 84.6 | 24 | 82.8 | 1.0 | | 0.9 | 1.0 | | 0.9 |
| 1-2 | 4 | 15.4 | 5 | 17.2 | 0.9 | 0.2-3.7 | | 0.9 | 0.2-5.5 | |
| No. of older siblings with positive HBeAg | | | | | | | | | | |
| 0 | 25 | 96.2 | 29 | 100 | 1.0 | | | 1.0 | | |
| 1 | 1 | 3.8 | 0 | 0 | N/A | | | N/A | | |

[1] Model included maternal HBsAg, number of older siblings with HBsAg, year of birth, and total sibship size.

4.4.3. Children of index women (3rd generation)

In total, 96, 102 and 101 children with HBeAg-positive, HBsAg-positive and HBeAg-negative, and HBsAg-negative mothers participated, respectively. Of whom, 38 (39.6%), 14 (13.7%) and 6 (5.9%) tested positive for HBsAg, respectively ($p<0.001$, figure 4.1). The characteristics of these HBsAg-positive children (n=58) by maternal HBV marker are presented in table 4.3. The median age was similar in the three groups. However, HBsAg-positive children with HBeAg positive mothers were more likely to be female, HBV vaccinated and to have three or fewer siblings than HBsAg-positive children born to HBeAg negative mothers.

The associations between familial HBV sero-status and HBeAg positivity in HBsAg-positive children are presented in table 4.3. There was a non-significant trend that HBeAg prevalence in children increased with increasing number of HBeAg-positive older siblings (64.1%, 69.2% and 83.3% in children with 0, 1, and ≥2 HBeAg-positive older siblings, respectively), but there was no trend after adjusting for confounding (table 4.4). In contrast, the presence of maternal HBeAg was a risk factor for HBeAg positivity after adjusting for confounding (adjusted OR 4.5, 95% CI: 1.0-19.5, p=0.04). In a bivariable model with only maternal HBeAg and number of HBeAg-positive older siblings, OR for the association between maternal HBeAg and e antigenaemia in children did not differ from the crude OR (adjusted OR 1.6, 95% CI: 0.4-5.6, p=0.5) whilst the ORs for the number of HBeAg-positive older siblings decreased; using no HBeAg-positive older siblings as the reference, the ORs were 1.1 (95% CI: 0.2-5.6) and 1.5 (0.5-4.8) for having one and two or more HBeAg-positive older siblings, respectively (p=0.7). The prevalence of e antigenaemia was similar between vaccinated (70.0%, 7/10) and unvaccinated (66.7%, 32/48, p=0.8) children.

Table 4.3. Characteristics of HBsAg-positive children according to maternal HBV sero-status

| Variables | | HBsAg(+) HBeAg(+) mother (n=38) | | HBsAg(+) HBeAg(-) mother (n=14) | | HBsAg(-) mother (n=6) | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | |
| Sex | Male | 12 | 31.6 | 8 | 57.1 | 5 | 83.3 | 0.03 |
| | Female | 26 | 68.4 | 6 | 42.9 | 1 | 16.7 | |
| Age group | 0-5 | 15 | 39.5 | 2 | 14.3 | 3 | 50.0 | 0.4 |
| | 6-10 | 16 | 42.1 | 9 | 64.3 | 2 | 33.3 | |
| | ≥11 | 7 | 18.4 | 3 | 21.4 | 1 | 16.7 | |
| Year of birth | 1973-1980 | 5 | 13.2 | 3 | 21.4 | 1 | 16.7 | 0.6 |
| | 1981-1985 | 18 | 47.4 | 9 | 64.3 | 3 | 50.0 | |
| | 1986-1991 | 15 | 39.4 | 2 | 14.3 | 2 | 33.3 | |
| Total sibship size | 1-3 | 10 | 26.3 | 1 | 7.1 | 1 | 16.7 | 0.03 |
| | 4-5 | 9 | 23.7 | 7 | 50.0 | 5 | 83.3 | |
| | 6-7 | 19 | 50.0 | 6 | 42.9 | 0 | 0.0 | |
| HBV vaccination | No | 28 | 73.7 | 14 | 100 | 6 | 100 | 0.04 |
| | Yes | 10 | 26.3 | 0 | 0.0 | 0 | 0.0 | |

Table 4.4. Risk factors for HBeAg positivity in HBsAg-positive children

| Variables | Prevalence of HBeAg (+) | Crude odds ratios | | | Adjusted odds ratios[2,3] | | |
|---|---|---|---|---|---|---|---|
| | | OR | 95% CI | P | OR | 95% CI | P |
| Maternal HBsAg | | | | | | | |
| Negative | 83.3% (5/6) | 1.0 | | 0.5 | 1.0 | | 0.5 |
| Positive | 65.4% (34/52) | 0.4 | 0.1-4.2 | | 0.4 | 0.1-5.3 | |
| Maternal HBeAg | | | | | | | |
| Negative | 60.0% (12/20) | 1.0 | | 0.5 | 1.0 | | 0.04 |
| Positive | 71.1% (27/38) | 1.6 | 0.4-6.1 | | 4.5 | 1.0-19.5 | |
| No of older siblings with positive HBsAg | | | | | | | |
| 0 | 65.6% (21/32) | 1.0 | | 0.7[1] | 1.0 | | 0.9[1] |
| 1 | 66.7% (10/15) | 1.0 | 0.3-3.8 | | 1.1 | 0.2-7.2 | |
| ≥2 | 72.7% (8/11) | 1.4 | 0.3-6.4 | | 1.2 | 0.0-47.5 | |
| No. of older siblings with positive HBeAg | | | | | | | |
| 0 | 64.1% (25/39) | 1.0 | | 0.4[1] | 1.0 | | 0.8[1] |
| 1 | 69.2% (9/13) | 1.3 | 0.3-4.9 | | 1.1 | 0.3-4.9 | |
| ≥2 | 83.3% (5/6) | 2.8 | 0.3-27.7 | | 2.0 | 0.1-42.7 | |

[1] Linear test for trend

[2] Model included maternal HBsAg, maternal HBeAg, number of older siblings with HBsAg, number of older siblings with HBeAg, year of birth, and total sibship size.

[3] Wald test from generalised estimating equations.

## 4.5. Discussion

The effect of HBV sero-markers in mothers and older siblings on hepatitis B e antigenaemia in children carrying HBsAg (3rd generation) was examined. Positive association was found between e antigenaemia in children and maternal HBeAg but not with maternal HBsAg. Likewise, the prevalence of e antigenaemia in children was higher if older siblings were HBeAg positive, although this result was not statistically significant. These relationships imply that the early establishment of chronic HBV infection through perinatal transmission from infectious mothers, as well as early postnatal transmission from infectious older siblings is associated with persistence of HBeAg. In addition, this is consistent with the observation that positive HBeAg confers greater infectivity than HBsAg positivity alone. For example, the risk of perinatal transmission is 10-17% in HBsAg-positive mothers without HBeAg and 63-67% in HBeAg positive mothers in sSA.[14,17] Indeed, in the current study, I observed that prevalence of HBsAg in children with HBeAg-positive mothers was significantly higher than that in children with HBsAg-positive but HBeAg-negative mothers (40% versus 15%).

The associations of familial HBeAg with e antigenaemia in index women (2nd generation) could not be confirmed because all of the mothers of index women tested negative for HBeAg and only one older sibling tested positive for HBeAg. The low prevalence of HBeAg in the mothers and older siblings of index women was expected since HBeAg is lost over time. In The Gambia, 85% of children who established chronic HBV infection during early childhood had lost HBeAg by the second decade of life.[185] This makes it difficult to investigate the effect of familial HBeAg status in adults.

A strength of this study was that I could assess the effect of familial HBV sero-status, rather than a family history of HBV infection. The latter is inaccurate because the absence of such a history does not necessarily indicate that the family member was sero-negative for HBsAg.[261] Second, I could control for the HBV markers of older siblings when assessing the association of maternal HBV sero-status with HBeAg positivity. And similarly the association between siblings' sero-status and HBeAg positivity was adjusted for maternal HBV markers. In other studies this has not been possible.[78,83,242,245,247,250,251] The mutual adjustment with siblings' sero-markers was particularly relevant in The Gambia, where sibling-to-sibling transmission during early childhood is the most frequent route.[42,60] After the mutual adjustment the OR for the association between maternal HBeAg and e antigenaemia in children did not change whilst the ORs for the number of HBeAg-positive older siblings decreased. This suggests that perinatal maternal transmission might be a more important in determining the risk of persistent viral replication than early horizontal transmission from older siblings in The Gambia. This is consistent with the hypothesis that earlier age at HBV infection is associated with higher risk of e antigenaemia.

Two African case-control studies have investigated the association between age at HBV infection and HCC, ultimate sequelae of chronic HBV infection. Larouze *et al.*, found that the prevalence of maternal HBsAg was higher in HCC cases (71.4%, 20/28) than in healthy controls (14.3%, 4/28, $P<0.0001$) while the prevalence of HBsAg in siblings was similar in cases (9.6%, 7/73) and controls (14%, 8/58).[186] Ryder et al., studied the association between birth order and HCC, and found strong evidence that higher birth order, and hence earlier age at infection, is associated with higher HCC risk ($P<0.005$).[193] In the absence of immunisation,

children with low birth order are exposed to HBV after they start schooling, whilst children with high birth order are infected much earlier by their older siblings who got the infection outside the household.[187] However, because individuals negative for HBsAg were included in both studies, the associations might be due to an increased risk of chronic HBV infection related with early age at infection. The results of these studies are therefore not directly comparable to the findings from the HBeAg Study.

The study has several limitations. First, the sample size was small, and the associations were therefore poorly estimated. Second, many family members of the index women did not participate in the HBeAg study, and this might have led to selection bias. Third, important confounding variables might have been omitted, in particular viral genotype.

HBV vaccination programmes in Africa and Asia have been successful in preventing postnatal horizontal transmission of HBV, thereby reducing the prevalence of chronic HBV infection.[238,264] However, they have had a limited impact on perinatal mother-to-infant transmission, especially when the mothers are HBeAg-positive or highly viraemic.[126,238] In East Asia, where perinatal maternal transmission is common, HBV vaccine is frequently given within 24 hours of delivery (i.e. timely birth dose).[265,266] By contrast a timely birth dose of HBV vaccine is rarely administered in Africa because logistical challenges seem to outweigh its potential impact due to the relatively low frequency of mother-to-infant transmission.[267] Despite a high coverage of hepatitis B vaccine, only six countries in sSA are undertaking the birth dose by the end of 2012 and The Gambia is one of them.[117] However, in two villages in rural Gambia where HBV vaccine efficacy was evaluated,[238] only 2.8% (63/2173) of children who were

vaccinated against HBV between 1984 and 2007 were given a dose within 24 hours of birth (unpublished data). This might be because in The Gambia, most children are born at home, and are usually not taken away from home in the first week.[113]

This study suggests that the risk of prolonged e antigenaemia, an important predictor of HCC,[268] may be higher in individuals who perinatally established chronic infection through infectious mothers than in those infected with HBV by horizontal transmission. This implies that interrupting mother-to-infant transmission in sub-Saharan Africa might help reduce the burden of liver disease. A timely dose of HBV vaccine within 24 hours of birth, as recommended by WHO,[116] needs to be considered in sub-Saharan Africa.

## 4.6. Summary

The effect of early age at infection with HBV on prolonged hepatitis B e antigenaemia was examined using maternal HBsAg and HBeAg as proxies for perinatal maternal transmission and number of older siblings with positive HBsAg and HBeAg as proxies for early postnatal transmission. Between 1986 and 1990, six cross-sectional HBV sero-surveys targeting mothers and children were conducted in The Gambia. All the mothers identified as being positive for HBeAg were invited to participate in the current study, as well as the same numbers of HBsAg-positive HBeAg-negative mothers and HBsAg-negative mothers who were randomly

selected from the databases. In addition, all the family members of these mothers (i.e. index women) were also asked to take part in the study. The associations of familial HBV markers with HBeAg positivity were examined in two successive generations: in index women and their children.

In index women, the effect of maternal HBeAg could not be examined because none of mothers of index women (i.e. grandmothers) were positive for HBeAg. In contrast, there was a positive association between maternal HBeAg and e antigenaemia in children. The increasing HBeAg prevalence was also observed with increasing number of HBeAg-positive older siblings although this association did not reach the statistical significance. After maternal sero-status and older siblings' sero-status were mutually adjusted, the effect of maternal HBeAg was stronger than the effect of older siblings' HBeAg, suggesting that perinatal maternal transmission might be a more important determinant of persistent viral replication than early horizontal transmission from older siblings in The Gambia. Although it was difficult to draw a robust conclusion due to small sample size, these findings emphasise the importance of interrupting the mother-to-infant transmission in sub-Saharan Africa.

# Chapter 5. The association of birth order with hepatocellular carcinoma and liver cirrhosis in people with chronic hepatitis B infection: a secondary data analysis of the Gambia Liver Cancer Study (GLCS), a case-control study in the Gambia, West Africa

## 5.1. Abstract

**Background**

Early age at hepatitis B virus (HBV) infection is a potential risk factor for liver cirrhosis and hepatocellular carcinoma (HCC) in individuals with chronic hepatitis B infection. However, this has never been studied in Africa. A historical dataset from the Gambia Liver Cancer Study (GLCS) was further exploited focusing on the effect of age at infection on HCC and liver cirrhosis using birth order as a proxy for age at infection, a question not originally answered. Because the major mode of HBV transmission in The Gambia is sibling-to-sibling during childhood, it is hypothesised that the risk of HCC increases with increasing birth order.

**Methods**

HCC cases and liver cirrhosis cases were recruited from three referral hospitals in The Gambia between 1997 and 2001. In the original analysis, for each HCC case, two control participants without clinical liver disease, frequency matched by age, gender and study site, were recruited.

Because this current analysis was restricted to participants with positive hepatitis B surface antigen (HBsAg), the original sampling scheme and thus matching were disrupted.

**Results**

The analysis included 129 HBV-related HCC, 67 HBV-related liver cirrhosis, and 64 HBsAg-positive controls. Compared to the first-born, the odds ratios for HCC were 0.62 (95% CI: 0.25-1.55), 0.99 (0.32-3.08), 0.87 (0.27-2.79) and 0.55 (0.18-1.67) in second-, third-, fourth- and fifth- or later-born participant, respectively, when the birth order excluded half-siblings (p=0.5). When the birth order counted both full- and half-siblings, the odds ratio for HCC was 0.37 (95% CI: 0.11-1.16), 1.03 (0.33-3.18), 0.37 (0.11-1.20) and 0.64 (0.18-2.29), in $2^{nd}$-, $3^{rd}$- and $4^{th}$-, $5^{th}$- and $6^{th}$-, and $7^{th}$- or later-born participant, respectively (p=0.5). A similar non-significant trend was observed for liver cirrhosis.

**Conclusion**

Although the association was not statistically significant, $1^{st}$ birth order was associated with the highest risk for cirrhosis and HCC, while the risk was lower for $2^{nd}$, $3^{rd}$, $4^{th}$ and $\geq 5^{th}$ birth order. This suggests, contrary to the original hypothesis, that mother-to-infant transmission of HBV might be an important determinant of liver cirrhosis and HCC in chronic HBV carriers in The Gambia.

## 5.2. Introduction

The systematic review of observational studies in Chapter 3 suggests that there is an association between early age at hepatitis B virus (HBV) infection and increased risk of liver cirrhosis and hepatocellular carcinoma (HCC) amongst chronic carriers of HBV. However, the review did not include any African study. In this chapter, an old dataset of the Gambia Liver Cancer Study (GLCS) was further exploited focusing on the effect of early age at infection on liver cirrhosis and HCC, an association that was not examined in the original study. The GLCS is a case-control study of liver cirrhosis and HCC conducted by Kirk *et al*. in The Gambia in 1997-2001.[169] In the analysis, birth order is used as a proxy for early exposure to HBV through sibling-to-sibling transmission as discussed in Chapter 2.[187,194]

## 5.3. Methods

5.3.1. The Gambia Liver Cancer Study (GLCS)

The GLCS is a case-control study which was conducted in The Gambia from 1997 to 2001. Cases had been diagnosed with either cirrhosis or liver cancer. The study was designed to estimate the population attributable fraction associated with HBV, hepatitis C virus (HCV), or aflatoxin.[156,169] Study participants were recruited from three tertiary care hospitals in The Gambia, namely: Royal Victoria Teaching Hospital (RVTH), the sole tertiary care hospital in The Gambia located at capital Banjul; Medical Research Council (MRC) Clinic, within the Greater Banjul Area; and Bansang General Hospital (BSG) in the upcountry. First, patients with suspected liver disease were identified by local physicians or through active surveillance of the outpatient clinics and inpatient wards by field staff. After informed consent, the patients

underwent a structured interview with a standardised questionnaire, clinical and ultrasound examination and collection of a blood sample. For each case, two control participants without clinical liver disease were recruited from the outpatient clinics of the same hospitals. They were frequency matched to patients with liver disease by age (using 10-year age bands), gender and study site. The same questionnaire, examination and blood collection was administered to the controls. The study was approved by the Gambia Government/MRC joint ethics committee.

### 5.3.2. Case and Control Definition

HCC cases were defined as patients with suspected liver disease who had either 1) pathological confirmation or 2) a serum alpha-fetoprotein (AFP) levels >100 ng/mL with space-occupying hepatic lesions (SOL) on ultrasound. Liver cirrhosis cases were defined as patients with suspected liver disease who had 1) no evidence of SOL by ultrasound; 2) no evidence of alternative diagnoses such as cardiac or renal disorder; and 3) a score of ≥7 in the cirrhosis diagnostic scale using ultrasound.[269] Control participants were defined as patients who had 1) no clinical evidence of liver disease and 2) AFP levels <20 ng/ml.

### 5.3.3. Birth order

The number of full-siblings (siblings who share both biological parents) and half-siblings who lived with the study participant when he or she was old enough to go to school was obtained by administering a standardised questionnaire. Twins and triplets were counted as independent births. Miscarriages and stillbirths were not counted. The number of siblings who were older than the participant at this time was used to derive birth order. Two different birth ranks were

assessed: birth order in full-siblings and birth order in all siblings (counting both full- and half-siblings).

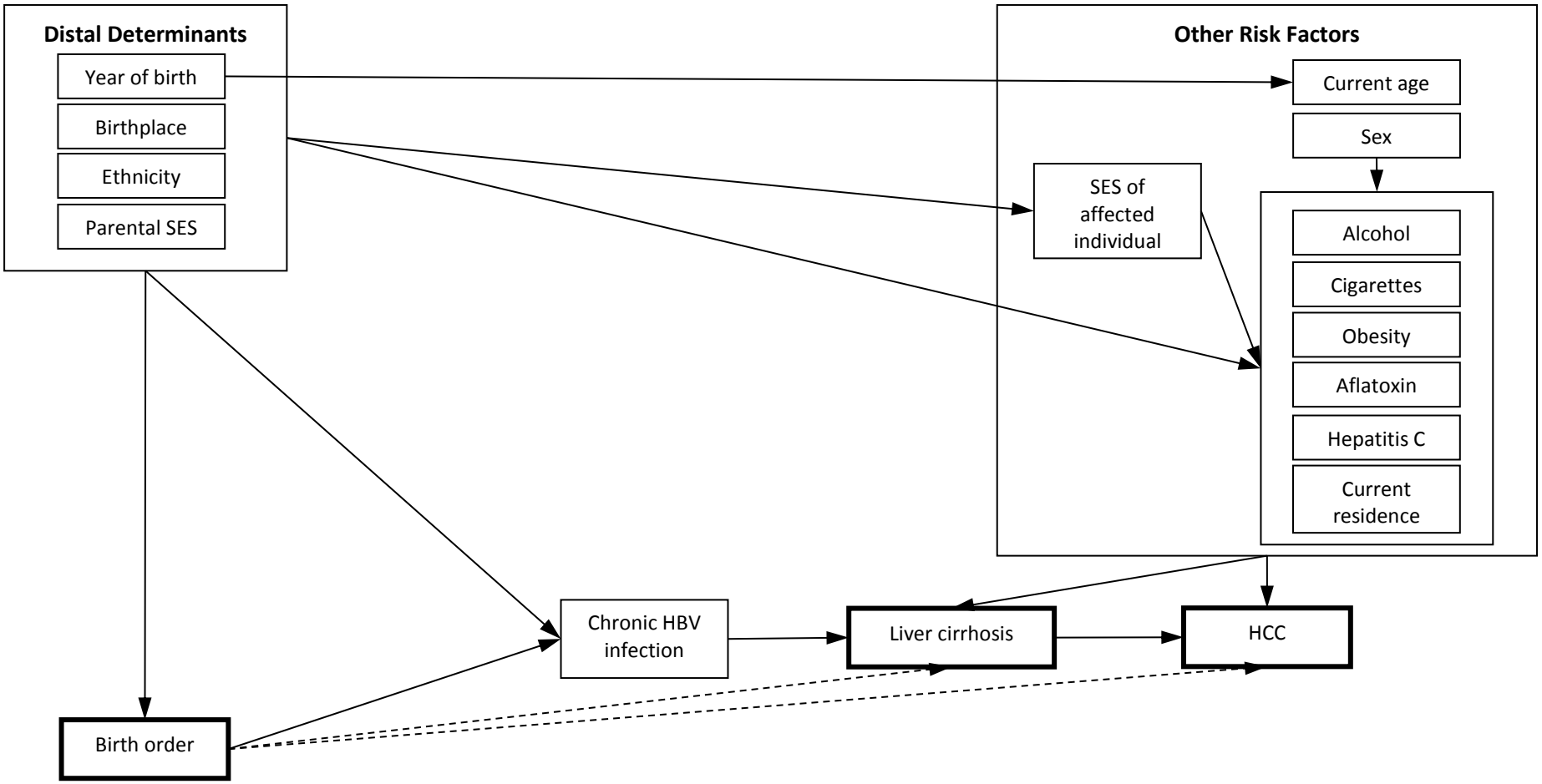
### 5.3.4. Statistical analyses

Analyses were restricted to study participants with positive HBsAg, because the objective was to examine the effect of early age at HBV infection on liver diseases beyond its effect of increasing the chance of chronic HBV infection. The restriction to HBsAg-positive individuals disrupted the original sampling scheme and thus matching. The prevalence of risk factors was compared between cirrhosis cases and controls, and between HCC cases and controls using the chi-squared test (sex, ethnic group, recruitment site, education level, family history of liver cancer, alcohol consumption, cigarettes smoking, HBeAg, anti-HCV and $249^{ser}$ TP 53 mutation), chi-squared test for trend (age group and number of siblings), or Wilcoxon rank-sum test (age). Logistic regression was used to estimate odds ratios for the association of birth order with liver cirrhosis and HCC adjusted for *a priori* confounders.

As discussed in details in Chapter 4, a minimally sufficient set of *a priori* confounders for the association between birth order and liver diseases was identified by applying the backdoor test in a causal diagram (figure 5.1).[263] The confounders identified from the causal diagram were: year of birth, birthplace, ethnic group and parental socioeconomic status (SES). Data for birthplace were unavailable and thus could not be included in the model. As discussed in Chapter 2, it is unclear whether to include the number of siblings (sibship size) as a confounder in the analysis of birth order.[202,218] Therefore, two different models were used to account for confounding due to SES: in model 1 schooling (of the study participant) was used as a proxy for

SES and in model 2 sibship size was used as the proxy. Because participants were recruited over a relatively short period (5 years), year of birth and age at study entry were approximately equivalent. Both models included age, sex and study site, as cases and controls were frequency matched on these variables. Recruitment date was included in the multivariable model in the original paper,[165] however this was excluded in the current analysis because it is unlikely that the recruitment date varies according to the exposure (i.e., birth order).

Figure 5.1. Causal diagram for the effect of birth order on the risk of liver cirrhosis and HCC in the Gambia Liver Cancer Study, 1997-2001



Early birth order is a proxy for perinatal mother-to-infant HBV transmission whilst late birth order is a proxy for early horizontal transmission. Early age at HBV infection is known to be associated with liver cirrhosis and HCC through increasing the risk of chronic HBV infection. The hypothesis of this analysis is that in addition to this effect, early age at infection further increases the risk of liver cirrhosis and HCC (presented as a dashed arrow). The exposure and outcome variables of interest are surrounded by lines in bold-type. Abbreviations: SES, socioeconomic status; HCV, hepatitis C virus.

## 5.4. Results

5.4.1. Characteristics of the study participants

Two-hundred sixteen HCC cases, 121 liver cirrhosis cases and 408 controls were recruited into the study, among which 129 (61.1%), 67 (56.3%), and 64 (15.9%) were HBsAg-positive, respectively (table 5.1). Characteristics of HBV-related HCC cases, HBV-related liver cirrhosis and HBsAg-positive controls are presented in table 5.2. The gender ratio was similar in each group. HCC cases tended to be older than cirrhosis or controls, but the difference was not significant. For ethnic group, cirrhosis and HCC cases were less likely to be Mandinka than controls and more likely to be Fula and Wollof. HCC cases were less educated than controls. Alcohol consumption, smoking cigarettes, anti-HCV and family history of liver cancer were similar in cases and controls. The prevalence of HBeAg was higher in cases than controls, as was the TP53 mutation.

Table 5.1 Prevalence of HBsAg in controls, liver cirrhosis and HCC cases in the Gambia Liver Cancer Study, 1997-2001

| HBsAg | Controls (N=408) | Liver cirrhosis (N=121) | HCC (N=216) |
|---|---|---|---|
| Negative | 338 (84.1%) | 52 (43.7%) | 82 (38.9%) |
| Positive | 64 (15.9%) | 67 (56.3%) | 129 (61.1%) |
| Missing | 6 | 2 | 5 |

Table 5.2 Characteristics of HBsAg-positive controls, HBV-related liver cirrhosis and HBV-related HCC in the Gambia Liver Cancer Study, 1997-2001

| | HBsAg (+) controls (N=64) | | HBV-related liver cirrhosis (N=67) | | | HBV-related HCC (N=129) | | |
|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | P | n | % | P |
| Male sex | 52 | 81.3 | 51 | 76.1 | 0.5 | 108 | 83.7 | 0.7 |
| Age (yrs), median (IQR) | 38.5 (27-50) | | 38 (29-48) | | 0.8[1] | 42 (32-48) | | 0.3[1] |
| Age group | | | | | 0.6[2] | | | 0.5[2] |
| <30 | 21 | 32.8 | 17 | 25.4 | | 24 | 18.6 | |
| 30 – 39 | 12 | 18.8 | 19 | 28.4 | | 30 | 23.3 | |
| 40 – 49 | 11 | 17.2 | 17 | 25.4 | | 44 | 34.1 | |
| 50 – 59 | 11 | 17.2 | 10 | 14.9 | | 16 | 12.4 | |
| ≥60 | 9 | 14.1 | 4 | 6.0 | | 15 | 11.6 | |
| Ethnic group | | | | | 0.1 | | | 0.05 |
| Mandinka | 21 | 34.4 | 16 | 24.2 | | 29 | 23.0 | |
| Fula | 11 | 18.0 | 22 | 33.3 | | 37 | 29.4 | |
| Wollof | 9 | 14.8 | 18 | 27.3 | | 28 | 22.2 | |
| Other | 20 | 32.8 | 10 | 15.2 | | 32 | 25.4 | |
| Number of full-siblings | | | | | | | | |
| 1-2 | 5 | 8.3 | 12 | 18.5 | 0.4[2] | 22 | 17.5 | 0.7[2] |
| 3-4 | 20 | 33.3 | 16 | 24.6 | | 32 | 25.4 | |
| 5-6 | 17 | 28.3 | 20 | 30.8 | | 29 | 23.0 | |
| ≥7 | 18 | 30.0 | 17 | 26.1 | | 43 | 34.1 | |
| Number of siblings (full + half) | | | | | | | | |
| 0-5 | 15 | 25.9 | 19 | 30.7 | 0.2[2] | 37 | 29.6 | 0.4[2] |
| 6-8 | 13 | 22.4 | 22 | 35.5 | | 39 | 31.2 | |
| 9-11 | 17 | 29.3 | 10 | 16.1 | | 18 | 14.4 | |
| ≥12 | 13 | 22.4 | 11 | 17.7 | | 31 | 24.8 | |
| Recruitment site | | | | | 0.07 | | | 0.2 |
| RVTH | 19 | 29.7 | 33 | 49.3 | | 54 | 41.9 | |
| MRC | 22 | 34.4 | 16 | 23.9 | | 40 | 31.0 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| BSG | 23 | 35.9 | 18 | 26.9 | | 35 | 27.1 | |
| Education | | | | | 0.07 | | | 0.03 |
| Ever attended school | 58 | 93.5 | 55 | 83.3 | | 103 | 81.8 | |
| None | 4 | 6.5 | 11 | 16.7 | | 23 | 18.2 | |
| Family history of liver cancer | | | | | 0.3 | | | 0.1 |
| Yes | 2 | 3.3 | 5 | 7.6 | | 13 | 10.3 | |
| No | 59 | 96.7 | 61 | 92.4 | | 113 | 89.7 | |
| Ever drunk alcohol | | | | | 0.9 | | | 0.3 |
| Yes | 6 | 9.7 | 7 | 10.6 | | 19 | 15.3 | |
| No | 56 | 90.3 | 59 | 89.4 | | 10 | 84.7 | |
| Ever smoked cigarettes | | | | | 0.4 | | | 0.4 |
| Yes | 32 | 51.6 | 29 | 43.9 | | 72 | 58.1 | |
| No | 30 | 48.4 | 37 | 56.1 | | 52 | 41.9 | |
| HBeAg | | | | | 0.001 | | | 0.006 |
| Positive | 2 | 3.5 | 16 | 25.0 | | 23 | 81.6 | |
| Negative | 56 | 96.5 | 48 | 75.0 | | 102 | 18.4 | |
| HCV infection | | | | | 0.3 | | | 0.2 |
| Yes | 1 | 1.7 | 0 | 0 | | 7 | 6.0 | |
| No | 59 | 98.3 | 57 | 100 | | 110 | 94.0 | |
| $249^{ser}$ TP53 mutation | | | | | 0.02 | | | <0.001 |
| Yes | 2 | 4.0 | 10 | 18.2 | | 41 | 40.6 | |
| No | 48 | 96.0 | 45 | 81.8 | | 60 | 59.4 | |

P-value was obtained using chi-squared test unless indicated.

[1] Wilcoxon rank-sum test

[2] Test for trend

5.4.2. Characteristics of HBsAg-positive controls by birth order in full siblings

The association between birth order in full siblings and risk factors for liver cirrhosis and HCC in HBsAg-positive controls is presented in table 5.3. The number of full siblings was lower in low birth order than in high birth order. Otherwise, there were no substantial imbalances in the distribution of risk factors according to birth order. Although none of *a priori* confounders (age, ethnic group, parental socioeconomic status, sex and study site) were associated with the exposure of interest (birth order in full-siblings), these factors were included in the multivariable analyses that assess the effect of birth order on liver cirrhosis and HCC. This is because small differences in the distribution of confounders between the exposed and unexposed group can still bias estimates even if the differences are not statistically significant.

5.4.3. Birth order and risk of liver cirrhosis and HCC

The results of univariable and multivariable analysis for the association of birth order with HCC and cirrhosis are presented in table 5.4 and 5.5, respectively. Although the association was not statistically significant, the risk of cirrhosis and HCC was generally greater for $1^{st}$ birth order than $2^{nd}$, $3^{rd}$, $4^{th}$ and $\geq 5^{th}$ birth order, that had a similar lower degree of risk. The exceptions are multivariable model 2 for the effect of birth order in full-siblings on HCC and model 1 and 2 for the effect of birth order in all (full- and half-) siblings on HCC; these found the highest risk in the $3^{rd}$ birth rank.

Table 5.3 Characteristics of HBsAg-positive controls by birth order in full-siblings in the Gambia Liver Cancer Study, 1997-2001

| | Birth order in full-siblings | | | | | P |
|---|---|---|---|---|---|---|
| | 1st (16) | 2nd (18) | 3rd (9) | 4th (7) | 5th (9) | |
| Male sex | 14 (88%) | 15 (83%) | 6 (67%) | 5 (71%) | 7 (78%) | 0.7 |
| Median age (range) | 37 (19, 75) | 48 (25, 70) | 33 (22, 40) | 30 (21, 70) | 30 (21, 80) | 0.2 |
| Ethnic group | | | | | | 0.6 |
| Mandinka | 5 (31%) | 5 (28%) | 3 (37%) | 2 (29%) | 4 (45%) | |
| Fula | 3 (19%) | 4 (22%) | 2 (25%) | 0 | 2 (22%) | |
| Wolof | 1 (6%) | 5 (28%) | 1 (13%) | 1 (14%) | 0 | |
| Others | 7 (44%) | 4 (22%) | 2 (25%) | 4 (47%) | 3 (33%) | |
| Number of full-siblings | | | | | | 0.09 |
| 1-2 | 1 (6%) | 4 (22%) | 0 | 0 | 0 | |
| 3-4 | 7 (44%) | 7 (39%) | 4 (44%) | 1 (14%) | 0 | |
| 5-6 | 2 (12%) | 5 (28%) | 3 (33%) | 3 (43%) | 4 (44%) | |
| ≥7 | 6 (38%) | 2 (11%) | 2 (22%) | 3 (43%) | 5 (56%) | |
| Recruitment site | | | | | | 0.8 |
| RVTH | 6 (38%) | 4 (22%) | 2 (22%) | 2 (29%) | 2 (22%) | |
| MRC | 6 (38%) | 5 (28%) | 3 (33%) | 2 (29%) | 5 (56%) | |
| BSG | 4 (25%) | 9 (50%) | 4 (45%) | 3 (43%) | 2 (22%) | |
| Never attended school | 0 | 1 (6%) | 2 (22%) | 0 | 1 (11%) | 0.3 |
| Family history of HCC | 5 (5%) | 5 (11%) | 1 (2%) | 0 | 5 (10%) | 0.3 |
| Ever drunk alcohol | 3 (19%) | 1 (6%) | 1 (11%) | 0 | 1 (11%) | 0.6 |
| Ever smoked cigarettes | 8 (50%) | 11 (61%) | 3 (33%) | 3 (43%) | 5 (56%) | 0.7 |
| Obesity (BMI≥30) | 0 | 0 | 0 | 0 | 0 | 1.0 |
| HBeAg positive | 0 | 1 (6%) | 1 (17%) | 0 | 0 | 0.4 |
| HCV positive | 0 | 0 | 0 | 0 | 1 (11%) | 0.3 |
| $249^{ser}$ TP53 mutation | 0 | 0 | 0 | 1 (20%) | 1 (13%) | 0.2 |

P-value was obtained using chi-squared test unless indicated.

[1] Kruskal-Wallis one-way analysis of variance

Table 5.4 Univariable and multivariable analysis for the association between birth order and HCC among HBsAg-positive individuals in the Gambia Liver Cancer Study, 1997-2001

| Variables | | HBsAg (+) controls (N=64) | | HBV-related HCC (N=129) | | Univariable analysis | | Model 1 (education level as proxy for parental SES) | | Model 2 (total sibship size as proxy for parental SES) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | OR (95% CI) | P | OR (95% CI) | P | OR (95% CI) | P |
| Birth order in full-siblings[2] | 1st | 16 | 27.1 | 43 | 34.4 | 1.00 | 0.5[1] | 1.00 | 0.5[1] | 1.00 | 0.6[1] |
| | 2nd | 18 | 30.5 | 32 | 25.6 | 0.66 (0.29-1.49) | | 0.62 (0.25-1.55) | | 0.71 (0.29-1.74) | |
| | 3rd | 9 | 15.3 | 20 | 16.0 | 0.83 (0.31-2.19) | | 0.99 (0.32-3.08) | | 1.05 (0.34-3.20) | |
| | 4th | 7 | 11.9 | 15 | 12.0 | 0.80 (0.27-2.31) | | 0.87 (0.27-2.79) | | 0.93 (0.29-3.02) | |
| | ≥5th | 9 | 15.3 | 15 | 12.0 | 0.62 (0.22-1.70) | | 0.55 (0.18-1.67) | | 0.58 (0.18-1.91) | |
| | Not reported | 5 | | 4 | | | | | | | |
| Birth order in siblings (full + half)[3] | 1st | 8 | 14.3 | 27 | 22.1 | 1.00 | 0.4[1] | 1.00 | 0.5[1] | 1.00 | 0.7[1] |
| | 2nd | 15 | 26.8 | 22 | 18.0 | 0.43 (0.16-1.21) | | 0.37 (0.11-1.16) | | 0.39 (0.12-1.24) | |
| | 3rd & 4th | 13 | 23.2 | 41 | 33.6 | 0.93 (0.34-2.55) | | 1.03 (0.33-3.18) | | 1.16 (0.38-3.61) | |
| | 5th & 6th | 11 | 19.6 | 16 | 13.1 | 0.43 (0.14-1.30) | | 0.37 (0.11-1.20) | | 0.39 (0.11-1.31) | |
| | ≥7th | 9 | 16.1 | 16 | 13.1 | 0.53 (0.17-1.64) | | 0.64 (0.18-2.29) | | 0.69 (0.18-2.67) | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Not reported | 8 | | 7 | | | | | | | |

[1] Test for trend

[2] Multivariable model included age, sex, ethnicity, recruitment site, birth order in full-siblings and education level (model 1) or number of full-siblings (model 2).

[3] Multivariable model included age, sex, ethnicity, recruitment site, birth order in siblings (full + half) and education level (model 1) or number of siblings (full + half) (model 2).

Table 5.5 Univariable and multivariable analysis for the association between birth order and liver cirrhosis among HBsAg-positive individuals in the Gambia Liver Cancer Study, 1997-2001

| Variables | | HBsAg (+) controls (N=64) | | HBV-related liver cirrhosis (N=67) | | Univariable analysis | | Model 1 (education level as proxy for parental SES) | | Model 2 (total sibship size as proxy for parental SES) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | OR (95% CI) | P | OR (95% CI) | P | OR (95% CI) | P |
| Birth order in full-siblings[2] | 1st | 16 | 27.1 | 27 | 41.5 | 1.00 | 0.4[1] | 1.00 | 0.6[1] | 1.00 | 0.7[1] |
| | 2nd | 18 | 30.5 | 14 | 21.5 | 0.46 (0.18-1.17) | | 0.39 (0.13-1.13) | | 0.33 (0.11-0.99) | |
| | 3rd | 9 | 15.3 | 7 | 10.8 | 0.46 (0.14-1.48) | | 0.50 (0.13-1.95) | | 0.52 (0.13-2.00) | |
| | 4th | 7 | 11.9 | 8 | 12.3 | 0.68 (0.21-2.22) | | 0.81 (0.19-3.38) | | 0.85 (0.20-3.69) | |
| | ≥5th | 9 | 15.3 | 9 | 13.9 | 0.59 (0.20-1.80) | | 0.63 (0.18-2.15) | | 0.80 (0.21-3.00) | |
| | Not reported | 5 | | 2 | | | | | | | |
| Birth order in siblings (full + half)[3] | 1st | 8 | 14.3 | 17 | 27.4 | 1.0 | 0.3[1] | 1.0 | 0.4[1] | 1.0 | 0.6[2] |
| | 2nd | 15 | 26.8 | 12 | 19.4 | 0.38 (0.12-1.17) | | 0.40 (0.11-1.44) | | 0.36 (0.10-1.26) | |
| | 3rd & 4th | 13 | 23.2 | 15 | 24.2 | 0.54 (0.18-1.67) | | 0.71 (0.19-2.72) | | 0.62 (0.17-2.32) | |
| | 5th & 6th | 11 | 19.6 | 7 | 11.3 | 0.30 (0.08-1.06) | | 0.24 (0.05-1.09) | | 0.26 (0.06-1.20) | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≥7th | 9 | 16.1 | 11 | 17.7 | 0.58 (0.17-1.94) | | 0.69 (0.16-3.02) | | 0.83 (0.18-3.91) | |
| | Not reported | 8 | | 5 | | | | | | | |

[1] Test for trend

[2] Multivariable model included age, sex, ethnicity, recruitment site, birth order in full-siblings and education level (model 1) or number of full-siblings (model 2).

[3] Multivariable model included age, sex, ethnicity, recruitment site, birth order in siblings (full + half) and education level (model 1) or number of siblings (full + half) (model 2).

## 5.5. Discussion

There was no statistically significant association between birth order and risk of HCC and cirrhosis in people with chronic HBV infection. However, the study found that first child in the family tended to have the highest risk of developing cirrhosis or HCC whilst later-born children had a lower degree of risk.

Of six studies of birth order that were included in the systematic review (Chapter 3), two from Greece[187,194] found that HBV-related HCC cases have higher birth orders than HBsAg-positive non-HCC controls while four from China and Taiwan found that lower birth order was associated with HCC[253–255] or positive HBeAg.[252] This heterogeneity in the direction of association was explained by the geographical difference in the main mode of HBV transmission between East Asia and Southern Europe (Chapter 2 & 3). In SSA, the major mode of HBV transmission and subsequent natural history of chronic HBV infection is similar to Mediterranean countries.[71] However, the current study did not reproduce the association observed in Greece.

There are several explanations for such a discrepancy. First, mother-to-infant transmission might be more common in SSA than in Greece. Indeed, the prevalence of HBeAg among HBsAg-positive women of child-bearing age was slightly higher in The Gambia (6.8-13.7%)[55,60,114] than in Greece (2.7%).[270] Second, the positive association observed in the Greek data might be due to differential study participation, as demonstrated by Grulich *et al*. in their pooled analysis of data from 14 case-control studies of birth order and risk of non-Hodgkin lymphoma (NHL).[208] The analysis revealed that statistically significant positive associations

between birth order and NHL were confined to studies which have lower response rates.[208] Because higher SES is related with smaller family size and thus lower birth order,[207] and people in higher SES are more likely to participate in a study,[271] there would be a significant excess of individuals with low birth order among participants than non-participants. Since response rates are generally higher in cases than in controls,[208] the differential participation between cases and controls according to SES and birth order would eventually lead to a false conclusion that having more elder siblings is a risk of the disease. Unfortunately there is no information on the participation rate in the current study or the Greek studies.

The study has several limitations. First, the sample size was small. Second, the birth order question in the current study was designed to quantify the probability of early horizontal transmission from older siblings rather than the probability of perinatal maternal transmission. Exclusion of miscarriages and stillbirths and counting twins and triplets independently might have contributed to the lack of significant association between birth order and HCC. Third, using hospital-controls may not be ideal,[208] as birth order has been suggested to be associated with many other morbidity and mortality,[191] such as other cancers,[189] allergic conditions,[190] and cardiovascular risk factors.[188]

Although the association was not statistically significant, the direction of the association is consistent with the findings from the HBeAg Study (Chapter 4). Together these studies suggest that mother-to-infant transmission might play a role in the persistence of viral replication and liver diseases in The Gambia. This needs to be further confirmed with a population-based case-control study of HCC or a longitudinal cohort study of people with chronic HBV infection.

## 5.6. Summary

The association between early age at HBV infection and liver cirrhosis and HCC was assessed using birth order as a proxy for the age at infection. Before the analysis a positive association between birth order and liver diseases was hypothesised, because most transmission in The Gambia occurs between siblings during childhood. Contrary to the hypothesis, the analysis showed that first birth order had the highest risk for HCC and liver cirrhosis, although the association was not statistically significant. This suggests that birth order might be a surrogate for maternal age at delivery.

# Chapter 6. The natural history of chronic hepatitis B infection in The Gambia, West Africa: a longitudinal population-based study of chronic carriers in three rural villages

## 6.1. Abstract

**Background**

The natural history of chronic hepatitis B (CHB) infection in sub-Saharan Africa (sSA) is poorly documented. In particular the effect of perinatal maternal transmission of hepatitis B virus (HBV) on progression of liver disease after establishing the chronic infection is unknown. An open cohort study of treatment-naïve people with CHB infection in The Gambia, was used to estimate the rates of hepatitis B e (HBeAg) and surface antigen (HBsAg) clearance, incidence of hepatocellular carcinoma (HCC), overall mortality and prevalence of active CHB disease, significant liver fibrosis and condition meeting the international treatment criteria for CHB infection. The association of maternal HBsAg, a proxy for perinatal mother-to-infant transmission with these outcomes was also examined.

**Methods**

Since 1973, HBV sero-surveys have been conducted every 4-5 years in three villages in the West Kiang District in The Gambia. In 2012-2013, as part of the PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa) project, we invited the cohort of chronic HBV carriers for a comprehensive liver assessment. CHB infection was defined as a serum HBsAg positivity on

two occasions at least 6 months apart for the people aged less than 10 years and HBsAg positivity at least once for those over 10 years old.

**Results**

414 chronic carriers were recruited. The median length of follow-up was 28 years (range: 4-39) and the median number of sero-surveys participated in was six (range: 2-10). The HBeAg and HBsAg seroclerance rate was 7.3% (95%CI: 6.1-8.7%) and 1.3% (1.1-1.6%) per annum, respectively. Six subjects died of HCC, which implies an HCC incidence of 54.9/100,000 carrier-years. In 2012-13, 264 individuals with CHB infection were assessed (41% male, median age 37 years, range 11-78 years). The prevalence of significant liver fibrosis (≥7.5 kPa by transient elastography) was 10% (95%CI: 6-14%), and 5% (95%CI: 2-8%) met the EASL (European Association for the Study of the Liver) treatment criteria. Having an HBsAg-positive mother was significantly associated with delayed HBeAg sero-clearance, higher viral load and ALT levels over time, active CHB disease, advanced liver fibrosis and meeting the EASL criteria. It was estimated that 71.4% (95% CI: 6.3-91.3%) of chronic carriers requiring antiviral therapy were attributable to perinatal mother-to-infant transmission.

**Conclusion**

Positive maternal HBsAg was associated with an increased risk of liver fibrosis through persistent viral replication and frequent hepatitis flare. Interventions to prevent perinatal maternal transmission, such as a dose of hepatitis B vaccination within 24 hours of birth, could have a substantial impact on the burden of complications associated with CHB infection in sub-Saharan Africa.

## 6.2. Introduction

In sub-Saharan Africa (sSA), prevalence of hepatitis B virus (HBV) infection in adults is still high and exceeds 8% in many countries.[2] Hepatocellular carcinoma (HCC) is ranked the second and third most common malignancy in African men and women, respectively, [5] and more than half of HCC cases are attributable to chronic HBV (CHB) infection.[169] Hepatitis B vaccine is highly effective in preventing CHB infection. It has been included in the national immunisation programmes in all African countries, with vaccine coverage ranging between 58% and 99%.[272] However, the vaccine is limited as a control measure. First, a large number of people who acquired the infection prior to the implementation of the vaccination programme are left with CHB infection.[273] Theses individuals will continue to be at high risk of developing cirrhosis and HCC.[274]

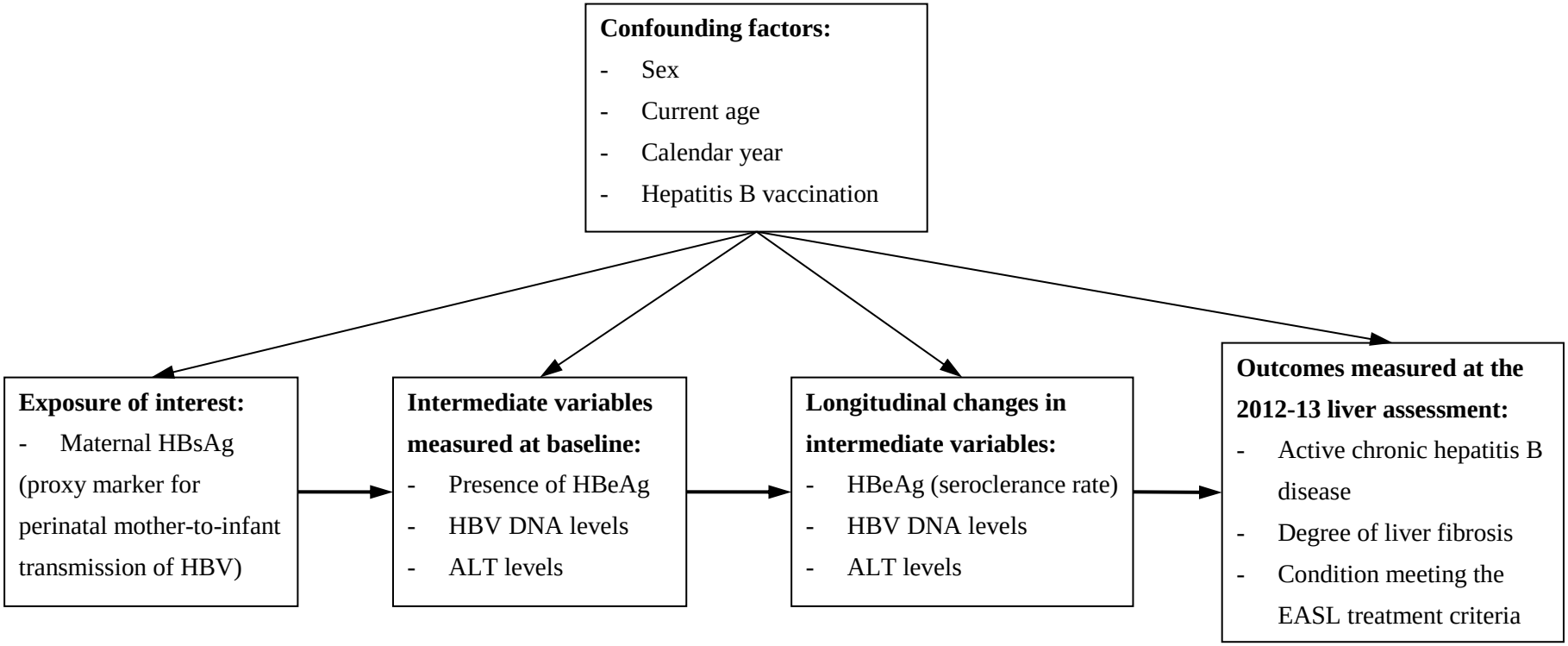
Second, hepatitis B vaccine does not always prevent mother-to-infant transmission,[238,126] especially when the vaccine was not given at birth.[121] In Asia where mother-to-infant transmission is common, HBV vaccine is frequently given within 24 hours of delivery to interrupt perinatal transmission.[265,266] In sSA, however, HBV vaccination usually occurs later because logistical challenges outweigh the potential impact on chronic HBV infection due to the relatively low frequency of mother-to-infant transmission.[267] It is well known that early age at HBV infection increases the risk of chronic infection.[69] Moreover, a systematic review (Chapter 3) suggests that mother-to-infant transmission, or early age at HBV infection, may further increase the risk of HCC and its predictors beyond its effect of increasing the risk of CHB infection.[261] However, this association has not been studied in Africa.

The Gambia is a small country in West Africa. The prevalence of HBV infection in adults is high (>8%),[275] with horizontal transmission during childhood being the major mode of spread,[60] and HCC is the most frequent cancer.[276] Community-based surveys of HBV sero-markers have been regularly conducted in Keneba and Manduar, two villages in rural Gambia, since 1973. A pilot hepatitis B vaccination programme started in 1984, and sero-surveys were undertaken between 1984 and 2008, primarily to measure vaccine efficacy. A cohort of chronic carriers was followed in parallel during this period. The PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa) project started in The Gambia in 2011; it is the first community-based HBV treatment programme in sSA. As part of this project, we invited the chronic carriers in the Keneba and Manduar cohort for a comprehensive liver assessment between 2012 and 2013.

The objectives of this study are to determine: i) the sero-clearance rate of HBeAg and HBsAg, ii) the incidence of HCC and end-stage liver disease (ESLD), iii) the overall mortality rate, iv) the prevalence of active CHB disease, significant liver fibrosis and conditions that meet the international antiviral treatment criteria, and v) whether these outcomes are associated with maternal HBsAg, a proxy marker for perinatal mother-to-infant transmission. The hypothesis is that in addition to increasing the risk of chronic infection (which is already well known), perinatal maternal transmission increases the risk of active CHB disease, progression of liver fibrosis and the risk of requiring antiviral treatment, through persistence of HBeAg, prolonged HBV viraemia and high frequency of alanine transaminase (ALT) flares (figure 6.1). The analyses were restricted to a comparison of chronic HBV carriers believed to be due to perinatal infection (i.e., those with HBsAg-positive mother) to chronic carriers believed to be due to horizontal infection (i.e., those with HBsAg-negative mother), in order to remove the effect of

perinatal transmission on chronic infection. To demonstrate that changes in HBeAg, HBV DNA and ALT mediate the effect of perinatal transmission on disease outcomes (i.e., active CHB disease, degree of liver fibrosis and condition requiring the antiviral therapy), the association of maternal HBsAg with these intermediate variables and the associations between the intermediate variables and the disease outcomes were evaluated. The proportion of chronic carriers requiring antiviral therapy due to perinatal transmission was estimated to quantify the impact of this mode of transmission on disease outcome.

Figure 6.1 Hypothetical causal pathway between perinatal maternal transmission of HBV and increased risk of advanced liver fibrosis and condition meeting the international antiviral treatment criteria



## 6.3. Methods

6.3.1. Study participants in historical open cohort

Keneba and Manduar are small villages located 8 km apart in the West Kiang District (figure 6.2). The combined population of the villages has increased from 1,099 in 1973 to 2,421 in 2013 (Tony Fulford, unpublished data). The inhabitants predominantly belong to the Madinka tribe and about half were under the age of 15 years in 1980.[42] The first sero-survey was undertaken in 1973 and 174 (13.2%) of 1,317 villagers had HBsAg.[277] Since 1980, the community-based HBV sero-surveys were conducted every 4-5 years (1980, 1984-1985, 1989, 1992-1993, 1998, 2003, and 2008).[42,60,115,238,278–280] In 1980, all children under the age of 15 years in the study villages and their mothers were asked to participate in the second sero-survey.[42] This included 67 of the 174 HBsAg-positive individuals in 1973 survey, and 109 persons who were newly identified as HBsAg positive (table 6.1). In 1984, all children aged <20 years in these villages were invited to participate in the third sero-survey; 191/936 (20.4%) were found to carry the virus and 147 were newly identified as HBsAg positive.[60] This survey was immediately followed by a vaccine trial. All non-immune children under 5 years in these villages were randomly allocated to one of the following three regimens of plasma derived hepatitis B vaccine: three intradermal doses of 2 μg at two-month intervals; 20 μg intramuscular dose followed by two intradermal doses of 2 μg at two-month intervals; and three intramuscular doses of 20 μg at 2-month intervals.[115] Subsequently, routine infant vaccination began. Since this vaccine trial, sero-surveys were undertaken primarily to measure the vaccine efficacy. In parallel, the serological markers (HBsAg and HBeAg) of people with positive HBsAg in Keneba and Manduar were also regularly assessed every 4-5 years (table 6.1 and figure 6.3). Since 2003, the study expanded to include Kantong Kunda, another village adjacent to Keneba.

Participation in the surveys was generally high, especially in small children. Between 1980 and 1998, attendance ranged from 92-94% in children aged 0-4 years, to 50-73% in those aged 10-14 and 81-85% in those aged 15-19.

Figure 6.2. Map of The Gambia showing Keneba and Manduar

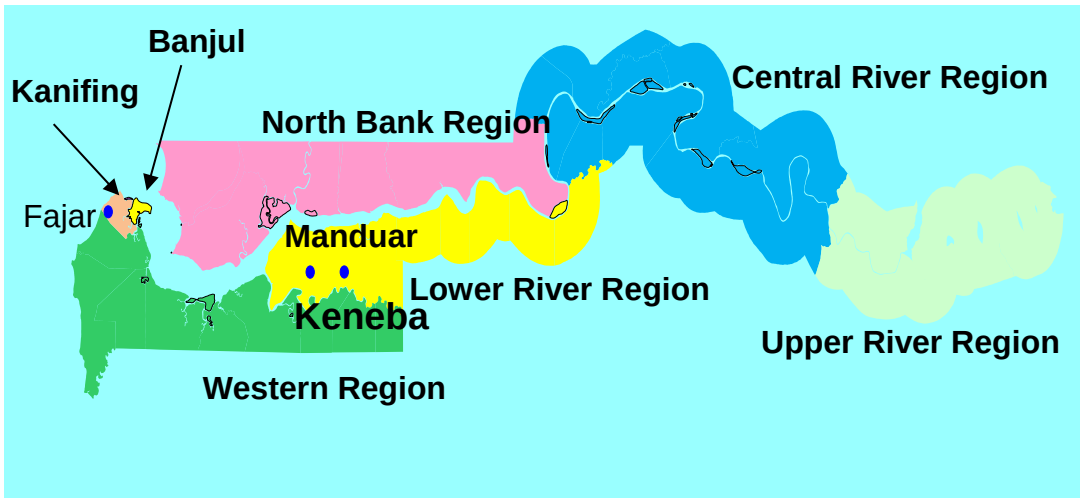


Table 6.1 Number of participants, number who tested HBsAg-positive (number of newly identified HBsAg-positive and number who were already found HBsAg-positive and who were followed-up) and type of laboratory tests at each sero-survey

| Year | Villages | Target population | Total tested | HBsAg(+) (a) | Tested HBsAg-positive at least once | | | | | Laboratory tests performed | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Newly identified (b) | Already in cohort who tested positive (c) | Already in cohort who tested HBsAg negative (d) | Number of HBsAg(+) who did not return to a current survey (%) (e) | Total cumulative number (f) | HBsAg | HBeAg | HBV DNA | ALT |
| 1973 | K & M | All | 1317 | 174 (13.2%) | 136[1] | - | - | - | 136 | RIA | - | - | - |
| 1980 | K & M | Children <15 yo & mothers | 790 | 144 (18.2%) | 109 | 35 | 32 | 69 (28.2%) | 245 | RPHA | RIA | - | - |
| 1984 | K & M | Children | 936 | 191 | 147 | 44 | 55 | 146 | 392 | RPHA | RIA | q-PCR | Cobas |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | <20 yo | | (20.4%) | | | | (37.2%) | | | | | Mira |
| 1985 | K & M | Vaccinated children | 221 | 5 (2.3%) | 3 | 2 | 5 | 385 (97.5%) | 395 | RPHA | RIA | - | - |
| 1989 | K & M | Children <20 yo & mothers | 1356 | 217 (16.0%) | 49 | 168 | 98 | 129 (29.1%) | 444 | RPHA | RIA | q-PCR | - |
| 1992 | K & M | Carriers | 271 | 187 (69.0%) | 1 | 186 | 84 | 174 (39.1%) | 445 | RPHA | RIA | Dot-blot Hybridisation | Cobas Mira |
| 1993 | K & M | Children <20 yo & mothers | 1478 | 132 (8.9%) | 27 | 105 | 71 | 269 (57.0%) | 472 | RPHA | RIA | q-PCR | - |
| 1998 | K & M | Carriers & vaccinees | 1343 | 126 (9.3%) | 11 | 115 | 56 | 301 (62.3%) | 483 | RPHA | RIA | - | - |
| 2003 | K, M & | All | 1637 | 251 | 63 | 188 | 103 | 192 | 546 | IC | EIA | q-PCR | - |

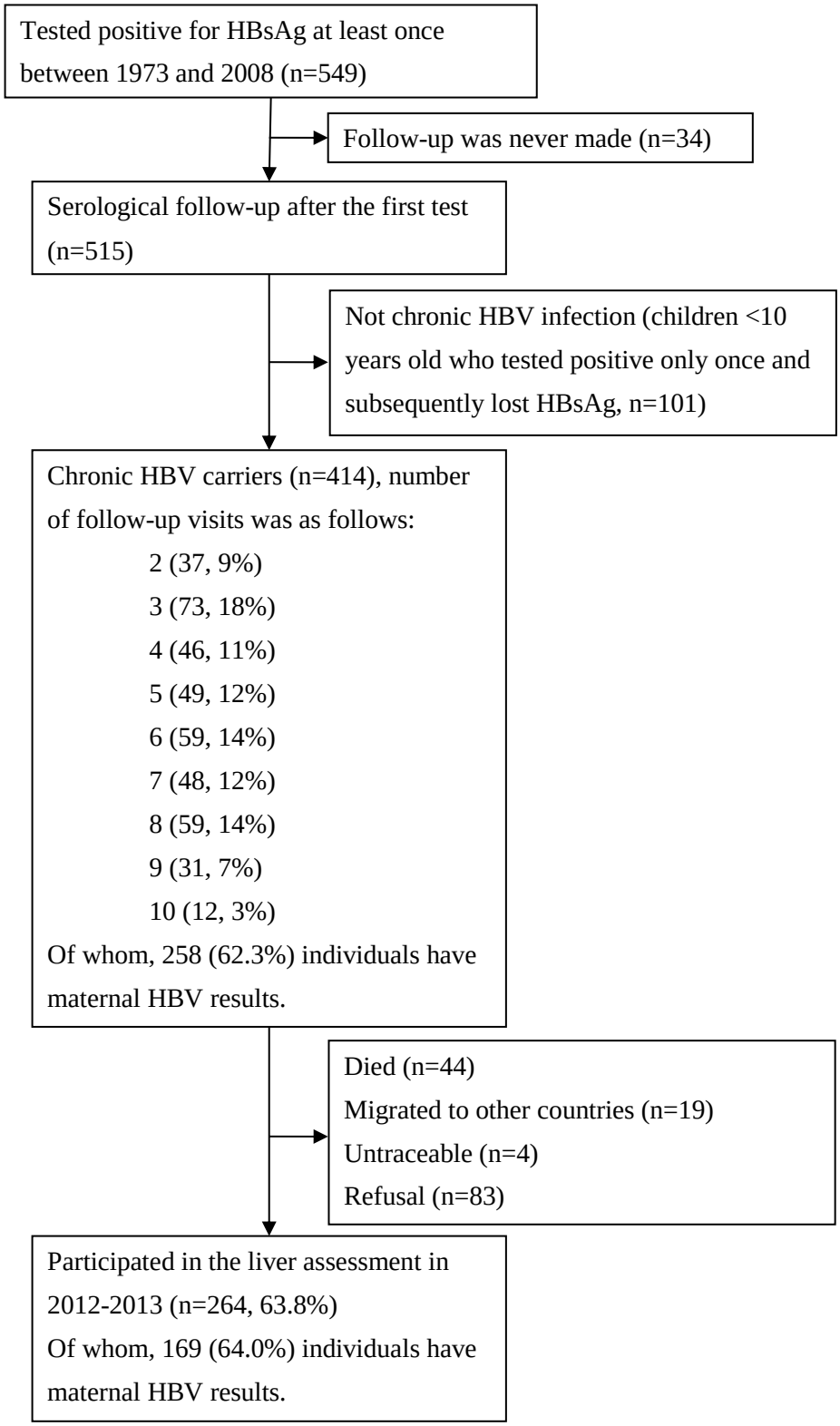
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | KK | | | (15.3%) | | | | (35.2%) | | | | | |
| 2008 | K, M & KK | Carriers & vaccinees | 2056 | 213 (10.4%) | 3 | 210 | 110 | 226 (41.2%) | 549 | IC | EIA | q-PCR | Vitros DT60-II |
| 2012 -13 | K, M & KK | Carriers | 284 | 247 (87.0%) | 0 | 247 | 37 | 265 (48.3%) | 549 | IC | EIA | q-PCR | Vitros 350 |

Abbreviations: K, Keneba; M, Manduar; KK, Kantong Kunda; RIA, radioimmunoassay; RPHA, reverse passive haemagglutination assay; PCR, polymerase chain reaction; IC, immunochromatography; EIA, enzyme immunoassay.

Total number of participants tested positive for HBsAg (a) is a sum of newly identified HBsAg-positive (b) and participants who were already in the cohort and who tested positive (c). Total cumulative number of people ever tested positive (f) in a previous survey equals to a sum of people already in the cohort and tested positive in the current survey (c), people in the cohort and tested negative in the current survey (d), and people in the cohort but lost in the current survey (e). Total cumulative number of people ever tested positive (f) in the current survey equals to a sum of (b), (c), (d) and (e) in the corresponding survey.

[1] Thirty-eight records are missing.

Figure 6.3 Flow diagram of study participants



6.3.2. Follow-up liver assessment in 2012-13

CHB infection was defined as serum HBsAg positivity on two occasions at least 6 months apart. However, in the pre-vaccination era, more than 80% of children in Keneba and Manduar acquired the infection (indicated by positive anti-HBc) by the age of 10 years, suggesting that new HBV infections in adults were uncommon.[60] Thus, for those over 10 years old, HBsAg positivity on only one occasion was considered sufficient to reflect chronic infection.

Between 1973 and 2008, 549 people tested positive for HBsAg at least once. There were no cases of HCC at enrolment into the cohort. After excluding 34 people who did not participate in further follow-up sero-surveys, and 101 children <10 years who only tested positive once and who were negative in their subsequent surveys, there were 414 people with CHB infection (figure 6.3). The chronic carriers were invited for a liver assessment as part of the PROLIFICA project in 2012-2013. A team of fieldworkers and I met the head of each village (Keneba, Manduar and Kantong Kunda) and explained the purpose of the follow-up study in April 2012. This was followed by a meeting with villagers. After community approval, trained fieldworkers identified eligible persons by verifying their name, date of birth, birth place and the names of their parents. Eligible individuals were asked to participate in a liver assessment at the clinic. The study was conducted in two phases. In May 2012, a team of fieldworkers, nurses, laboratory technicians and hepatologists organised a specialist liver clinic at Medical Research Council (MRC) Keneba Field Station to assess the severity of the liver disease for persons with CHB infection resident in the study villages (phase 1). Those who had migrated from the study villages to another part of The Gambia were invited to the liver clinic at MRC Fajara, close to the capital Banjul, from June 2012 to December 2013 (phase 2). After written consent, the

participants underwent a structured interview, and a standardised clinical examination which included abdominal ultrasound and collection of a blood and urine sample. Liver stiffness measurement (LSM) was performed using transient elastography (Fibroscan FS402, Echosens, France) following the manufacturer's instructions.[104] As food intake increases the frequency of unreliable measurements by transient elastography and overestimates the liver stiffness, participants were asked not to have breakfast on the day of liver assessment.[281] After the first liver assessment, those who met any of the study criteria: HBV DNA levels ≥2,000 IU/ml, LSM ≥6.5 kPa or serum alanine transaminase (ALT) ≥40 IU/L, were invited for ultrasound-guided percutaneous liver biopsy. Liver fibrosis was scored according to the Metavir system[282] by one liver pathologist in The Gambia and another in UK. The study was approved by the Gambia Government/MRC Joint Ethics Committee.

### 6.3.3. Laboratory assays

HBsAg was detected by radioimmunoassay (RIA) (Ausria I, Abbott, USA) in 1973 survey,[42] reverse passive haemagglutination assay (RPHA) (Wellcotest, Wellcome Diagnostics, UK) from 1980 to 1998,[278] and immunochromatography (IC) (Determine, Abbott, USA) from 2003 to 2013.[185] When the samples were positive using RPHA, the test was repeated after neutralisation with rabbit anti-HBs. The manufacturer of Determine HBsAg changed to Inverness, UK (currently Alere) in 2005. Samples positive for HBsAg were tested for HBeAg by RIA (Sorin Biochemica, Italy) from 1980 to 1998[278] and later by enzyme immunoassay (EIA) (Diasorin, Biomedica, Italy).[185] A good correlation was observed among the IC, RIA and RPHA for HBsAg detection, and between the EIA and RIA for HBeAg.[185] HBV DNA levels were measured by dot-blot hybridisation with a phosphorus-32 label (detection limit of $3 \times 10^6$ IU/ml)

for samples collected in 1992[283] and quantitative real-time polymerase chain reaction (q-PCR) (detection limit of 5 x 10 IU/ml)[284] for those collected in 1984, 1989, 1993, 2003 and 2008. In 2013, we used another in-house q-PCR. These assays for viral load measurement were calibrated against an international HBV DNA standard[284] and thus should be correlated with each other. Serum transaminases were measured by Cobas Mira Autoanalyser (Roche, Switzerland) in 1984 and 1992, by Vitros DT60-II analyser (Johnson & Johnson, USA) in 2008 and by Vitros 350 analyser in 2013. The tests used are summarised in table 6.1. In addition, we tested samples collected in 2012 and 2013 for alpha-fetoprotein and other co-infection (anti-HCV (hepatitis C virus), anti-HDV (hepatitis D virus) and anti-HIV (human immunodeficiency virus)) (see details in Chapter 7).

### 6.3.4. Ascertainment of hepatocellular carcinoma (HCC), end-stage liver disease (ESLD) and death

New cases of HCC were identified either through a follow-up examination, by reviewing the medical records in the Keneba MRC Clinic, or by data linkage with the Gambia National Cancer Registry. The diagnosis was based on the identification of a focal hepatic lesion consistent with HCC on the ultrasound and elevated serum alpha-fetoprotein. ESLD includes HCC and non-malignant ESLD; the latter was defined as a clinical diagnosis of liver cirrhosis without HCC and the presence of one of the following: ascites, hepatic encephalopathy, or haematemesis. Non-malignant ESLD was ascertained through a follow-up examination or a review of the medical records in Keneba Clinic. The vital status of study participants was determined by fieldworkers, and the date of death was ascertained through a review of medical

chart in Keneba or data linkage with the West Kiang Demographic Surveillance System (DSS). When the linkage was unsuccessful, the date reported by a family member was used.

### 6.3.5. Statistical analyses

#### *6.3.5.1. HBeAg and HBsAg Sero-clearance*

The person-years of follow-up for HBeAg or HBsAg loss were calculated from the date they were identified as HBsAg-positive test to the date of sero-clearance or the date of last follow-up sero-survey, whichever came first. Some individuals were initially HBsAg-negative. For these individuals the date they became HBsAg-positive was assumed to be the midpoint between their last HBsAg-negative and their first HBsAg-positive sample. The date of sero-clearance was defined as the midpoint between the last positive HBsAg test and the first negative result. Incidence rates were calculated by dividing the number of events by person-years, and are presented stratified by sex. The cumulative incidence was estimated as a function of age using the Kaplan-Meier Method. Age was used rather than time since entry into the study because most infections occur perinatally, or during early childhood, and therefore age approximates the duration of HBV infection.[83] Poisson regression models were used to estimate the effect of having an HBsAg-positive mother on sero-clearance. The analysis used maternal HBsAg status as recorded at the survey in which a child entered the study. The association of maternal HBsAg was adjusted for current age, calendar year, sex and HBV vaccine (figure 6.1). To account for clustering in children that share the same mother, p-values and confidence intervals were based on robust standard errors. The association of HBeAg, HBV DNA ($<$2,000, 2,000-$10^8$ and $\geq 10^8$ IU/ml) and ALT levels ($<$40 or $\geq$40 IU/L) measured at baseline with subsequent sero-clearance was also assessed; the multivariable models included current age, calendar year, sex, HBV

vaccine and maternal HBsAg in addition to HBeAg, HBV DNA and ALT levels at baseline. Baseline refers to when a participant had the first positive result for HBsAg. When HBV DNA or ALT was unavailable at the baseline survey, the value recorded in the subsequent survey was taken. Baseline HBeAg was not included in the analysis when the outcome was HBeAg sero-clearance.

*6.3.5.2. Incidence of HCC, ESLD and death*

The person-years for HCC, ESLD or death were calculated from the date when individuals were identified as HBsAg-positive to the date of endpoint (HCC or ESLD diagnosis or death) or the date of last contact, whichever was earliest. The incidence of HCC and mortality are approximately equivalent because all HCC patients died within one year after the diagnosis. As the number of the event was small, the association between maternal HBsAg and HCC, ESLD and death was examined using Fisher's exact test and results from multivariate models are not presented.

*6.3.5.3. Longitudinal change in viral load and ALT*

HBV viral load and ALT levels were treated as continuous variables, and the former was $\log_{10}$ transformed. The detection limit of the quantitative HBV DNA assay was assigned to samples with undetectable viral load. Locally weighted regression (LOWESS) was used to present the trajectories of HBV DNA levels and ALT levels over time by maternal sero-status. The association of maternal HBsAg status with HBV DNA and ALT was quantified using generalised estimating equations, with an autoregressive correlation structure to account for the

multiple measurements made on the same individuals over time. The associations were adjusted for current age, calendar year, sex and HBV vaccine.

*6.3.5.4. Liver fibrosis and condition meeting the international treatment criteria*

The 2012-2013 liver assessment was used to determine the phase of the natural history of CHB infection, degree of liver fibrosis and whether individuals required antiviral therapy according to the EASL (European Association of the Study of the Liver) criteria.[70] The proportion of participants in each phase of the natural history was described based on the definitions presented in table 6.2. Significant liver fibrosis was defined as a LSM ≥7.5 kPa by transient elastography based on a recent validation study in The Gambia which demonstrated that this cut-off distinguished significant liver fibrosis (≥F2 with Metavir) from no or mild fibrosis (sensitivity 88.1% and specificity 80.3% ) (Lemoine et al., unpublished data). The EASL criteria for antiviral therapy are: i) HBV viral load ≥2,000 IU/ml and significant liver fibrosis (LSM ≥7.5 kPa or ≥F2 by Metavir fibrosis score), or ii) viral load ≥2,000 IU/ml and moderate/severe active necroinflammation (≥A2 by Metavir activity grade), or iii) viral load ≥20,000 IU/ml and serum ALT ≥80 IU/L or iv) detectable serum viral load and cirrhosis (LSM ≥9.0 kPa or F4 by Metavir fibrosis score). The effect of maternal HBsAg on diseases (active CHB diseases including both HBeAg-positive and –negative CHB disease, significant liver fibrosis, and requiring antiviral therapy) was estimated using logistic regression to control for age, sex and HBV vaccination. The effect of persistent HBeAg, frequent high HBV DNA and ALT levels on diseases was also assessed controlling for current age, sex, HBV vaccination and maternal HBsAg. The categories for "persistence of HBeAg" were: 1) negative HBeAg at baseline, 2) HBeAg sero-clearance during the follow-up, and 3) HBeAg positive at the last follow-up. High viral load (≥2,000

IU/ml) and elevated ALT (≥40 IU/L) were only examined in participants who had at least two results available, and they were categorised as: 1) never, 2) <50% of visits, and 3) ≥50% of visits.[139]

Table 6.2 Phases of the natural history of CHB infection (adapted from EASL/AASLD guidelines)[70,7]

| Phase | | HBsAg | HBeAg | HBV DNA (IU/ml) | ALT (U/L) |
|---|---|---|---|---|---|
| Immune tolerant phase | | Positive | Positive | ≥20,000 | <40 |
| Chronic hepatitis B disease | HBeAg-positive chronic hepatitis B | Positive | Positive | Any | ≥40 |
| | HBeAg-negative chronic hepatitis B | Positive | Negative | ≥2,000 | ≥40 |
| Inactive HBV carrier state | | Positive | Negative | <2,000 | <40 |
| Occult HBV infection | | Negative | Negative | Detectable | Any |
| Resolved hepatitis B | | Negative | Negative | Undetectable | <40 |
| Unclassified | HBeAg-positive | Positive | Positive | <20,000 | <40 |
| | HBeAg-negative | Positive | Negative | ≥2,000 | <40 |
| | | | | <2,000 | ≥40 |

*6.3.5.5. Population attributable fraction for perinatal maternal transmission*

As discussed in the Chapter 1, in The Gambia preventive measures that may eliminate the risk of perinatal maternal HBV transmission (e.g., timely birth dose of hepatitis B vaccine within 24 hours of birth, provision of hepatitis B immunoglobulin, or treating pregnant women with antiviral therapy) have not been well implemented. Therefore, it is important to estimate the proportion of high-risk chronic HBV carriers in the total population that would be avoided if the risk of perinatal maternal transmission was removed. Here, the proportion of chronic carriers requiring antiviral treatment (based on EASL criteria) due to mother-to-infant transmission was estimated using the formula for the population attributable fraction (PAF):[285]

$$PAF = p_c (OR - 1) / OR,$$

where $p_c$ is the proportion of cases whose mothers were HBsAg-positive, and OR is the odds ratio for the effect of maternal HBsAg status on liver disease (needing treatment based on the EASL criteria) adjusted for current age and sex. This analysis included all the survey participants in Keneba and Manduar between 1973 and 2008 with available maternal HBsAg status. It was not restricted to chronic carriers in order to consider the whole effect of perinatal transmission: both the increased risk of chronic infection and the increased risk of liver disease progression in those who have established chronic infection. Ideally, the PAF should be derived from people who received hepatitis B vaccine as most of children are now immunised against HBV in The Gambia. However, the analysis included both vaccinees and non-vaccinees because there were few cases that required antiviral treatment in the vaccinated group. All analyses were conducted using STATA 11.0 (Stata Corporation, College Station, Texas).

## 6.4. Results

6.4.1. Baseline characteristics

Table 6.3 shows the baseline characteristics of 414 individuals with CHB infection. Half were men and 261 (63%) were children <15 years at baseline. Most were residents of Keneba or Manduar. Thirty chronic HBV carriers had been vaccinated for hepatitis B: four were vaccinated after HBsAg-positivity was confirmed, six were HBsAg-negative when the vaccine was given but subsequently became positive for HBsAg (vaccine failure), and the timing of infection in relation to vaccination was unknown in 20. About half of the participants were HBeAg-positive at baseline and viral load exceeded 2,000 IU/ml in 43%. Abnormal ALT was seen only in 6%. There were no data for maternal HBV sero-status in 156 participants, and the exact reasons for this are unknown. However, because the participants without maternal sero-status tend to be older at the study entry than those with maternal sero-status (table 6.3), it is possible that their mothers have already died before these children's study entry. This might be a source of bias as mortality may be higher in HBV-positive than HBV-negative mothers. Of 258 participants with available maternal HBV sero-status, mothers of 175 (68%) were HBsAg-negative, 63 (24%) were HBsAg-positive and HBeAg-negative, and 20 (8%) were HBsAg-positive and HBeAg-positive at enrolment. There was no difference in the distribution of sex and age between those with HBsAg-positive and –negative mothers. However, children of HBsAg-positive mothers were more likely to live in Manduar or Kantong Kunda (p=0.05). The proportion of carrier children who had received vaccine was significantly higher in those with HBsAg-positive mothers (17% vs. 5%, p=0.005). Children of positive mothers tended to

carry HBeAg more often (69% vs. 60%) and were more likely to have a viral load exceeding 2,000 IU/ml (68% vs. 54%), but neither of these differences was statistically significant. The proportion with abnormal ALT levels was higher in children with carrier mothers (10% vs. 3%, p=0.04). The amount of follow-up was similar in the two groups.

Table 6.3 Baseline characteristics of people with chronic HBV infection by maternal HBsAg status (N=414)

| | All (N=414) | Unknown maternal sero-status (n=156) | With HBsAg(+) mother (n=83) | With HBsAg(-) mother (n=175) | p-value[1] |
|---|---|---|---|---|---|
| Sex | | | | | 1.0 |
| Male | 205 (50%) | 62 (40%) | 46 (55%) | 97 (55%) | |
| Female | 209 (50%) | 94 (60%) | 37 (45%) | 78 (45%) | |
| Age group | | | | | 0.9[2] |
| <5 | 108 (26%) | 4 (3%) | 39 (47%) | 65 (37%) | |
| 5 – 9 | 82 (20%) | 8 (5%) | 20 (24%) | 54 (31%) | |
| 10 – 14 | 71 (17%) | 24 (15%) | 9 (11%) | 38 (22%) | |
| 15 – 19 | 38 (9%) | 22 (14%) | 5 (6%) | 11 (6%) | |
| ≥20 | 115 (28%) | 98 (63%) | 10 (12%) | 7 (4%) | |
| Birth place | | | | | 0.05 |
| Keneba | 199 (48%) | 75 (48%) | 33 (40%) | 91 (52%) | |
| Manduar | 177 (43%) | 50 (32%) | 44 (53%) | 83 (47%) | |
| Kantong Kunda | 38 (9%) | 31 (20%) | 6 (7%) | 1 (1%) | |
| Maternal sero-status | | | | | |
| HBsAg(-) | 175 (68%) | | | | |
| HBsAg(+) HBeAg(-) | 63 (24%) | | | | |
| HBsAg(+) HBeAg(+) | 20 (8%) | | | | |
| HBV vaccine | | | | | 0.005 |
| No vaccination | 384 (93%) | 149 (95%) | 69 (83%) | 166 (95%) | |
| Infected before vaccination | 4 (1%) | 1 (1%) | 0 (0%) | 3 (2%) | |

| | | | | | |
|---|---|---|---|---|---|
| Infected after vaccination | 6 (2%) | 0 (0%) | 2 (2%) | 4 (2%) | |
| Time of infection relative to vaccination unknown | 20 (5%) | 6 (4%) | 12 (15%) | 2 (1%) | |
| HBeAg | | | | | 0.2 |
| Positive | 165 (47%) | 15 (14%) | 51 (69%) | 99 (60%) | |
| Negative | 183 (53%) | 93 (86%) | 23 (31%) | 67 (40%) | |
| HBV DNA (IU/ml) | | | | | 0.1[2] |
| Undetectable | 151 (40%) | 93 (66%) | 16 (21%) | 42 (26%) | |
| 50-2,000 | 68 (18%) | 26 (18%) | 9 (12%) | 33 (20%) | |
| 2,000-$10^5$ | 30 (8%) | 9 (6%) | 7 (9%) | 14 (9%) | |
| $10^5$-$10^8$ | 58 (15%) | 10 (7%) | 13 (17%) | 35 (21%) | |
| $\geq 10^8$ | 75 (20%) | 4 (3%) | 32 (42%) | 39 (24%) | |
| ALT (IU/L) | | | | | 0.04 |
| <40 | 369 (94%) | 137 (93%) | 70 (90%) | 162 (97%) | |
| ≥40 | 24 (6%) | 11 (7%) | 8 (10%) | 5 (3%) | |
| Median no. of follow-up sero-surveys (range) | 6 (2, 10) | 4 (2, 8) | 6 (2, 10) | 7 (2, 10) | 0.1[2] |
| Median years of follow-up (range) | 28.1 (3.9, 38.9) | 19.0 (3.9, 38.9) | 28.1 (3.9, 38.3) | 28.2 (3.9, 38.9) | 0.1[2] |

[1] Comparison was made between participants with HBsAg-positive mothers and HBsAg-negative mothers. P-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] Linear test for trend

### 6.4.2. HBeAg sero-clearance

One hundred and sixty-five individuals were positive for HBeAg at baseline and the mean duration of their follow-up was 10.0 years. HBeAg was cleared in 120, and the annual rate of clearance was 7.3% (95% CI: 6.1-8.7, table 6.4). Half had cleared HBeAg by 14 years of age (figure 6.4). Table 6.5 presents factors associated with HBeAg sero-clearance obtained by Poisson regression models. The rate of loss was slowest in children between 0-9 years and during the period 1973-92. Having an HBsAg-positive mother was associated with a delayed HBeAg loss (figure 6.5 and table 6.5). Higher viral load at baseline was also associated with a slower rate of loss, but this was not statistically significant (p=0.09).

### 6.4.3. HBsAg sero-clearance

There were 7,880 person-years of follow-up, and the annual rate of HBsAg sero-clearance was 1.3% (95% CI: 1.1-1.6, table 6.4). Half had cleared HBsAg by 53 years of age (figure 6.4). Male sex, younger age and recent period (1993-2013) were associated with delayed HBsAg sero-clearance (table 6.6). The rate of sero-clearance was slower in individuals with an HBsAg-positive mother, but this was not statistically significant (figure 6.5 and table 6.6).

Table 6.4 Rate of HBeAg and HBsAg sero-clearance, incidence of HCC and ESLD and all-cause mortality in people with chronic HBV infection by gender

| Event | No. of subjects | Person-years at risk | No. of events | Rate | 95% CI |
|---|---|---|---|---|---|
| HBeAg clearance | 165 | 1653 | 120 | 7.3 / 100 | 6.1 – 8.7 |
| Male | 106 | 1046 | 74 | 7.1 | 5.6 – 8.9 |
| Female | 59 | 607 | 46 | 7.6 | 5.7 – 10.1 |
| HBsAg clearance | 414 | 7880 | 103 | 1.3 / 100 | 1.1 – 1.6 |
| Male | 205 | 3687 | 35 | 0.9 | 0.7 – 1.3 |
| Female | 209 | 4193 | 68 | 1.6 | 1.3 – 2.1 |
| HCC | 414 | 10925 | 6 | 54.9 / 100,000 | 24.7 – 122.3 |
| Male | 205 | 5141 | 6 | 116.7 | 52.4 – 259.8 |
| Female | 209 | 5784 | 0 | 0.0 | N/A |
| ESLD (including HCC) | 414 | 10925 | 8 | 73.2 / 100,000 | 36.6 – 146.4 |
| Male | 205 | 5141 | 7 | 136.2 | 64.9 – 285.6 |
| Female | 209 | 5784 | 1 | 17.3 | 2.4 – 122.7 |
| All-cause mortality | 414 | 10925 | 44 | 402.8 / 100,000 | 299.7 – 541.2 |
| Male | 205 | 5141 | 25 | 486.3 | 328.6 – 719.7 |
| Female | 209 | 5784 | 19 | 328.5 | 209.5 – 515.0 |

Figure 6.4 Proportion of participants with CHB infection who cleared HBeAg and HBsAg as a function of age

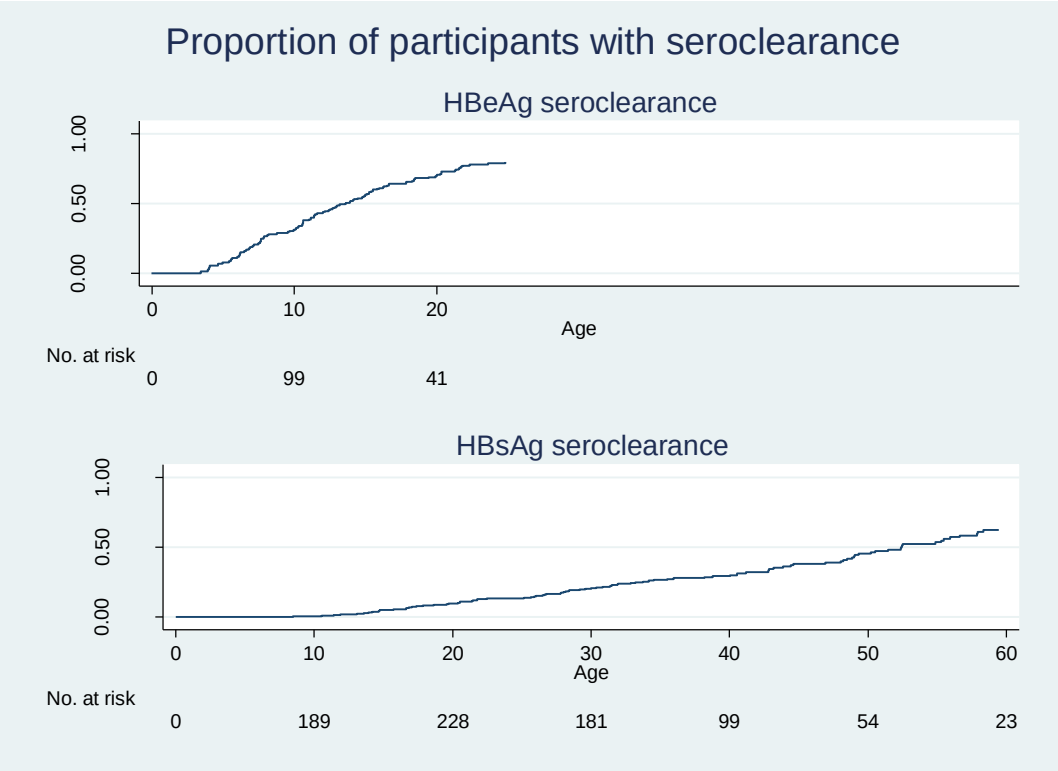
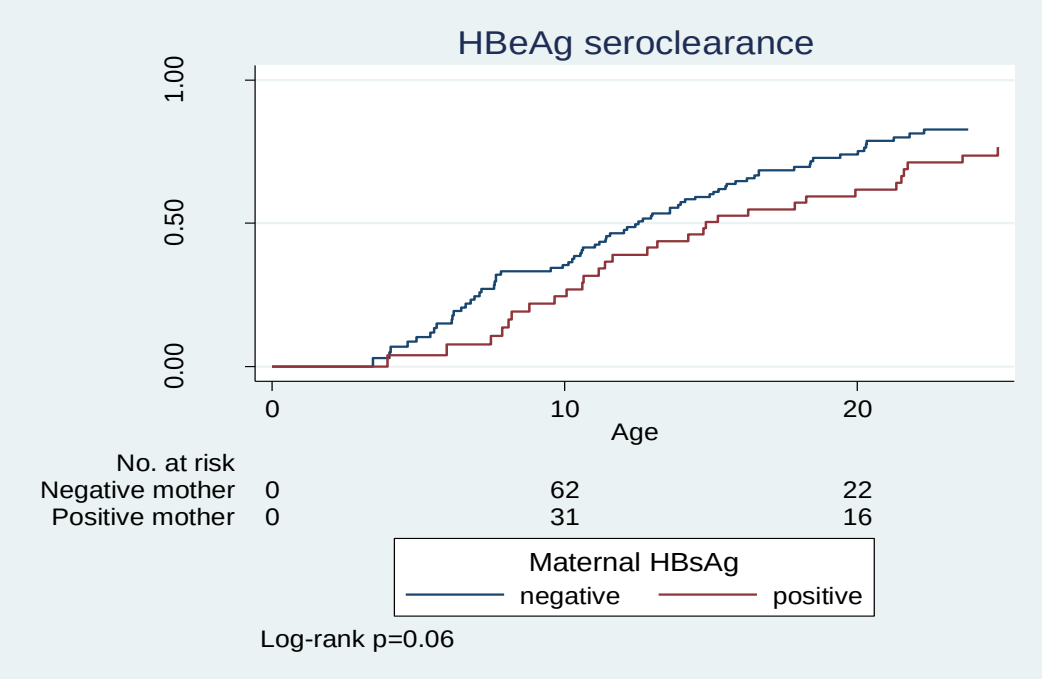


Figure 6.5 Proportion of participants with CHB infection who cleared HBeAg and HBsAg as a function of age according to maternal HBsAg positivity



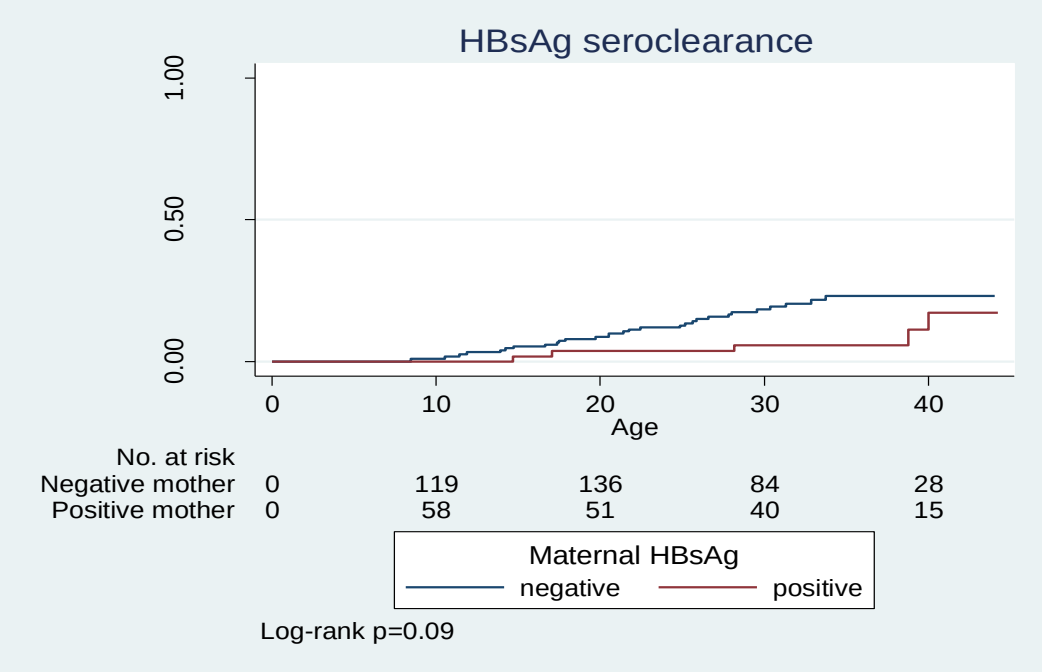



Table 6.5 Factors associated with HBeAg sero-clearance (n=165)

| Variables | | Person-years at risk | No. of subjects cleared HBeAg | Rate (% per annum) | Crude RR (95% CI) | p-value[1] | Adjusted RR (95% CI) | p-value[1] |
|---|---|---|---|---|---|---|---|---|
| Sex | Male | 1046 | 74 | 7.1 | 1.0 (ref) | 0.7 | | |
| | Female | 607 | 46 | 7.6 | 1.1 (0.8 – 1.5) | | | |
| Current age | 0-9 | 628 | 32 | 5.1 | 1.0 (ref) | 0.03[2] | | |
| | 10-19 | 687 | 59 | 8.6 | 1.7 (1.1 – 2.6) | | | |
| | ≥20 | 337 | 29 | 8.6 | 1.7 (1.0 – 2.8) | | | |
| Calendar year | 1973-92 | 1140 | 69 | 6.1 | 1.0 (ref) | 0.01 | | |
| | 1993-2013 | 513 | 51 | 9.9 | 1.6 (1.1 – 2.4) | | | |
| Maternal HBsAg | Negative | 922 | 79 | 8.6 | 1.0 (ref) | 0.03 | 1.0 (ref) | 0.04[3] |
| | Positive | 566 | 34 | 6.0 | 0.7 (0.5 – 0.9) | | 0.7 (0.4 – 0.9) | |
| Hepatitis B vaccination | None | 1545 | 114 | 7.3 | 1.0 (ref) | 0.6 | 1.0 (ref) | 0.4[3] |
| | Yes | 108 | 7 | 6.5 | 0.9 (0.5 – 1.5) | | 0.8 (0.4 – 1.4) | |
| HBV DNA (IU/ml) at | -2,000 | 258 | 22 | 8.5 | 1.0 (ref) | 0.1[2] | 1.0 (ref) | 0.09[2,4] |
| | 2000-$10^8$ | 546 | 44 | 8.1 | 0.9 (0.6 – 1.4) | | 0.9 (0.5 – 1.4) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| baseline | ≥$10^8$ | 785 | 51 | 6.5 | 0.8 (0.5 – 1.1) | | 0.7 (0.4 – 1.1) | |
| ALT (IU/L) at baseline | <40 | 1536 | 115 | 7.5 | 1.0 (ref) | 0.1 | 1.0 (ref) | 0.3[4] |
| | ≥40 | 79 | 3 | 3.8 | 0.5 (0.2 – 1.2) | | 0.6 (0.2 – 1.6) | |

[1] p-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] Test for linear trend.

[3] Model included sex, current age, calendar year, maternal HBV status and hepatitis B vaccination.

[4] Model included sex, current age, calendar year, maternal HBV status, hepatitis B vaccination, HBV DNA and ALT.

Table 6.6 Factors associated with HBsAg sero-clearance (n=414)

| Variables | | Person-years at risk | No. of subjects cleared HBsAg | Rate (% per annum) | Crude RR (95% CI) | p-value[1] | Adjusted RR (95% CI) | p-value[1] |
|---|---|---|---|---|---|---|---|---|
| Sex | Male | 3686 | 35 | 0.95 | 1.0 (ref) | 0.008 | | |
| | Female | 4193 | 68 | 1.62 | 1.7 (1.1 – 2.5) | | | |
| Current age | 0-9 | 948 | 1 | 0.11 | 1.0 (ref) | <0.0001[3] | | |
| | 10-19 | 2188 | 21 | 0.96 | 9.1 (1.3 – 61.8) | | | |
| | 20-29 | 2153 | 27 | 1.25 | 11.9 (1.6 – 87.0) | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 30-39 | 1306 | 16 | 1.22 | 11.6 (1.5 – 88.0) | | | |
| | 40-49 | 750 | 19 | 2.53 | 24.0 (3.2 – 179.4) | | | |
| | 50-70 | 535 | 19 | 3.55 | 33.7 (4.5 – 252.7) | | | |
| Calendar year | 1973-92 | 3457 | 61 | 1.76 | 1.0 (ref) | 0.004 | | |
| | 1993-2013 | 4423 | 42 | 0.95 | 0.5 (0.4 – 0.8) | | | |
| Maternal HBsAg | Negative | 3773 | 32 | 0.85 | 1.0 (ref) | 0.3 | 1.0 (ref) | 0.1[3] |
| | Positive | 1745 | 9 | 0.52 | 0.6 (0.3 – 1.4) | | 0.5 (0.2 – 1.2) | |
| Hepatitis B vaccination | None | 7493 | 100 | 1.33 | 1.0 (ref) | 0.6 | 1.0 (ref) | 0.4[3] |
| | Yes | 387 | 3 | 0.78 | 0.6 (0.2 – 1.8) | | 2.0 (0.4 – 9.5) | |
| HBeAg at baseline | Negative | 3699 | 40 | 1.08 | 1.0 (ref) | 0.0004 | 1.0 | 0.8[4] |
| | Positive | 3742 | 13 | 0.35 | 0.3 (0.2 – 0.6) | | 0.9 (0.3 – 2.5) | |
| HBV DNA (IU/ml) at baseline | -2,000 | 4105 | 79 | 1.92 | 1.0 (ref) | <0.0001[2] | 1.0 (ref) | 0.4[2,4] |
| | 2000-$10^8$ | 1904 | 10 | 0.53 | 0.3 (0.1 – 0.5) | | 0.9 (0.3 – 3.2) | |
| | ≥$10^8$ | 1660 | 3 | 0.18 | 0.1 (0.0 – 0.3) | | 0.5 (0.1 – 2.6) | |
| ALT (IU/L) at baseline | <40 | 7421 | 95 | 1.28 | 1.0 (ref) | 0.6 | 1.0 (ref) | 0.5[4] |
| | ≥40 | 303 | 5 | 1.65 | 1.3 (0.5 – 3.4) | | 2.0 (0.2 – 20.1) | |

[1] p-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] Test for linear trend.

[3] Model included sex, current age, calendar year, maternal HBV status and hepatitis B vaccination.

[4] Model included sex, current age, calendar year, maternal HBV status, hepatitis B vaccination, HBeAg, HBV DNA and ALT.

6.4.4. HCC, ESLD and death of any cause

Forty-four people died and the all-cause mortality rate was 402.8/100,000 person-years (95% CI: 299.7-541.2) (table 6.4). In 25 men who died, HCC was the most common cause (24%), followed by bacterial infection (12%) and neurological diseases (12%). In the 19 women who died, bacterial infection was the most frequent cause (21%) followed by cardiac diseases (16%) (table 6.7). There were two patients with non-malignant ESLD - one male and one female. The cause of death was unknown in 32% of the patients.

All patients with HCC and non-malignant ESLD died within one year of diagnosis, and none survived to the end of follow-up. Incidence rates (per 100,000 person-years) of HCC and ESLD (including HCC and non-malignant ESLD) were 54.9 (95% CI: 24.7-122.3) and 73.2 (95% CI: 36.6-146.4), respectively (table 6.4). All HCC patients were men aged between 38 and 67 years old at diagnosis (table 6.8). One patient with a non-malignant ESLD was a woman who died at the age of 19 years. Maternal HBV status was known in three ESLD patients, and all had HBsAg-positive mothers. Crude mortality rate according to maternal HBsAg status was as follows; all-cause mortality rate (288.9/100,000 person-years (95% CI: 129.8-642.9) in individuals with an HBsAg-positive mother *versus* 203.4/100,000 person-years (109.4-378.0) in individuals with an HBsAg-negative mother, p=0.5) and ESLD mortality rate (144.4/100,000 person-years (95% CI: 46.6-447.8) in those with an HBsAg-positive mother *versus* 0/100,000 person-years in those with an HBsAg-negative mother, p<0.001). Two (25%) of ESLD patients were HBeAg-negative at baseline. Amongst six ESLD patients who participated in the 1992 survey, HBV DNA measured by dot-blot hybridisation was undetectable in five and ALT was

normal in five patients. HBsAg sero-clearance was confirmed in one patient before the diagnosis of ESLD.

Table 6.7 Causes of death (n=44)

| | Males (n=25) | Females (n=19) | Total (n=44) |
|---|---|---|---|
| HCC | 6 (24%) | 0 | 6 (14%) |
| Non-malignant ESLD | 1 (4%) | 1 (5%) | 2 (5%) |
| Bacterial infection | 3 (12%) | 4 (21%) | 7 (16%) |
| Tuberculosis | 1 (4%) | 2 (11%) | 3 (7%) |
| AIDS | 1 (4%) | 1 (5%) | 2 (5%) |
| Cardiac diseases | 2 (8%) | 3 (16%) | 5 (11%) |
| Neurological diseases | 3 (12%) | 0 | 3 (7%) |
| Other neoplasms | 0 | 2 (11%) | 2 (5%) |
| Unknown | 8 (32%) | 6 (32%) | 14 (32%) |

Table 6.8 Characteristics of individuals who died of ESLD (includes HCC and non-malignant ESLD)

| Cause of death | Age at enrolment | Age at diagnosis | Sex | Birth place | Maternal HBsAg | HBeAg at baseline | HBV DNA in 1992 (IU/ml) | ALT in 1992 (IU/L) | HBsAg loss during F/U | Source of diagnosis |
|---|---|---|---|---|---|---|---|---|---|---|
| HCC | 43 | 45 | M | Keneba | N/A | (-) | N/A | N/A | No | KMN & NCR |
| HCC | 29 | 67 | M | Manduar | N/A | (-) | $<3 \times 10^6$ | 43 | No | KMN & NCR |
| HCC | 23 | 57 | M | Manduar | N/A | (-) | $<3 \times 10^6$ | 13 | No | KMN & NCR |
| HCC | 20 | 50 | M | Manduar | (+) | (-) | $<3 \times 10^6$ | 10 | No | KMN |
| HCC | 21 | 42 | M | Manduar | (+) | (+) | $3 \times 10^6$ | 15 | No | KMN |
| HCC | 21 | 38 | M | Manduar | N/A | (-) | N/A | N/A | Yes | NCR |
| Non-malignant ESLD | 21 | 57 | M | Keneba | N/A | (-) | $<3 \times 10^6$ | 6 | No | KMN |
| Non-malignant ESLD | 7 | 19 | F | Keneba | (+) | (+) | $<3 \times 10^6$ | 5 | No | KMN |

Abbreviations: KMN, Keneba medical notes; NCR, National Cancer Registry.

### 6.4.5. Mean HBV DNA and ALT levels over time

Table 6.9 and 6.10 present factors associated with elevated HBV DNA levels and ALT levels respectively. Both were derived from generalised estimating equations to account for the repeat measurements made on the same individuals over time. High viral load was associated with male sex, younger age group, recent years (1993-2013), and having an HBsAg-positive mother (table 6.9). Mean ALT level was positively associated with older age, recent calendar years (1993-2013), and having an HBsAg-positive mother (table 6.10). The trajectories of HBV DNA levels and ALT levels over time are presented in figures 6.6 and 6.7. HBVDNA declined with increasing age whilst ALT levels increased. The positive maternal HBsAg group was consistently higher for both HBV DNA and ALT levels.

Table 6.9 Predictors of geometric mean HBV DNA (IU/ml) (n=414)

| Variables | | Geometric mean viral load (IU/ml) | Ratio of geometric mean viral load (95% CI)[1] | p-value[1] |
|---|---|---|---|---|
| Sex | Male | 6,310 | 1.0 (ref) | <0.001 |
| | Female | 631 | 0.08 (0.04 – 0.2) | |
| Current age | 0-9 | 2,511,886 | 1.0 (ref) | <0.001[2] |
| | 10-19 | 6.310 | $2x10^{-3}$ ($6x10^{-4}$ – $6x10^{-3}$) | |
| | 20-29 | 794 | $3x10^{-4}$ ($9x10^{-5}$ – $8x10^{-4}$) | |
| | 30-39 | 251 | $1x10^{-4}$ ($3x10^{-5}$ – $3x10^{-4}$) | |
| | 40-49 | 158 | $7x10^{-5}$ ($2x10^{-5}$ – $2x10^{-4}$) | |
| | 50-70 | 126 | $6x10^{-5}$ ($2x10^{-5}$ – $2x10^{-4}$) | |
| Calendar year | 1973-92 | 7.943 | 1.0 (ref) | <0.001 |
| | 1993-2013 | 794 | 0.11 (0.06 – 0.19[2]) | |
| Maternal HBsAg | Negative | 2,511 | 1.0 (ref) | 0.005[3] |
| | Positive | 10,000 | 3.8 (1.5 – 9.9) | |
| Hepatitis B vaccination | None | 1,585 | 1.0 (ref) | 0.3[3] |
| | Yes | 10,000 | 0.3 (0.1 – 2.3) | |

[1] Ratio of geometric means, p-value and 95% CI estimated using generalised estimating equations with an autoregressive correlation structure to account for dependence of repeated measurements within participants

[2] Test for linear trend.

[3] Model included sex, current age, calendar year, maternal HBV status and hepatitis B vaccination.

Table 6.10 Predictors of mean ALT levels (IU/L) (n=414)

| Variables | | Mean ALT (IU/ml) | Difference (95% CI)[1] | p-value[1] |
|---|---|---|---|---|
| Sex | Male | 20.6 | | 0.2 |
| | Female | 18.9 | -2.2 (-5.4 – 0.9) | |
| Current age | 0-9 | 10.8 | | <0.001[2] |
| | 10-19 | 15.0 | 3.2 (0.1 – 6.3) | |
| | 20-29 | 24.9 | 13.2 (7.6 – 18.8) | |
| | 30-39 | 23.5 | 12.9 (9.4 – 16.4) | |
| | 40-49 | 21.1 | 9.6 (6.8 – 12.5) | |
| | 50-70 | 21.6 | 12.9 (5.9 – 19.9) | |
| Calendar year | 1973-92 | 12.3 | | <0.001 |
| | 1993-2013 | 25.9 | 12.9 (9.7 – 16.1) | |
| Maternal HBsAg | Negative | 17.4 | | 0.02[3] |
| | Positive | 22.9 | 4.4 (0.7 – 8.1) | |
| Hepatitis B vaccination | None | 19.1 | | 0.2[3] |
| | Yes | 28.3 | 3.2 (-1.3 – 7.8) | |

[1] Mean difference, p-value and 95% CI estimated using generalised estimating equations with an autoregressive correlation structure to account for dependence of repeated measurements within participants

[2] Test for linear trend.

[3] Model included sex, current age, calendar year, maternal HBV status and hepatitis B vaccination.

Figure 6.6 Changes with age in serum HBV DNA levels by maternal HBsAg status using samples tested between 1984 and 2013 (n=414), the best fit line was obtained using locally weighted regression (LOWESS)

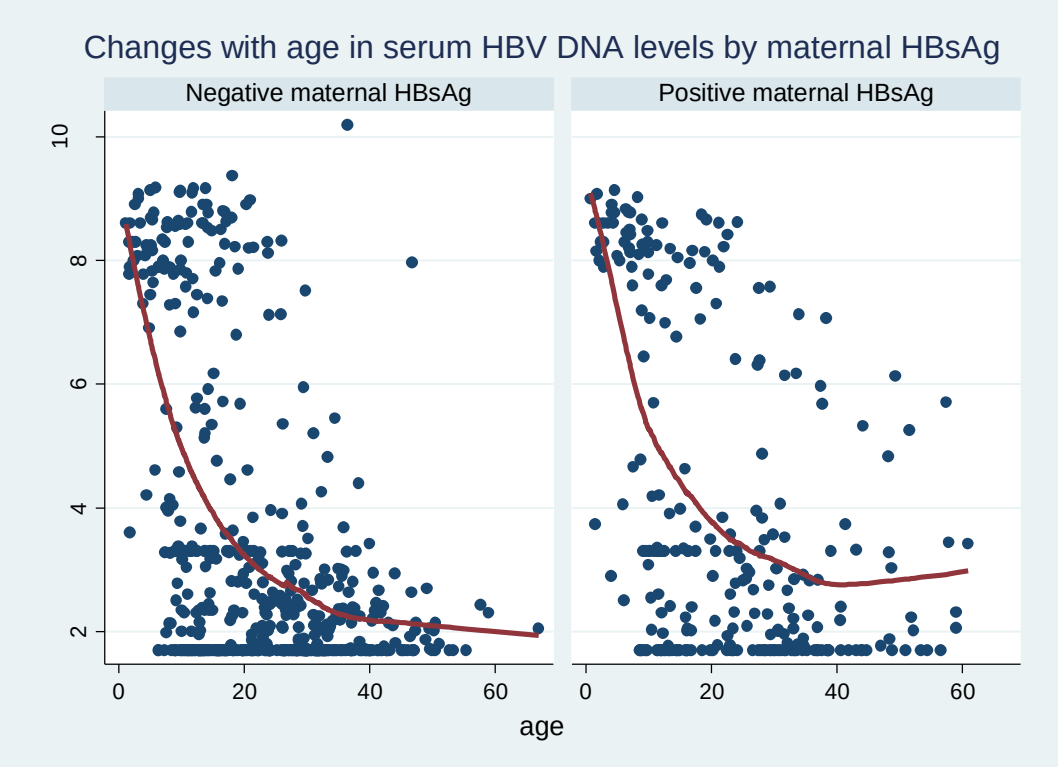
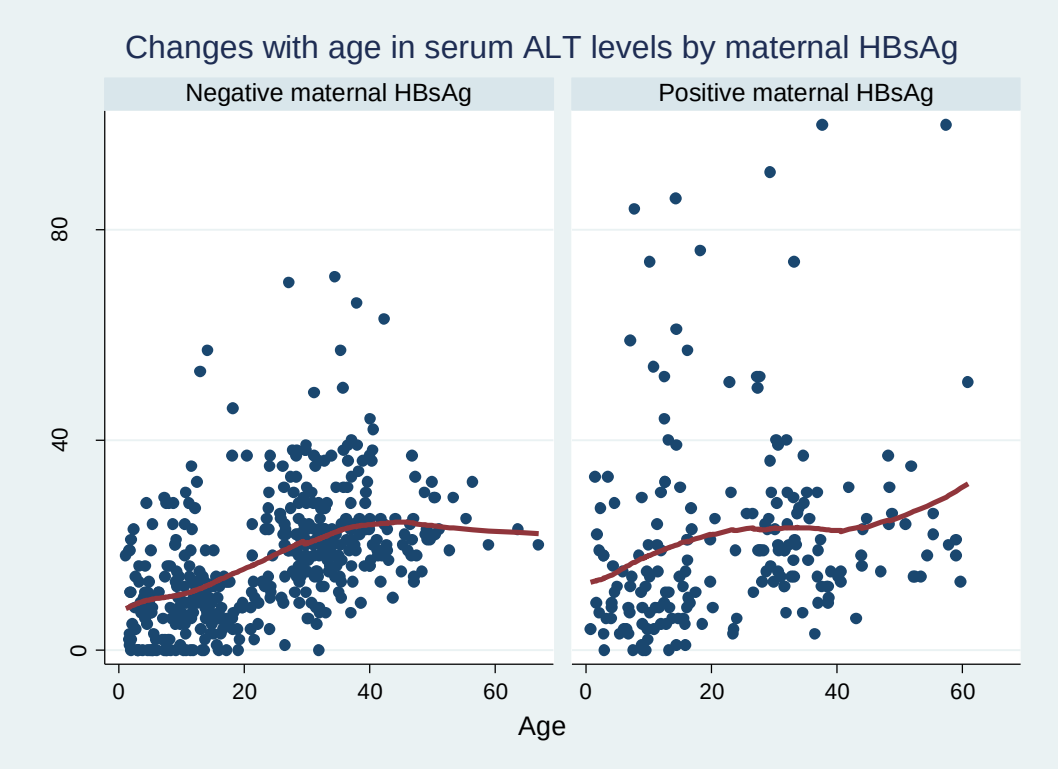


Figure 6.7 Changes with age in serum ALT levels by maternal HBsAg status using samples tested between 1984 and 2013 (n=414), line was generated using locally weighted regression (LOWESS)[1]




[1] Two outliers (ALT: 166 and 351 IU/L) in positive maternal HBsAg group are not presented in the figure.

6.4.6. Liver assessment (2012-13)

Of 414 people with CHB infection who were identified between 1973 and 2008, 44 died, 19 migrated to other countries, four were untraceable and 83 refused to participate in the liver assessment (figure 6.3). The remaining 264 (63.8%) people were enrolled in the PROLIFICA study and had a comprehensive liver assessment in 2012-13. Table 6.11 shows the baseline characteristics of attenders and non-attenders. Attenders were more likely to be women, originally from Kantong Kunda and HBeAg negative at baseline.

Of 264 chronic carriers who participated in the PROLIFICA, 107 (41%) were male, and the median age was 37 years (table 6.12). Twenty-three participants (8%) received hepatitis B vaccine. The median duration of follow-up was 32 years (range: 4-39). Only five (2%) have ever drunk alcohol, three (1%) were co-infected with HIV and none were positive for anti-HCV or anti-HDV. Twenty-three (10%) had ALT ≥40 IU/L and 32 (15%) had a platelet count <150,000/microL. Fifty-five (21%) had lost HBsAg by 2012-13. Twenty-eight individuals (12%) had viral load ≥2,000 IU/ml. More than half were negative for HBeAg at the baseline survey. Amongst 102 participants who were HBeAg-positive at the baseline, 79 (78%) lost HBeAg during the follow-up, and 23 (22%) remained HBeAg-positive in 2012-13. Of 217 participants whose HBV DNA levels were measured at least twice during the follow-up (median number of assays: 3, range: 2-7), 96 (44%) had persistently low viral load (<2,000 IU/ml), 71 (33%) exceeded 2,000 IU/ml on less than 50% of visits and 50 (23%) had high viral load at ≥50% of visits. Of 235 participants whose ALT was measured at least twice (median: 3, range: 2-4), 197 (83%) had persistently normal ALT, 13 (6%) had abnormal ALT but on less than <50% of visits and 25 (11%) had abnormal ALT at ≥50% of visits.

Table 6.12 shows the characteristics of participants in the liver assessment 2012-13 by maternal HBsAg. The distributions of age and sex were similar in the two groups. Vaccination was more common in people with carrier mothers. Viral load measured in 2012-13 as well as the frequency of high viral load measurements and abnormal ALT were significantly higher in carriers whose mothers were also HBV carriers.

Table 6.11 Baseline characteristics of those who attended and those who did not attend the liver assessment 2012-13 (n=414)

| | Attenders (n=264) | Non-attenders (n=150) | p-value[1] |
|---|---|---|---|
| Sex | | | <0.001 |
| Male | 107 (41%) | 98 (65%) | |
| Female | 157 (59%) | 52 (35%) | |
| Age group | | | 0.8[2] |
| <5 | 74 (28%) | 34 (23%) | |
| 5 – 9 | 52 (20%) | 30 (20%) | |
| 10 – 14 | 38 (14%) | 33 (22%) | |
| 15 – 19 | 24 (9%) | 11 (9%) | |
| ≥20 | 76 (29%) | 39 (26%) | |
| Birth place | | | 0.003 |
| Keneba | 123 (46%) | 76 (51%) | |
| Manduar | 105 (40%) | 72 (48%) | |
| Kantong Kunda | 36 (14%) | 2 (1%) | |
| Maternal sero-status | | | 0.1[2] |
| HBsAg(-) | 111 (66%) | 64 (72%) | |
| HBsAg(+) HBeAg(-) | 42 (25%) | 21 (24%) | |
| HBsAg(+) HBeAg(+) | 16 (9%) | 4 (4%) | |
| HBV vaccine | | | 0.1 |
| None | 241 (91%) | 143 (95%) | |
| Yes | 23 (9%) | 7 (5%) | |
| HBeAg | | | 0.02 |
| Positive | 102 (43%) | 63 (56%) | |
| Negative | 134 (57%) | 49 (44%) | |
| HBV DNA (IU/ml) | | | 0.7[2] |
| Undetectable | 96 (38%) | 55 (43%) | |

| | | | |
|---|---|---|---|
| 50-2,000 | 55 (22%) | 13 (10%) | |
| 2,000-$10^5$ | 19 (7%) | 11 (9%) | |
| $10^5$-$10^8$ | 37 (14%) | 21 (17%) | |
| $\geq 10^8$ | 48 (19%) | 27 (21%) | |
| ALT (IU/L) | | | 0.1 |
| <40 | 239 (92%) | 130 (97%) | |
| ≥40 | 20 (8%) | 4 (3%) | |

[1] p-value from Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] Linear test for trend

Table 6.12 Characteristics of people with chronic HBV infection who participated in the liver assessment 2012-13 by maternal HBsAg status (n=264)

| | All (n=264) | With HBsAg(+) mother (n=58) | With HBsAg(-) mother (n=111) | p-value[1] |
|---|---|---|---|---|
| Sex | | | | 0.9 |
| Male | 107 (41%) | 28 (48%) | 52 (47%) | |
| Female | 157 (59%) | 30 (52%) | 59 (53%) | |
| Age group | | | | 0.9[2] |
| 11 – 29 | 41 (17%) | 14 (25%) | 18 (17%) | |
| 30 – 39 | 101 (43%) | 26 (47%) | 56 (53%) | |
| 40 – 49 | 45 (19%) | 7 (13%) | 24 (23%) | |
| 50 – 78 | 48 (20%) | 8 (15%) | 7 (7%) | |
| Birth place | | | | 0.1 |
| Keneba | 123 (46%) | 21 (36%) | 58 (52%) | |
| Manduar | 105 (40%) | 32 (55%) | 52 (47%) | |
| Kantong Kunda | 36 (14%) | 5 (9%) | 1 (1%) | |
| HBV vaccine | | | | 0.005 |
| No vaccination | 241 (91%) | 48 (83%) | 103 (93%) | |
| Infected before vaccination | 4 (1%) | 0 (0%) | 3 (3%) | |
| Infected after vaccination | 4 (1%) | 0 (0%) | 4 (4%) | |
| Time of infection relative to vaccination unknown | 15 (6%) | 10 (17%) | 1 (1%) | |
| Median no. of follow-up sero-surveys (range) | 6 (2, 10) | 7 (2, 10) | 7 (2, 10) | 0.1 |
| Median years of follow-up (range) | 32 (4, 39) | 32 (4, 38) | 32 (10, 39) | 0.1 |
| ALT in 2012/13 | | | | 0.07 |

| | | | | |
|---|---|---|---|---|
| <40 IU/L | 201 (90%) | 43 (81%) | 92 (92%) | |
| ≥40 IU/L | 23 (10%) | 10 (19%) | 8 (8%) | |
| Platelet count in 2012/13 | | | | 0.4 |
| <150,000/microL | 32 (15%) | 5 (10%) | 15 (16%) | |
| ≥150,000/microL | 180 (85%) | 43 (90%) | 80 (84%) | |
| HBV marker in 2012/13 | | | | 0.3[2] |
| HBsAg(+), HBeAg(+) | 23 (9%) | 9 (16%) | 10 (9%) | |
| HBsAg(+), HBeAg(-) | 186 (70%) | 42 (72%) | 84 (76%) | |
| HBsAg(-) | 55 (21%) | 7 (12%) | 17 (15%) | |
| HBV DNA (IU/ml) in 2012/13 | | | | 0.002[2] |
| Undetectable | 81 (39%) | 16 (30%) | 48 (49%) | |
| 50-200 | 61 (27%) | 13 (25%) | 26 (26%) | |
| 200-2,000 | 48 (21%) | 11 (21%) | 17 (17%) | |
| 2,000-100,000 | 14 (6%) | 5 (9%) | 4 (4%) | |
| ≥100,000 | 14 (6%) | 8 (15%) | 4 (4%) | |
| Persistence of HBeAg | | | | 0.1[2] |
| Negative at baseline | 134 (57%) | 17 (33%) | 48 (44%) | |
| Cleared during follow-up | 79 (33%) | 26 (50%) | 50 (46%) | |
| Still carrier of HBeAg | 23 (10%) | 9 (17%) | 10 (9%) | |
| Frequency of having high HBV DNA levels (≥2,000 IU/ml)[3] | | | | 0.001[2] |
| Never | 96 (44%) | 10 (20%) | 44 (39%) | |
| <50% of visits | 71 (33%) | 18 (36%) | 48 (43%) | |
| ≥50% of visits | 50 (23%) | 22 (44%) | 20 (18%) | |
| Frequency of having ALT elevation (≥40 IU/L)[3] | | | | 0.002[2] |
| Never | 197 (83%) | 36 (71%) | 94 (90%) | |
| <50% of visits | 13 (6%) | 4 (8%) | 7 (7%) | |

| | | | | |
|---|---|---|---|---|
| ≥50% of visits | 25 (11%) | 11 (21%) | 4 (4%) | |

[1] p-value from Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] Linear test for trend

[3] This only includes subjects who had at least two measurements during the follow-up.

6.4.7. Phase of natural history of CHB infection

Table 6.13 presents the characteristics of the participants by phase of the natural history of CHB infection. Five (2%) were immunotolerant, three (1%) were HBeAg-positive CHB disease, 15 (6%) were HBeAg-positive unclassified, six (2%) were HBeAg-negative CHB disease, 153 (58%) were inactive carriers, 27 (10%) were HBeAg-negative unclassified, 14 (5%) were occult HBV and 41 (16%) were resolved hepatitis B. Although most people in the inactive phase did not meet the EASL criteria for requiring antiviral treatment (99%, 206/208), significant liver fibrosis was observed in 8% (13/162) in this group. Chronic carriers with active CHB disease were more likely to have an HBsAg-positive mother, persistent HBeAg, high viral replication and abnormal ALT than in carriers in other phases (table 6.14).

Table 6.13 Characteristics of participants in the liver assessment 2012-13 by phase of natural history (n=264)

| Variables | HBsAg(+) and HBeAg(+) | | | HBsAg(+) and HBeAg(-) | | | HBsAg(-) | |
|---|---|---|---|---|---|---|---|---|
| | Immune tolerant N=5 | Unclassified N=15 | HBeAg(+) CHB disease N=3 | HBeAg(-) CHB disease N=6 | Unclassified N=27 | Inactive carrier N=153 | Occult HBV N=14 | Resolved hepatitis B N=41 |
| Male sex | 4 (80%) | 9 (60%) | 3 (100%) | 2 (33%) | 16 (59%) | 63 (41%) | 0 (0%) | 10 (24%) |
| Median age (range) | 32 (26-46) | 28 (11-44) | 28 (18-30) | 38 (29-61) | 35 (12-58) | 37 (16-78) | 51 (30-67) | 42 (31-73) |
| Positive maternal HBsAg | 2/4 (50%) | 4/12 (33%) | 3/3 (100%) | 3/4 (75%) | 8/20 (40%) | 31/102 (30%) | 4/8 (50%) | 3/16 (19%) |
| Significant fibrosis by TE | 1/5 (20%) | 0/10 (0%) | 1/3 (33%) | 1/6 (17%) | 4/21 (19%) | 10/132 (8%) | 1/11 (9%) | 2/19 (11%) |
| Meeting EASL criteria | 1/5 (20%) | 0/15 (0%) | 1/3 (33%) | 5/6 (83%) | 4/27 (15%) | 2/153 (1%) | 0/14 (0%) | 0/41 (0%) |

Table 6.14 Factors associated with active CHB disease (HBeAg-positive and –negative CHB disease) among 264 people with CHB infection who participated in the liver assessment 2012-13

| Variables | | Proportion with outcome | Crude OR (95% CI) | P[1] | Adjusted OR (95% CI) | P[1] |
|---|---|---|---|---|---|---|
| Sex | Male | 5/107 (5%) | 1.0 (ref) | 0.4 | | |
| | Female | 4/157 (3%) | 0.5 (0.1-2.0) | | | |
| Current age | 11 – 29 | 3/41 (7%) | 1.0 (ref) | 0.2[3] | | |
| | 30 – 39 | 4/101 (4%) | 0.5 (0.1-2.4) | | | |
| | 40 – 80 | 2/93 (2%) | 0.3 (0.1-1.7) | | | |
| Maternal HBsAg | Negative | 1/111 (1%) | 1.0 (ref) | 0.02 | 1.0 (ref) | 0.03[4] |
| | Positive | 6/58 (10%) | 12.7 (1.5-107.1) | | 18.5 (1.3-249.9) | |
| Hepatitis B vaccination | None | 8/241 (3%) | 1.0 (ref) | 0.8 | 1.0 (ref) | 0.2[4] |
| | Yes | 1/23 (4%) | 1.3 (0.2-10.2) | | 0.2 (0.0-1.9) | |
| Persistence of HBeAg | Negative at baseline | 2/134 (1%) | 1.0 (ref) | 0.01[3] | 1.0 (ref) | 0.02[3,4] |
| | Cleared during follow-up | 4/79 (5%) | 3.5 (0.6-19.5) | | $7 \times 10^6$ ($1 \times 10^6$-$5 \times 10^7$) | |
| | Still carrier | 3/23 (13%) | 9.9 (1.5-63.4) | | $2 \times 10^7$ ($3 \times 10^6$-$2 \times 10^8$) | |
| Percent samples with viral | Never | 0/96 (0%) | N/A | 0.003[3] | N/A | 0.001[3,5] |

| | | | | | | |
|---|---|---|---|---|---|---|
| load ≥2,000 IU/ml[2] | <50% | 1/71 (1%) | 0.08 (0.0 – 0.6) | | 0.07 (0.0 – 0.5) | |
| | ≥50% | 8/50 (16%) | 1.0 (ref) | | 1.0 (ref) | |
| Percent samples with ALT elevation ≥40 IU/L[2] | Never | 0/197 (0%) | N/A | <0.001[3] | N/A | <0.001[3,4] |
| | <50% | 4/13 (31%) | 1.8 (0.3 – 9.3) | | 4.5 (0.4 – 47.8) | |
| | ≥50% | 5/25 (20%) | 1.0 (ref) | | 1.0 (ref) | |

[1] p-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] This only includes subjects who had at least two measurements during the follow-up.

[3] Test for linear trend.

[4] OR adjusted for sex, current age, maternal HBV status and hepatitis B vaccination.

[5] OR adjusted for sex and current age.

6.4.8. Significant liver fibrosis

Measurements of liver fibrosis based on transient elastography were classified as a failure or unreliable in 1% (3/227) and 8% (17/227), respectively. Of the remaining 207 (91%) with a successful measurement, 20 (9.7%, 95% CI: 5.6-13.7%) were found to have significant liver fibrosis (≥7.5 kPa), and five (2.4%, 95% CI: 0.3-4.5%) were over 9.0 kPa which are in cirrhotic range. Male sex, having HBsAg-positive mothers, having ever had high viral load (≥ 2,000 IU/ml), and abnormal ALT measurements were associated with significant liver fibrosis (table 6.15).

A liver biopsy was performed in 30 of the 65 patients who were eligible for the procedure. Six (25%) had moderate or severe necroinflammation (≥A2) and four (15%) had significant or severe liver fibrosis (≥F2). The prevalence of Metavir ≥A2 was similar in carriers with HBsAg-positive mothers (29%, 2/7) and –negative mothers (22%, 2/9, p=0.8). However, the prevalence of Metavir ≥F2 was higher in those with carrier mothers (43%, 3/7 *versus* 9%, 1/11, p=0.1).

Table 6.15 Factors associated with significant liver fibrosis (≥7.5 kPa by TE) among 207 people with CHB infection with a successful liver stiffness measurement (excluding participants with failure or unreliable measurements)

| Variables | | Proportion with outcome | Crude OR (95% CI) | P[1] | Adjusted OR (95% CI) | P[1] |
|---|---|---|---|---|---|---|
| Sex | Male | 16/91 (18%) | 1.0 (ref) | 0.002 | | |
| | Female | 4/116 (3%) | 0.2 (0.1-0.5) | | | |
| Current age | 11 – 29 | 4/37 (11%) | 1.0 (ref) | 1.0[3] | | |
| | 30 – 39 | 7/92 (8%) | 0.7 (0.2-2.2) | | | |
| | 40 – 49 | 6/37 (16%) | 1.6 (0.4-6.7) | | | |
| | 50 – 80 | 3/41 (7%) | 0.7 (0.1-3.1) | | | |
| Maternal HBsAg | Negative | 7/94 (7%) | 1.0 (ref) | 0.08 | 1.0 (ref) | 0.01[4] |
| | Positive | 9/49 (18%) | 2.8 (0.9-9.0) | | 4.8 (1.4-17.1) | |
| Hepatitis B vaccination | None | 19/190 (10%) | 1.0 (ref) | 0.6 | 1.0 (ref) | 0.3[4] |
| | Yes | 1/17 (6%) | 0.6 (0.1-4.6) | | 0.3 (0.1-4.1) | |
| Persistence of HBeAg | Negative at baseline | 5/108 (5%) | 1.0 (ref) | 0.02[3] | 1.0 (ref) | 0.6[3,4] |
| | Cleared during follow-up | 11/73 (15%) | 3.7 (1.2-10.7) | | 2.6 (0.5-13.3) | |
| | Still carrier | 2/18 (11%) | 2.6 (0.5-14.5) | | 0.9 (0.1-7.8) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Percent samples with viral load ≥2,000 IU/ml[2] | Never | 3/81 (4%) | 1.0 (ref) | 0.03[3] | 1.0 (ref) | 0.05[3,4] |
| | <50% | 10/65 (15%) | 4.7 (1.2-18.7) | | $1 \times 10^7$ ($4 \times 10^6$-$4 \times 10^7$) | |
| | ≥50% | 6/43 (14%) | 4.2 (0.9-19.1) | | $1 \times 10^7$ ($2 \times 10^6$-$4 \times 10^7$) | |
| Percent samples with ALT elevation ≥40 IU/L[2] | Never | 12/159 (8%) | 1.0 (ref) | 0.02[3] | 1.0 (ref) | 0.05[3,4] |
| | <50% | 2/12 (17%) | 2.5 (0.5-12.8) | | 0.9 (0.1-8.7) | |
| | ≥50% | 5/21 (24%) | 3.8 (1.2-12.3) | | 4.7 (1.0-21.4) | |

[1] p-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] This only includes subjects who had at least two measurements during the follow-up.

[3] Test for linear trend.

[4] OR adjusted for sex, current age, maternal HBV status and hepatitis B vaccination.

### 6.4.9. EASL treatment criteria

Thirteen participants (4.9%, 95% CI: 2.3-7.6%) met the EASL treatment criteria. Participants with HBsAg-positive mother and higher frequency of high viral load and abnormal ALT levels were more likely to require the antiviral therapy (table 6.16).

PAF for meeting the EASL criteria was calculated from the data including all the survey participants in Keneba and Manduar (1973-2008) with available maternal sero-status (n=2,760). The proportion of chronic carriers meeting the criteria with an HBsAg-positive mother ($p_c$) was 77.8% (7/9) and the adjusted OR for the effect of maternal HBsAg status was 12.7 (table 6.17). Therefore 71.4% (95% CI: 6.3-91.3%) of the chronic carriers requiring treatment are attributable to maternal transmission. Using the same data, the proportion of chronic carriers attributable to maternal perinatal transmission was 13.9% (95% CI: 5.5-21.5%).

Table 6.16 Factors associated with condition fulfilling the EASL treatment criteria among 264 people with CHB infection who participated in the liver assessment 2012-13

| Variables | | Proportion with outcome | Crude OR (95% CI) | P[1] | Adjusted OR (95% CI) | P[1] |
|---|---|---|---|---|---|---|
| Sex | Male | 6/107 (6%) | 1.0 (ref) | 0.7 | | |
| | Female | 7/157 (4%) | 0.8 (0.2-2.4) | | | |
| Current age | 11 – 29 | 3/41 (7%) | 1.0 (ref) | 0.3[3] | | |
| | 30 – 39 | 7/101 (7%) | 0.9 (0.2-4.0) | | | |
| | 40 – 49 | 1/45 (2%) | 0.3 (0.1-2.9) | | | |
| | 50 – 80 | 2/48 (4%) | 0.6 (0.1-3.4) | | | |
| Maternal HBsAg | Negative | 2/111 (2%) | 1.0 (ref) | 0.01 | 1.0 (ref) | 0.01[4] |
| | Positive | 7/58 (12%) | 7.5 (1.5-37.5) | | 8.9 (1.5-52.1) | |
| Hepatitis B vaccination | None | 12/241 (5%) | 1.0 (ref) | 0.9 | 1.0 (ref) | 0.3[4] |
| | Yes | 1/23 (4%) | 0.9 (0.1-7.1) | | 0.2 (0.0-3.4) | |
| Persistence of HBeAg | Negative at baseline | 4/134 (3%) | 1.0 (ref) | 0.1[3] | 1.0 (ref) | 0.2[3,4] |
| | Cleared during follow-up | 5/79 (6%) | 2.2 (0.6-8.4) | | 3.2 (0.2-53.0) | |
| | Still carrier | 2/23 (9%) | 3.1 (0.5-18.1) | | 6.9 (0.2-238,4) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Percent samples with viral load ≥2,000 IU/ml[2] | Never | 2/96 (2%) | 1.0 (ref) | 0.007[3] | 1.0 (ref) | 0.03[3,4] |
| | <50% | 2/71 (3%) | 1.4 (0.2-9.9) | | $7 \times 10^6$ ($1 \times 10^6$-$5 \times 10^7$) | |
| | ≥50% | 9/50 (18%) | 10.3 (2.1-51.9) | | $4 \times 10^7$ ($3 \times 10^6$-$4 \times 10^8$) | |
| Percent samples with ALT elevation ≥40 IU/L[2] | Never | 4/197 (2%) | 1.0 (ref) | <0.001[3] | 1.0 (ref) | 0.005[3,4] |
| | <50% | 2/13 (15%) | 8.8 (1.4-53.7) | | 10.1 (0.8-122.0) | |
| | ≥50% | 6/25 (24%) | 15.2 (3.9-59.2) | | 12.8 (2.0-83.1) | |

[1] p-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] This only includes subjects who had at least two measurements during the follow-up.

[3] Test for linear trend.

[4] OR adjusted for sex, current age, maternal HBV status and hepatitis B vaccination.

Table 6.17 Factors associated with condition fulfilling the EASL treatment criteria among 2,760 sero-survey participants (1973-2008) with available maternal sero-status

| Variables | | Proportion with outcome | Crude OR (95% CI) | P[1] | Adjusted OR (95% CI) | P[1] |
|---|---|---|---|---|---|---|
| Sex | Male | 6/1,387 (0.36%) | 1.0 (ref) | 0.8 | | |
| | Female | 4/1,367 (0.29%) | 0.8 (0.2-3.0) | | | |
| Current age | 11 – 29 | 3/1,771 (0.17%) | 1.0 (ref) | 0.03[2] | | |
| | 30 – 39 | 4/683 (0.59%) | 3.5 (0.8-15.6) | | | |
| | 40 – 49 | 1/223 (0.45%) | 2.7 (0.3-25.8) | | | |
| | 50 – 80 | 1/75 (1.33%) | 8.0 (0.8-74.8) | | | |
| Maternal HBsAg | Negative | 2/2,146 (0.09%) | 1.0 (ref) | 0.002 | 1.0 (ref) | 0.001[3] |
| | Positive | 7/614 (1.14%) | 12.4 (2.6-59.1) | | 12.7 (2.8-57.3) | |
| Hepatitis B vaccination | None | 8/975 (0.81%) | 1.0 (ref) | 0.01 | 1.0 (ref) | 0.01[3] |
| | Yes | 1/1,777 (0.06%) | 0.07 (0.01-0.55) | | 0.06 (0.01-0.53) | |

[1] p-value and 95% CI were obtained by Wald test with robust standard error to take account of clustering among individuals who share the same mother.

[2] Test for linear trend.

[3] Model included sex, current age, maternal HBV status and hepatitis B vaccination.

## 6.5. Discussion

This study used data from an open cohort to estimate incidence rates of major endpoints in the natural history of CHB infection: HBeAg and HBsAg sero-clearance, HCC, and death. These parameters have been poorly described in sSA.[73,71] In addition, a comprehensive liver assessment, including transient elastography and liver biopsy, was used to estimate the prevalence of significant liver fibrosis and the requirement for antiviral treatment according to international guidelines.

### 6.5.1. Incidence of HCC

The risk of HCC among people with CHB infection differs considerably by geographical location. HCC incidences estimated from population-based cohort studies of HBsAg-positive men vary from 34/100,000 carrier-years in Europe[99,100] to 230/100,000 in Alaska[81] and 530-1,030/100,000 in East Asia.[98,101] In sSA, the incidence was 68.3/100,000 carrier-years in a cohort of men in the Senegalese army.[97] In the current study the incidence of HCC in HBsAg-positive men was 116.7/100,000 carrier-years. This figure might be an underestimate because the HCC cases were ascertained through linkage with the Gambia National Cancer Registry database, which is estimated to contain only 50% of cases.[286] However, it is unlikely to be a substantial underestimate because we also reviewed the medical records at the primary care clinic in Keneba to improve the ascertainment of HCC cases.

### 6.5.2. Risk factors for HCC and its predictors

The geographical variation in HCC risk amongst HBsAg-positive persons suggests that factors other than HBsAg affect HCC risk. Certain HBV genotypes,[146] alcohol consumption,[157] diabetes

mellitus[165] or co-infection with other viruses (HCV,[167] HDV,[72] and HIV[182]) have been shown to increase the risk of HCC among chronic HBV carriers in other parts of the world. In The Gambia, the majority of people are Muslim and alcohol consumption is rare (2%). The prevalence of diabetes in adults was 0.2% in a nationwide survey.[166] And in our cohort, co-infection with HIV, HCV and HDV was almost zero. The distribution of HBV genotypes was studied in this cohort using the sample from 2003 sero-survey and almost all (96.0%, 95/99) were genotype E.[148]

Having an HBsAg-positive mother was associated with known predictors of HCC (delayed HBeAg sero-clearance, persistence of high viral replication and high ALT levels over time, higher prevalence of active CHB disease, significant liver fibrosis, and condition meeting the EASL treatment criteria). Moreover, all the patients who died of ESLD (including HCC) and whose maternal sero-status was known had HBsAg-positive mothers, although the numbers were small. These findings suggest that mother-to-infant transmission may increase the risk of HCC beyond its effect of increasing the risk of CHB infection. The findings are also consistent with previous reports from East Asia suggesting the effect of positive maternal HBV sero-markers on delayed HBeAg and HBsAg sero-clearance,[78,83,245,246] high ALT levels after HBeAg loss,[76] and paediatric HCC.[242,243] The chance of perinatal transmission from an HBsAg-positive mother to her infant is especially high when the mother is also HBeAg-positive.[30] The transplacental passage of HBeAg or HBeAg-derived peptides from mother to foetus is thought to induce a tolerance in newborns against the virus.[75] Such a mechanism may lead to CHB infection, and prolong the persistence of high viral replication, which can cause HCC.[101,139]

The current study found an association between high viral load and abnormal ALT during the follow-up and the disease outcomes (active CHB disease, significant liver fibrosis and condition requiring antiviral therapy). Similar findings were reported in a study conducted in Hong Kong.[111] This is consistent with our hypothesis that it is through prolonged viral replication and frequent hepatitis flare that perinatal mother-to-infant transmission leads to liver fibrosis.

### 6.5.3. Limitations of the study

There are several limitations to this study. First, the date of the HBeAg and HBsAg loss was not known and was estimated as the midpoint between the last positive result and the first negative result. The estimated date is less accurate than in other studies because the interval between follow-up sero-surveys (4-5 years) was longer than in other studies of the natural history of CHB infection (6 months to 1 year).[81,83,88] Nonetheless, the results obtained in this study are within a range that has been reported from other parts of the world (HBeAg loss per year: 3-9%,[80,81,83] and HBsAg loss per year: 0.5-2.3%).[81,88,89] Second, the effect of maternal HBsAg was examined rather than maternal HBeAg. Maternal HBeAg positivity is a stronger predictor of perinatal transmission than HBsAg positivity. However, maternal sero-status in this study was determined when the child entered the cohort, and by this time HBeAg is likely to have been lost.[261] Third, the associations between maternal HBsAg and factors predictive for HCC may have been confounded by factors that were not measured in this study. For example, an environmental risk factor for HCC such as aflatoxin exposure[156] could confound the association because the amount of dietary consumption of crops contaminated by this fungal toxin could vary between households. Moreover, aflatoxin can transfer from mother to child in utero[287] and

through the breast milk.[288] However, cross-sectional studies that examined the samples from the current cohort failed to identify the association of indicators for aflatoxin exposure (i.e. aflatoxin-albumin adducts levels in plasma and tumour protein 53 (TP53) mutation at codon 249 in circulating cell-free DNA in plasma) with hepatitis B viraemia.[148,153,156]

### 6.5.4. Population impact of perinatal maternal transmission

In the pre-vaccine era, mothers in sSA had similar seroplevalence of HBsAg (10%) to mothers in East Asia. However, the prevalence of HBeAg among HBsAg-positive mothers was lower in sSA (10% *versus* 40%),[63,289] and consequently the chance of mother-to-infant transmission was smaller in sSA than in East Asia. Previous mathematical modelling estimated that 40% of chronic HBV carriers in East Asia acquired the infection through the perinatal route while this was only 10% in sSA.[63] Indeed, the current study estimated that 13.9% of chronic HBV carriers were attributable to this mode of transmission. However, in people with established CHB infection, only few (5%) develop liver disease requiring antiviral therapy, and having an HBsAg-positive mother is a strong predictor of requiring antiviral therapy. Consequently, 71.4% of chronic carriers requiring antiviral therapy were estimated to be attributable to perinatal maternal HBV transmission (assuming that the estimated association reflects a causal relation). This figure may be conservative where there is high hepatitis B vaccine coverage (e.g. >95% in The Gambia), for the following reasons. First, the majority (50-90%) of children with vaccine failure resulting in CHB infection have HBV-positive mothers.[238,173] Second, as this study has shown, the vaccine does not seem to prevent progression of liver disease amongst people established the CHB infection. Nevertheless, there is considerable uncertainty in the estimate presented given the wide confidence interval and possibility of residual confounding.

The high PAF emphasises the potential importance of interrupting perinatal maternal transmission in sSA. The WHO recommends that the first dose of hepatitis B vaccine should be administered as soon as possible after the birth, ideally within 24 hours.[116] Despite a relatively high coverage of hepatitis B vaccine worldwide, the timely birth dose (<24 hours) is not always given. By the end of 2012, only half of the countries with national hepatitis B vaccine programmes recommended the first dose within 24 hours.[117] In sSA, only six countries are undertaking the birth dose and The Gambia is one of them. However, in Keneba and Manduar, of 2,173 persons who were vaccinated against HBV between 1984 and 2007, only 2.8% (63/2173) were given the first dose within 24 hours (unpublished data). This might be because in The Gambia, only half of births are in a health facility,[290] and the children are usually not taken away from home for at least seven days.[113] Currently there is no community-based delivery of hepatitis B vaccination in the country. A cost-effective analysis of birth dose in sSA that takes account of the additional risk of maternal transmission on disease progression is needed.

## 6.6. Summary

Sero-surveys of HBV markers have been conducted regularly in small villages in the West Kiang District of The Gambia since 1973. By the latest survey in 2008, 414 chronic HBV carriers have been identified and they were invited for the comprehensive liver assessment as part of the PROLIFICA project in 2012-13. This open cohort study allowed me to assess the effect of maternal HBV sero-marker when child entered the study. Positive maternal HBsAg (a

proxy for the presence of perinatal maternal transmission) was associated with predictors of HCC (delayed HBeAg seroclearance, high HBV DNA and high ALT levels over time, active CHB disease, significant liver fibrosis and requirement of antiviral treatment) in chronic HBV carriers. Moreover, all of the three individuals who died of ESLD and whose maternal sero-status was known had mothers carrying HBsAg. These results suggest that the effect of mother-to-infant transmission is two-fold: 1) it increases the risk of CHB infection (which is already established) and 2) it increases the risk of HCC in people with CHB infection. The proportion of chronic HBV carriers attributable to perinatal maternal transmission is small (13%) in The Gambia as the frequency of this mode of transmission is low. However, the proportion of chronic carriers requiring antiviral treatment that are attributable to perinatal maternal transmission is high (71%). Although there is much uncertainty in these estimates due to small number of cases requiring antiviral therapy and potential residual confounding, the study suggests that a timely birth dose of hepatitis B vaccine in sSA might help to reduce the burden of disease associated with CHB infection more efficiently than postponing hepatitis B vaccine.

# Chapter 7. The association between birth order and hepatocellular carcinoma in people with chronic hepatitis B infection: a case-control study in the Gambia, West Africa

## 7.1. Abstract

**Background**

In Chapter 5, the effect of age at hepatitis B virus (HBV) infection on hepatocellular carcinoma (HCC) amongst chronic carriers of HBV was assessed using a historical case-control study in The Gambia. However, the association was not significant and the study was limited by a small sample size, unknown participation rate, and having hospital-based controls. In this chapter data from PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa) project in The Gambia are used to examine the association between age at infection and HCC using birth order as a proxy measure for the age at infection (or mode of transmission).

**Methods**

PROLIFICA consists of two main studies: WATCH (West African Treatment Cohort for Hepatitis B) which aims to reduce HCC incidence through community-based screening for HBV infection, clinical assessment and antiviral therapy; and HC4 (Hepato-Celllular Carcinoma Case-Control study) which is a hospital-based HCC case-control study. The screening targeted all individuals ≥30 years old in 27 urban and 27 rural areas randomly selected within the Western Gambia. The distribution of birth order in hepatitis B surface antigen (HBsAg)-positive

HCC cases recruited from the HC4 study was compared with two different control groups. The first group comprised of people identified as HBsAg-positive without HCC in a community-based HBV screening of the WATCH. The second group was HBsAg-positive patients without HCC recruited from the HC4 study. The analysis was weighted for non-attendance in the first case-control comparison to better represent the distribution of birth order in the population.

**Results**

Between December 2011 and January 2014, 5,980 of 8,170 eligible people (68.9%, 95% CI: 65.1-72.6%) participated in the screening. HBsAg prevalence was 8.8% (495/5980, 95% CI: 7.9-9.7%). The first control group consisted of 392 HBsAg-positive subjects without HCC. From June 2012 to November 2013, 242 patients referred for suspected liver disease were enrolled in the HC4 study from which 72 HBsAg-positive HCC cases and 63 HBsAg-positive controls (second control group) were identified. After adjusting for age, ethnicity, birthplace and parental education levels, odds ratios decreased with increasing birth order in full-siblings in the first case-control analysis: 1.00 (reference), 0.55 (95% CI: 0.23-1.30), 0.61 (0.21-1.73), 0.55 (0.17-1.86) and 0.18 (0.05-0.68) in $1^{st}$, $2^{nd}$, $3^{rd}$, $4^{th}$, and $\geq 5^{th}$ birth order, respectively ($p = 0.01$). There was a similar inverse association between birth order in full-siblings and HCC in the second case-control comparison ($p = 0.05$).

**Conclusion**

HCC cases had lower birth order than controls. Low birth order is associated with perinatal transmission because low birth order children have young mothers who are more likely to have high viral replication. This is consistent with the findings described in the previous chapters that perinatal mother-to-infant transmission might increase the risk of HCC and its predictors

beyond its effect of increasing the risk of chronic HBV infection. The incidence of HBV-related HCC in The Gambia might be reduced by interrupting this mode of transmission.

## 7.2. Introduction

The studies of maternal hepatitis B virus (HBV) markers in The Gambia (Chapters 4 and 6) found associations between having an HBV-positive mother and predictors of hepatocellular carcinoma (HCC) in people chronically infected with HBV. In Chapter 5 (historical case-control study of HCC), the first birth order was associated with higher risk of liver cirrhosis and HCC than higher birth order, although the association did not reach statistical significance. These results are consistent with the hypothesis that perinatal mother-to-infant transmission of HBV is an important determinant of progression of liver diseases in The Gambia.[42,60] However, the study linking birth order with HCC (Chapter 5) was limited by a small sample size, unknown participation rate, and particularly by having hospital-based controls. In this chapter the same association was examined by using data from a new case control study of HCC in The Gambia. I attempted to improve the study design by comparing with population-based controls who may better represent the distribution of birth order of the population which derived the cases than hospital-based controls whose birth order may be biased because birth order is often associated with other diseases.[188–191]

The PROLIFICA (Prevention of Liver Fibrosis and Cancer in Africa) Programme, funded by European Union FP 7 grant and lead by Prof. Mark Thursz, started in 2011 in The Gambia, Senegal and Nigeria. In The Gambia, the principle investigator is Dr. Ramou Njie. PROLIFICA consists of two main studies: a cohort study of people with chronic hepatitis B (CHB) infection (WATCH: West African Treatment Cohort for Hepatitis B) and a case-control study of HCC (HC4: Hepato-Celllular Carcinoma Case-Control study). The former aims to reduce HCC incidence through screening for HBV infection, clinical assessment and antiviral therapy using

tenofovir (nucleotide analogue). Because people aged <30 years are likely to have been vaccinated against HBV in The Gambia, screening targeted people over 30 years old. The HC4 study was designed to develop a research platform for scientific studies of proteomics, metabonomics, molecular diagnostics, host genetics, HBV viral genetics of HCC in West Africa. HCC cases are recruited from the referral hospitals in The Gambia.

The primary objective of this chapter is to assess the association between birth order and HCC using data from the WATCH and HC4 studies. HCC cases recruited in the referral hospitals are compared with two different sets of HBsAg-positive controls; namely, population-based controls from the community screening, and hospital-based controls. The hypothesis is that HCC cases are associated with lower birth order, which is correlated with perinatal transmission because low birth order children have young mothers with high viral replication. The secondary objectives of this analysis are to determine: i) the attendance rate for community-based screening and factors associated with non-attendance, ii) the prevalence of HBsAg in adults over 30 years old in the Western part of The Gambia and factors associated with positive HBsAg, iii) the attendance rate at the liver clinic in people who tested positive for HBsAg and factors associated with non-attendance at the clinic, iv) the association between birth order and HBsAg positivity, and v) the associations between birth order and predictors of HCC (active CHB diseases, significant liver fibrosis and condition requiring antiviral treatment according to the international guidelines).

# 7.3. Methods

## 7.3.1. WATCH study

The WATCH study uses two methods of screening people for HBV infection: community-based screening and blood donor screening at blood donation centre of the Edward Francis Small Teaching Hospital (EFSTH). Data from the blood donor screening were not used for this analysis, as the recruitment of the study participants is still ongoing.

### *7.3.1.1. Stratification*

The WATCH study aimed to screen 5,500 people in The Gambia and 8,000 in Senegal. In The Gambia, the study was conducted in the Western part of the country (figure 7.1), which includes Banjul (capital), Kanifing and nine districts of the Western Region. Foni Kansala was excluded from the sample for political reasons. Because the natural history of CHB infection and risk factors for HCC are likely to differ between urban and rural populations due to environmental factors such as aflatoxin exposure,[153] the sample was stratified by urban and rural districts and the study aimed to screen equal numbers of participants (2,750) in each. Banjul, Kanifing, and two districts in the Western Region (Kombo North and Kombo Central) were classified as urban, and the others were classified as rural (figure 7.1, table 7.1).[286]

Figure 7.1 Districts of the Western Gambia (area surrounded by blue boundary indicates urban area. Foni Kansala was excluded.)

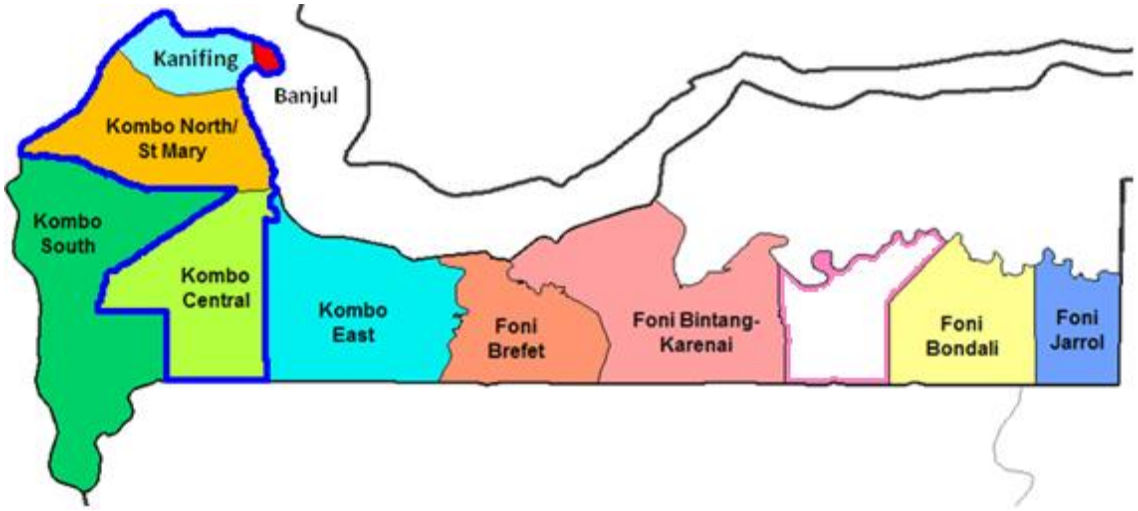


Table 7.1 Number of people aged over 30 years in each district of the Western Gambia (data from the national census in 2003)

| Region | District | Number of enumeration areas (EAs) | Total population size | Population size (≥30 years)[1] |
|---|---|---|---|---|
| **Urban** | | 1,197 | 608,633 | 164,331 |
| Banjul | | 92 | 35,061 | 9,467 |
| Kanifing | | 634 | 322,735 | 87,138 |
| Western Region | Kombo North | 316 | 166,715 | 45,013 |
| | Kombo Central | 155 | 84,122 | 22,713 |
| **Rural** | | 253 | 138,437 | 37,378 |
| Western Region | Kombo South | 112 | 61,247 | 16,537 |
| | Kombo East | 52 | 27,944 | 7,545 |
| | Foni Brefet | 22 | 10,822 | 2,922 |
| | Foni Bintang Karanai | 25 | 15,136 | 4,087 |
| | Foni Kansala (excluded) | 20 | 11,353 | 3,065 |
| | Foni Bondali | 11 | 6,080 | 1,641 |
| | Foni Jarrol | 11 | 5,855 | 1,581 |
| **Total** | | 1,450 | 747,070 | 201,709 |

[1] Estimates were obtained by multiplying the total population size by 0.27, which is the proportion of people aged ≥30 years in the whole country in 2003.

*7.3.1.2. Two-stage sampling*

The Western Gambia is divided into 1,450 enumeration areas (EAs) defined by Gambia Bureau of Statistics (table 7.1). One EA could be an entire village, or part of large village, or a cluster of small villages. According to the previous national census in 2003, 747,070 people reside in the Western Gambia, which is 55% of the total population. The population size per EA varies from 16 to 1,246 in the Western Gambia, and the mean is 516. The proportion of people aged ≥30 years in the whole country in 2003 was 27%, therefore the average population ≥30 years old in one EA is estimated to be 140. Twenty-seven EAs were randomly selected from each of the list of urban EAs and rural EAs. All inhabitants in the selected EAs were assessed for eligibility (eligibility criteria listed below).

*7.3.1.3. Sensitisation of the population*

The selected communities were informed about the study before the survey. A team of fieldworkers and I visited the head of the village (alkalo) with kola nuts as a gift. We explained the study purpose, delineated the study area by referring to a map from the Gambia Bureau of Statistics, and asked the alkalo to invite the community for a meeting. We visited the village again for the community meeting, at which point the study was described to the community. We used a poster and leaflet to help explain the study (Appendix 6).

*7.3.1.4. Pre-screening registration*

Following community approval, a team of fieldworkers registered people in the EA. Because there are no lists of village residents in The Gambia, the team conducted a rapid census by visiting each compound and registering everyone eligible for screening. Eligibility criteria were:

1) age ≥30 years, and 2) having spent the previous night in the compound. A number was assigned to each compound, and names, sex, age or year of birth, and phone numbers of eligible persons were recorded. Information was obtained from the head of the compound on people not present at the time of registration. We obtained year of birth from the national identity card. Eligible persons were invited to participate in the screening.

*7.3.1.5. Screening*

While some of the team registered eligible individuals, the rest set up a screening site at the centre of the EA. This could be at a health centre, school, mosque, bantaba (community gathering space under a large tree) or someone's compound depending on what was agreed with the community. At the screening site, the team confirmed the eligibility of the person who came by checking against the list of registered people. After obtaining written consent for the screening, a point-of-care test for HBsAg using immunochromatography (Determine, Alere, USA) and a standardised questionnaire (Appendix 7) were administered. The diagnostic accuracy of Determine was studied in The Gambia; sensitivity was 88.5% (95% CI: 80.7-93.9%) and specificity was 100% (95% CI: 99.5-100%) using a reference standard of enzyme-linked immunosorbent assay (ELISA) for HBsAg using AxSYM (Abbott, USA) (unpublished data). Responses to the questionnaire and test results were recorded using a Tablet PC (a small laptop with touch screen, Samsong Galaxy, Samsong, Korea). The electronic questionnaire included functions to reduce errors (automated range, consistency checks and skip patterns).[291]

The result of the rapid test was available within 15 minutes and post-test counselling was provided on site. All individuals who tested positive were given an appointment at the liver

clinic at the Medical Research Council (MRC), Fajara, for further investigations. This usually occurred 1-2 weeks after the survey. Once the screening in an EA was complete, the same number of HBsAg-negative people was randomly selected from the EA and invited to the liver clinic.

From August to November 2013, we conducted an add-on field validation study of point-of-care tests within the community screening programme. During this period, we used an additional point-of-care test (Vikia, Biomerieux, France) to compare diagnostic accuracy of Determine and Vikia against the AxSYM ELISA for HBsAg as the gold standard test which was performed on dried-blood spots collected at the field. The survey participants during this period were also asked whether they had been ever tested for HBV infection.

*7.3.1.6. Reminder and reason for non-attendance*

People who were registered but who did not come to the screening were reminded by fieldworkers by a phone call or visiting the compound. Up to three attempts either by phone or visit were made to follow the person up. The reason for non-attendance was selected from a list of possible choices; multiple choices were not allowed. The reasons were categorised as absence or refusal. The reasons for absence included: absence due to work, travelling or social gathering. The reasons for refusal included: being afraid of bleeding, too busy to participate, feeling ill, no benefit, already tested before for HBsAg, no trust in MRC, or husband refusal (women only). When the answer did not fit any of these a specific reason was recorded.

*7.3.1.7. Team of fieldworkers*

The team of fieldworkers consisted of one field supervisor, one nurse and six field assistants. All had had previous experience of fieldwork, but the length of experience before joining the project ranged from 2 to 29 years. A one-week workshop for fieldworkers was held before starting the project. This included an introduction to the epidemiological and clinical aspects of HBV infection, and training for administering a finger prick, use of the point-of-care test, for HBsAg, administering the questionnaires using the Tablet PC, and pre- and post-test counselling of HBsAg test.

In all the screening sessions, a field supervisor was available to lead the team. The availability of a nurse and field assistants for the fieldwork was determined by the amount of clinical activity at the liver clinic at the MRC, Fajara. However, at least four of the field workers including the supervisor were involved in screening at all times. Whenever possible, a village health worker (under the Regional Public Health Office) was asked to help the screening. In addition, the community often allocated a few volunteers to help with the project.

*7.3.1.8. Clinical assessment at the liver clinic at MRC, Fajara*

People identified to be positive for HBsAg and randomly selected HBsAg-negative people were invited for a comprehensive liver assessment at the liver clinic at the MRC, Fajara. After written consent for the study participation, urine and blood sample were collected. An interview using standardised epidemiological questionnaire and standardised clinical examination including abdominal ultrasound and liver stiffness measurement (LSM) with transient elastography (Fibroscan FS402, Echosens, France)[104] were performed. Participants fasted overnight as the ingestion of food hampers reliable measurements by transient elastography.[281] After the first

liver assessment, those who met any of the study criteria: HBV DNA levels ≥2,000 IU/ml, LSM ≥6.5 kPa or serum alanine transaminase (ALT) ≥40 IU/L, were invited for a liver biopsy. Liver fibrosis was scored according to the Metavir system[282] by one liver pathologist in The Gambia and another in the UK. The study was approved by the Gambia Government/MRC Joint Ethics Committee.

### 7.3.2. HC4 study

#### *7.3.2.1. Setting*

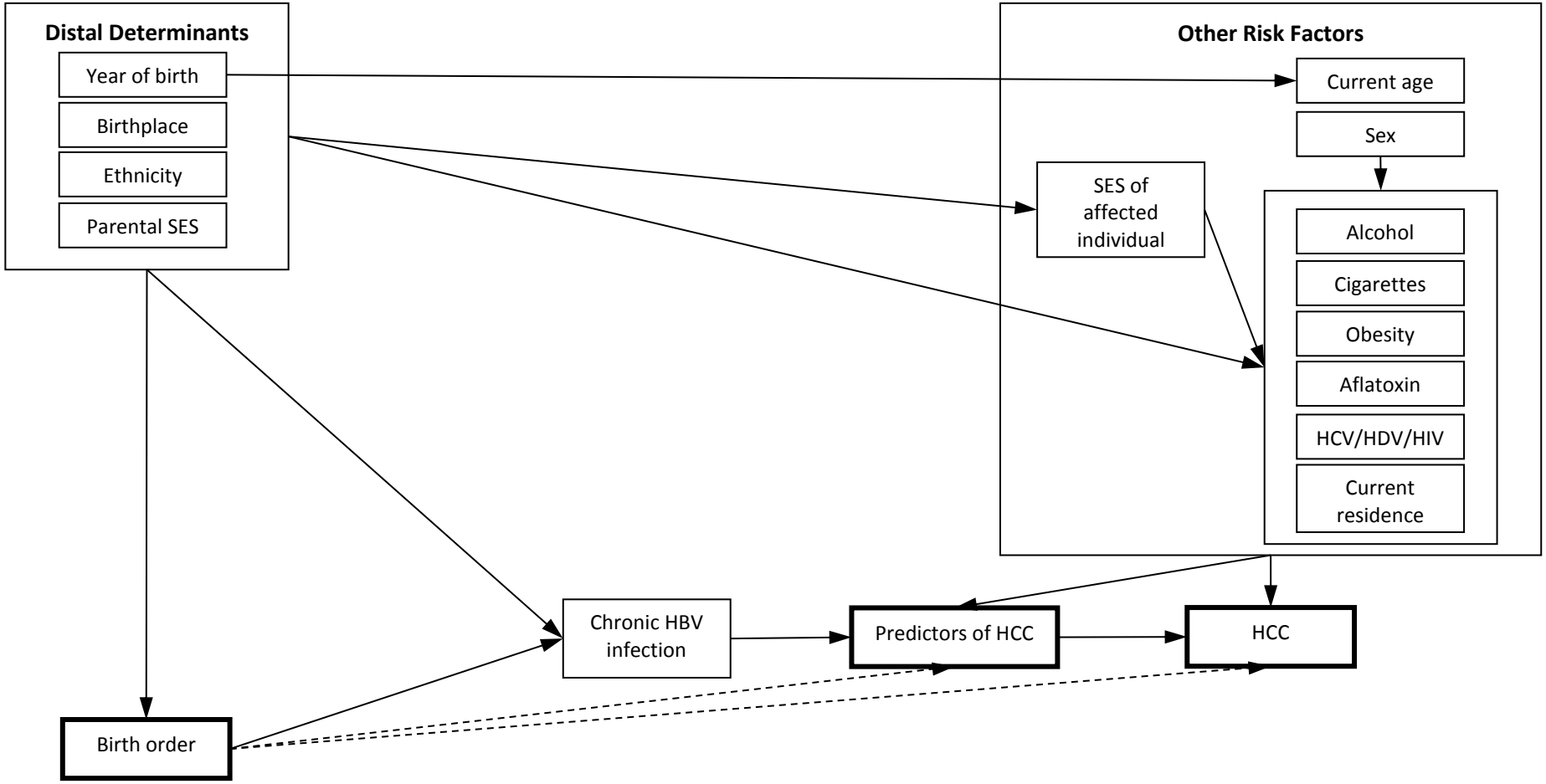
The study started in June 2012, and the current analysis included the patients recruited until November 2013. Participants were recruited from referral hospitals in The Gambia, namely: Edward Francis Small Teaching Hospital (EFSTH, formally known as Royal Victoria Teaching Hospital), the sole tertiary care hospital in The Gambia located at capital Banjul; MRC Clinic, in Fajara, Kanifing; Bwiam Hospital located in a rural part of the Western Gambia, Farafenni Hospital and Bansang General Hospital in the upcountry. Patients with suspected liver disease were identified by local physicians or through active surveillance of the outpatient clinics and inpatient wards by research nurses. All the patients referred for clinically suspected liver disease were eligible for the study. Consequently, the study included not only HCC or liver cirrhosis patients, but also patients with other diseases of non-hepatic origin such as metastatic liver cancer or tuberculous peritonitis, which are difficult to distinguish from liver diseases on a clinical basis alone. After informed consent, all the patients underwent a structured interview with a standardised questionnaire and a clinical examination which included ultrasound and transient elastography. Blood and urine samples were also collected. The questionnaires and case report forms used in the HC4 study were identical to those used in the WATCH study.

Unless contraindicated (e.g. because of coagulopathy), a liver biopsy was performed in all the study participants with a hepatic nodule on ultrasound who agreed to have the procedure. Those without a hepatic nodule were invited for the liver biopsy when they met one or more of the study criteria (which are the same as the one used in the WATCH study).

*7.3.2.2. Case Definition*

To estimate the effect of early age at HBV infection on HCC and its predictors beyond its effect of increasing the risk of CHB infection (figure 7.2), both cases and controls were restricted to people with positive HBsAg. Three different definitions for HCC were used for this analysis: 1) histopathologically confirmed; 2) clinically confirmed; and 3) clinically probable HCC cases. Clinically confirmed cases of HCC met all of the criteria: i) focal liver lesion ≥2 cm consistent with HCC by ultrasound; ii) alpha-fetoprotein levels ≥200 ng/ml; and iii) liver cirrhosis.[292] Clinically probable HCC cases met two of the criteria above. As the distribution of birth order and potential confounding factors for the association of birth order with HCC was similar between the three groups (table 7.2), confirmed and probable cases were combined.

Figure 7.2 Causal diagram for the effect of birth order on the risk of HCC and its predictors



Early birth order is a proxy for perinatal mother-to-infant HBV transmission whilst late birth order is a proxy for early horizontal transmission. Early age at HBV infection is known to be associated with liver cirrhosis and HCC through increasing the risk of chronic HBV infection. The hypothesis of this analysis is in addition to this effect, early age at infection further increases the risk of HCC and its predictors (presented as a dashed arrow). The exposure and outcome variables of interest are surrounded by lines in bold-type. SES denotes socio-economic status

Table 7.2. Distribution of birth order and *a priori* confounders for the association between birth order and HCC in HBV-related HCC according to the basis of diagnosis (histopathologically confirmed HCC cases, clinically confirmed HCC cases and clinically suspected HCC cases)

| | | Histo-pathologically confirmed HCC (n=16) | | Clinically confirmed HCC (n=17) | | Clinically suspected HCC (n=39) | | P |
|---|---|---|---|---|---|---|---|---|
| Male sex | | 14 | 88% | 13 | 77% | 27 | 71% | 0.4 |
| Median age (range) | | 36 (21, 60) | | 39 (23, 60) | | 40 (24, 78) | | 0.5 |
| Ethnic group | Mandinka | 4 | 25% | 3 | 19% | 10 | 27% | 0.3 |
| | Jola | 0 | 0 | 3 | 19% | 2 | 5% | |
| | Others | 12 | 75% | 10 | 63% | 25 | 68% | |
| Birth place | Urban | 2 | 13% | 0 | 0 | 6 | 16% | 0.4 |
| | Rural | 13 | 81% | 13 | 81% | 28 | 76% | |
| | Foreigners | 1 | 6% | 3 | 19% | 3 | 8% | |
| Parental schooling | None | 6 | 37% | 11 | 65% | 23 | 59% | 0.4 |
| | One parent only | 4 | 25% | 4 | 23% | 6 | 15% | |
| | Both parents | 6 | 37% | 2 | 12% | 10 | 26% | |
| Number of full siblings | 1-2 | 3 | 19% | 1 | 7% | 5 | 14% | 0.5 |
| | 3-4 | 3 | 19% | 3 | 20% | 8 | 22% | |
| | 5-6 | 6 | 37% | 6 | 40% | 6 | 16% | |
| | 7-8 | 3 | 19% | 4 | 27% | 9 | 24% | |
| | ≥9 | 1 | 6% | 1 | 6% | 9 | 24% | |
| Number of siblings (full + half) | 0-5 | 1 | 7% | 1 | 7% | 6 | 18% | 0.1 |
| | 6-8 | 9 | 64% | 3 | 20% | 7 | 21% | |
| | 9-11 | 1 | 7% | 5 | 33% | 9 | 26% | |
| | 12-14 | 0 | 0 | 1 | 7% | 4 | 12% | |
| | ≥15 | 3 | 21% | 5 | 33% | 8 | 23% | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Birth order in full siblings | 1st | 5 | 31% | 7 | 47% | 13 | 35% | 0.9 |
| | 2nd | 4 | 25% | 2 | 13% | 6 | 16% | |
| | 3rd | 1 | 6% | 3 | 20% | 5 | 14% | |
| | 4th | 3 | 19% | 1 | 7% | 7 | 19% | |
| | ≥5th | 3 | 19% | 2 | 13% | 6 | 16% | |
| Birth order in siblings (full + half) | 1st | 2 | 13% | 2 | 13% | 11 | 30% | 0.7 |
| | 2nd | 4 | 27% | 3 | 20% | 3 | 8% | |
| | 3rd & 4th | 2 | 13% | 4 | 27% | 7 | 19% | |
| | 5th & 6th | 4 | 27% | 3 | 20% | 7 | 19% | |
| | ≥7th | 3 | 20% | 3 | 20% | 9 | 24% | |

P-value was obtained using chi-squared test unless indicated.

[1] Kruskal-Wallis test

*7.3.2.3. First control group: population-based controls*

Two HBsAg-positive control groups were considered for the comparison with the HBV-related HCC cases. The first control group was HBsAg-positive people identified at the community-based screening who did not meet the HCC criteria listed above at the first clinical assessment at the liver clinic. One HBsAg-positive participant in the control group without evidence of HCC at the enrolment developed HCC during the course of follow-up. This control group consisted of people over 30 years old from the Western part of The Gambia. Consequently, HBV-related HCC cases less than 30 years old and those recruited at the hospitals outside the Western Gambia (i.e. Farafenni and Bansang Hospital) were excluded from this case-control analysis.

The strength of this population-based control group is that they represent the birth order in the general population; by contrast the distribution of birth order in hospital controls might be affected by other diseases.[188–191]

A possible disadvantage of the population-based is that the ascertainment of HCC cases in the Western Gambia is unlikely to be complete because not everyone can afford medical care when they develop HCC.[286] For this reason, hospital controls might better represent the population that generated the cases and another case-control analysis using hospital controls was performed.

*7.3.2.4. Second control group: hospital-based controls*

The second control group consisted of patients enrolled in the HC4 study who were referred to the liver clinics for suspected liver disease. The patients were HBsAg positive and without HCC at enrolment. In addition they were selected to be over 20 years old, because the youngest HBV-related HCC case was 21 years old.

7.3.3. Definition of birth order

When the questionnaire was designed it was assumed that birth order is a proxy for the number of infectious older siblings which might determine the probability of early childhood HBV infection. However, during the course of my PhD, I found that mother-to-infant transmission might determine the risk of liver diseases (Chapter 4, 5 and 6). The role of birth order has therefore changed from a proxy for early horizontal HBV transmission to a proxy for perinatal mother-to-infant transmission. Accordingly an inverse association between birth order and HCC

is predicted. To reflect perinatal transmission, birth order should include miscarriages and stillbirths, and multiple pregnancies should be counted as one (Chapter 2). Unfortunately in the WATCH and HC4 study we did not count miscarriages and stillbirths and did not record whether the siblings included twins or triplets. Although the current primary interest is the effect of birth order in full siblings, the effect of birth order counted in all siblings (full and half siblings) is also presented.

### 7.3.4. Blinding

The study hypothesis was not disclosed to fieldworkers who administered the birth order questions to avoid information bias. In addition, laboratory technicians were blinded to the demographic information of participants, including their birth order. In the WATCH study, birth order was determined at the community screening before clinical assessment occurred. Therefore, study clinicians were blinded to the birth order of the participants. Consistency check programmed in the electronic questionnaire avoided entering of implausible values (e.g. fifth-born in a family with sibship of three).

### 7.3.5. Laboratory assays

HBsAg status was determined using the point-of-care test (Determine, Alere, USA) in the field for the WATCH study and in the laboratory for the HC4 study. HBsAg-positive samples were tested for hepatitis B e antigen (HBeAg) using ELISA (ETI-EBK Plus, Diasorin, Italy), and quantified for HBV DNA using in-house real time quantitative polymerase chain reaction (qPCR). Alanine transaminase (ALT) was measured using VITROS 350 analyser (Ortho, USA). Alpha-fetoprtein level was quantified using Microparticle Enzyme Immunoassay (MEIA,

AxSYM AFP, Abbott, USA). Antibodies to Hepatitis C virus (anti-HCV) and Hepatitis D Virus (anti-HDV) were detected using MEIA (AxSYM, anti-HCV, Abbott, USA) and ELISA (ETI-AB-DELTAK-2, Diasorin, Italy). Antibodies to HIV-1 and HIV-2 and HIV p24 antigen were detected using enzyme immunoassay (EIA, Genscreen ULTRA HIV Ag-Ab, Bio-Rad, USA).

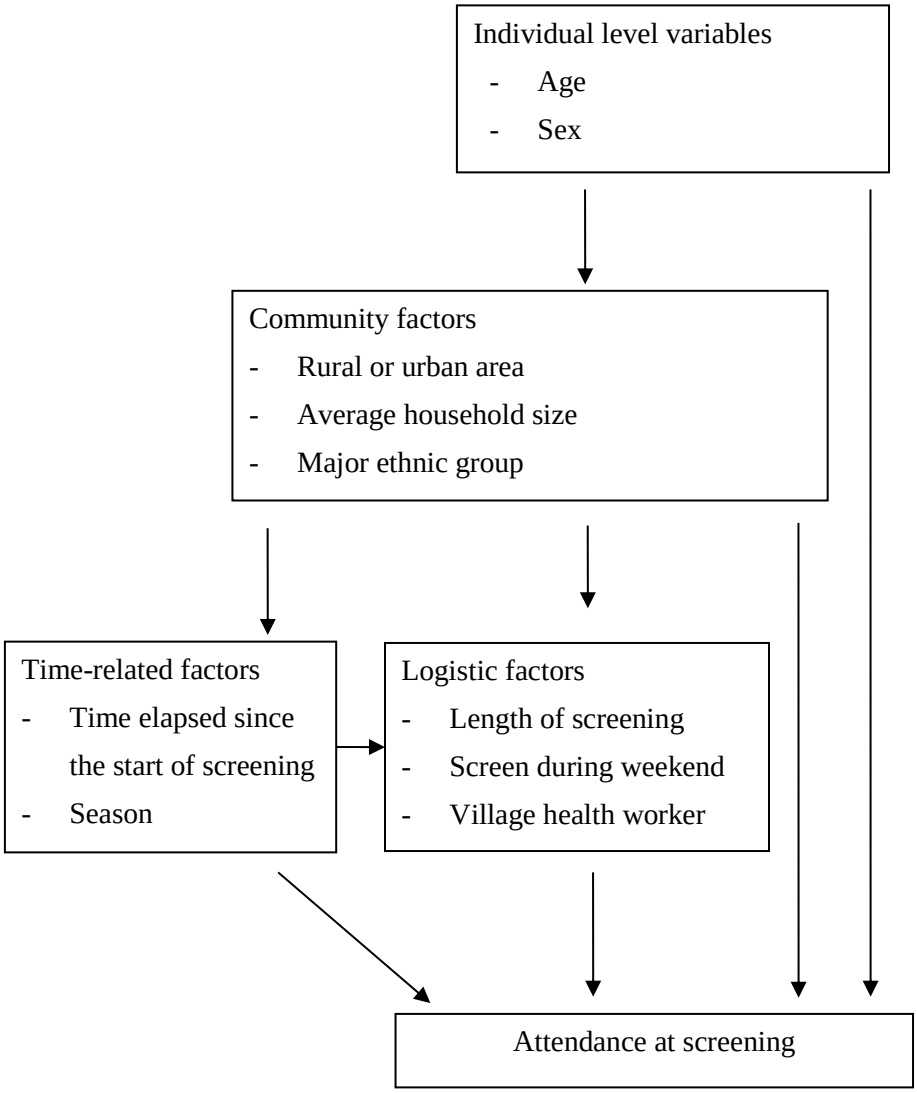
### 7.3.6. Statistical analyses

#### *7.3.6.1. Attendance at screening*

The attendance rate at screening was estimated by dividing the number of screening participants by the number registered as eligible. The estimates and confidence intervals accounted for survey design (stratification and clustering). The stratification weight for people from rural EAs was obtained by dividing the population in whole rural Western Gambia in 2003 census by the total number of eligible people in 27 rural EAs that were selected. Similarly, the urban weight was the population of urban Western Gambia in 2003 divided by the total number of eligible people in 27 urban EAs that were selected. The effect of each individual-level variable (sex and age) and each area-level variables (variables defined at each EA level: urban or rural area, average household size, major ethnic group, time elapsed since the start of the screening project, season, length of screening, screening during weekend and involvement of village health worker) on screening attendance was estimated using logistic regression, which allowed for clustering and stratification. A minimally sufficient set of *a priori* confounders of the association between each of the area-level variables and attendance was identified from a causal diagram (figure 7.3) by applying the backdoor test.[263] Based on the causal diagram the odds ratio (OR) for the association between each community factor (urban or rural area, average household size and

major ethnic group in the EA) and screening participation was adjusted for sex, age and the other community factors. ORs for time-related factors (whether the screening took place in the first year or second year of the project, and in the dry or rainy season) were adjusted for community factors and for the other time-related factors. Factors related to logistics (length of screening and number of days in weekend in the EA, and involvement of village health worker) were adjusted for community factors, time-related factors and other logistic factors. The major ethnic group in the EA was defined to be the one with more than 50% of participants. When no ethnic group constituted ≥50%, the EA was categorised as "mixture".

Figure 7.3 Causal diagram for the factors associated with attendance at screening



*7.3.6.2. HBsAg prevalence in the Western Gambia*

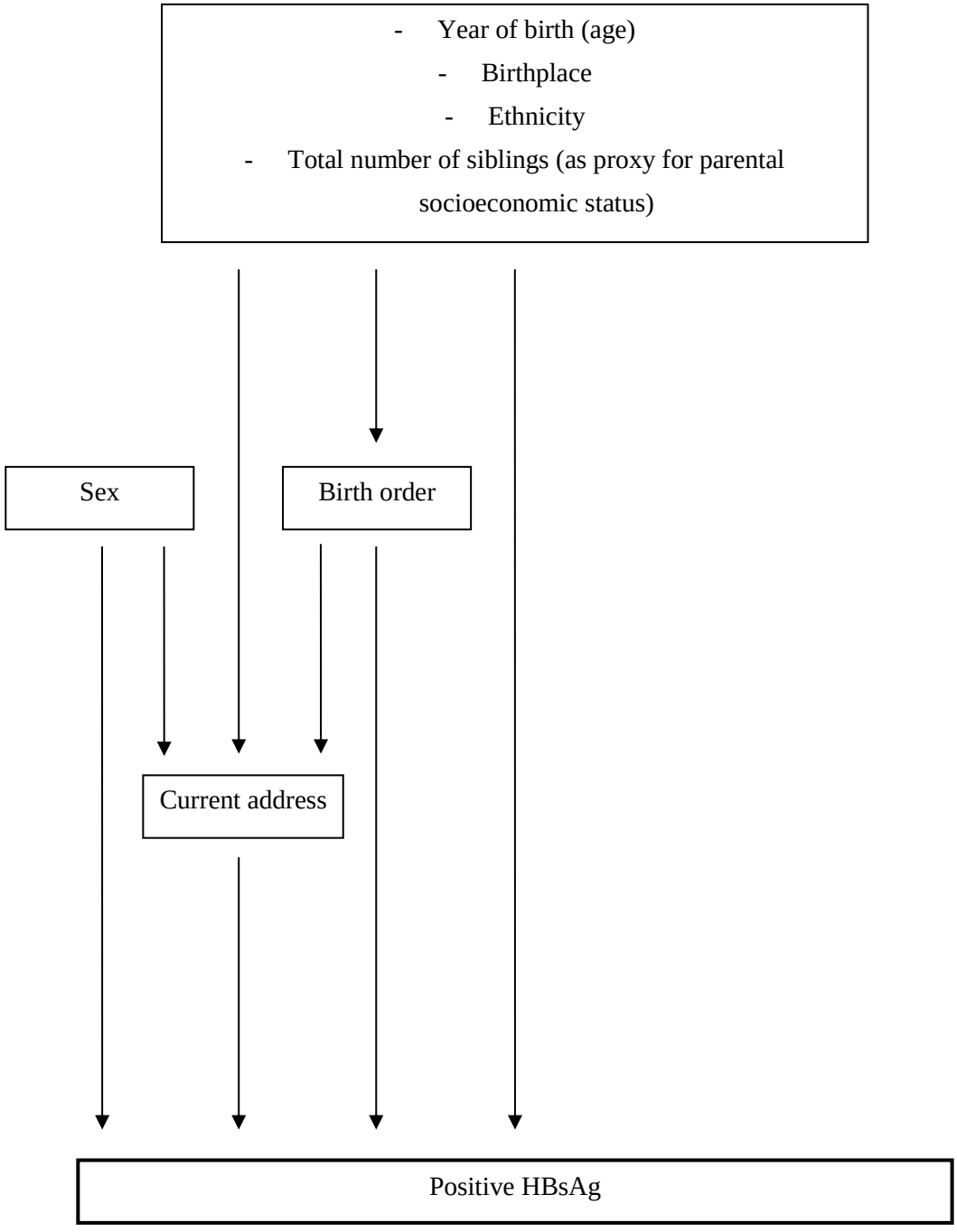
The prevalence of positive HBsAg in the Western Gambia was estimated by dividing the number of HBsAg-positive people by the number of screening participants. The estimates accounted for stratification and clustering in the survey design, and non-attendance at screening. The non-attendance weight was the reciprocal of the attendance probability obtained from logistic regression in which age group, sex and EA were predictors. A weighted logistic regression was used to estimate ORs for the associations between potential risk factors and presence of HBsAg. The weights were used to adjust for stratification and non-attendance, and confidence intervals were adjusted for clustering by EA. Potential confounders were identified using the causal diagram (figure 7.4). ORs for the association between determinants (age, birthplace, ethnic group and total number of siblings from the biological parents as proxy for parental socioeconomic status) and positive HBsAg were mutually adjusted for the other determinants. The OR for birth order was adjusted for age, birthplace, ethnic group and total number of siblings. And the OR for current address (urban or rural) was adjusted for age, birthplace, ethnic group, total number of siblings, sex and birth order.

To explore whether a change in HBsAg prevalence with increasing age can be explained by a spontaneous HBsAg loss over time and death related with CHB infection, the age- and sex-specific HBsAg sero-clearance rate and sex-specific mortality rate with end-stage liver disease derived from the cohort study in three rural villages (Chapter 6) was applied to the HBsAg prevalence in the lowest age group (30-39 years). The prevalence, $p_n$, in age group n was estimated from the prevalence in preceding age group n-1, separately for males and females using the equation:

$$p_n = p_{n-1} * (1 - q_{n-1} - r) / (1 - p_{n-1} * r)$$

where $q_{n-1}$ is HBsAg sero-clearance rate (per one carrier in 10 years) in previous age group, and r is mortality rate with HBV-related end-stage liver disease (per one carrier in 10 years).

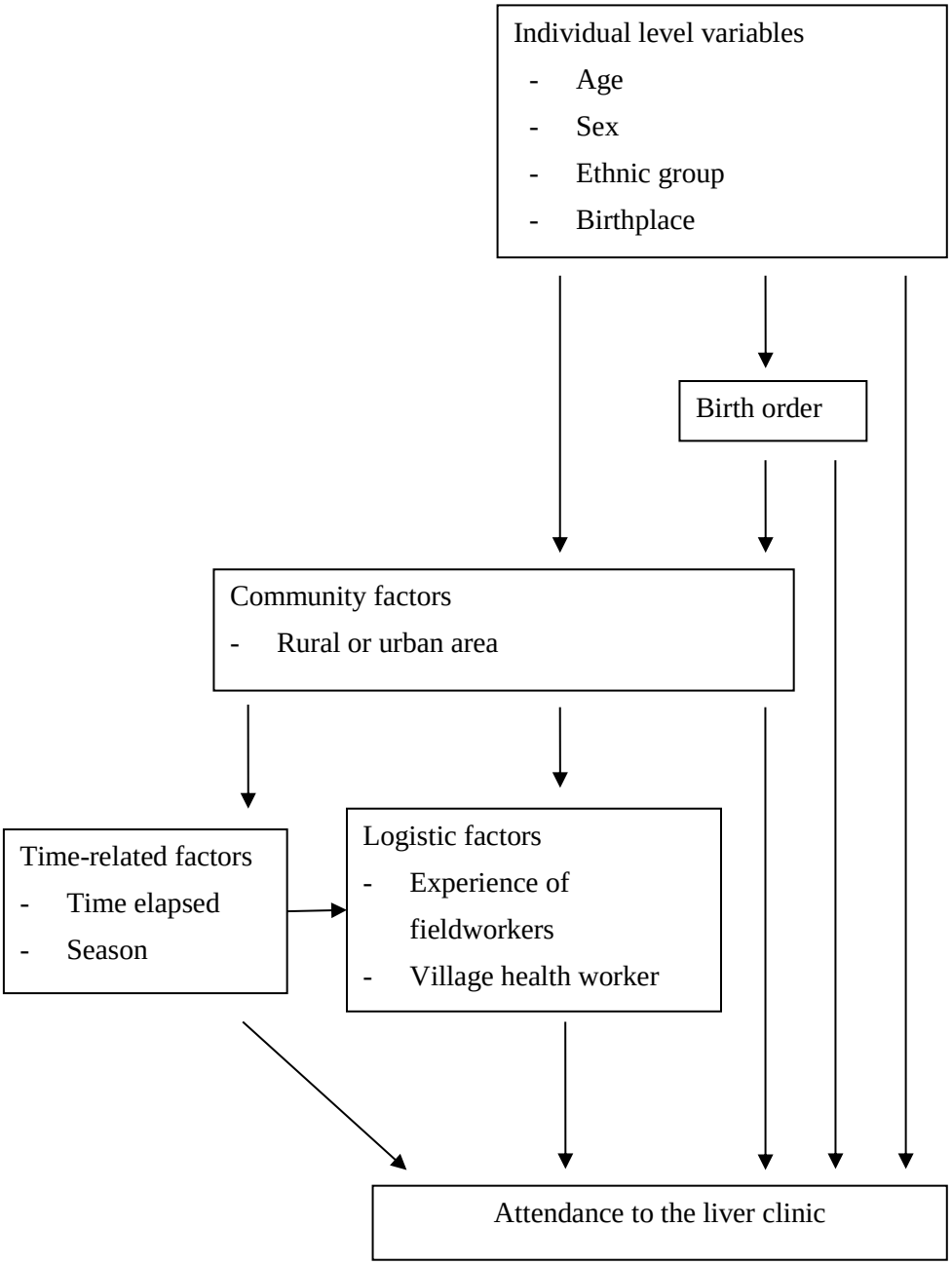
Figure 7.4 Causal diagram for the factors associated with positive HBsAg



*7.3.6.3. Attendance at liver clinic at MRC*

The rate of attendance at the liver clinic in people who tested positive for HBsAg in the Western Gambia was estimated by dividing the number who consented to take part in the liver assessment by the number tested positive at the community-screening. The estimates of the attendance rate and ORs for the association between potential predictive factors and clinic attendance accounted for clustering and stratification in the survey design, and non-attendance at screening. Logistic regression was used to estimate ORs for the effect of individual-level variables (sex, age, ethnic group, birthplace and total number of siblings from the biological parents as proxy for parental socioeconomic status) on the clinic attendance, and was adjusted for other individual level variables. To estimate the effect of birth order on participation,[208] the OR was adjusted for the other individual-level variables (sex, age, ethnic group, birthplace and total number of siblings) based on the causal diagram (figure 7.5). The OR for current residence (rural or urban) was adjusted for the individual-level variables including birth order. ORs for time-related factors (first or second year of the project, season of the screening) were adjusted for the current residence and for the other time-related factors. And the ORs for logistic factors (fieldwork experience of staff who provided the post-test counselling and involvement of village health worker in the screening) were adjusted for the current residence, time-related factors and for the other logistic factors.

Figure 7.5 Causal diagram for the factors associated with attendance to the liver clinic at the MRC, Fajara



*7.3.6.4. Association of birth order with the predictors of HCC*

The association between birth order and predictors of HCC (active CHB disease, significant liver fibrosis defined as LSM ≥7.5 kPa with transient elastography and condition requiring antiviral therapy according to the EASL (European Association of the Study of the Liver) guidelines[70]) was estimated using logistic regression. The analysis was conducted in HBsAg-positive people from the community-based screening who had clinical assessment at the liver clinic. The survey design (cluster sampling and stratification) and non-attendance at screening (see above) were accounted for in the analysis. In addition, the non-attendance weight to the clinic, which was the reciprocal of the attendance probability obtained from logistic regression in which age group, sex and current residence were predictors, was taken account. The definitions of active CHB disease, significant liver fibrosis and antiviral treatment criteria were the same as those used in the cohort study (Chapter 6).

The effect of birth order on the predictors of HCC was adjusted for *a priori* confounders by applying the backdoor test in a causal diagram (figure 7.2).[263] {Year of birth, birthplace, ethnic group and parental socioeconomic status (SES)} was identified as a minimally sufficient set of *a priori* confounders for the association between birth order and liver diseases (causal diagram is the same as the one in Chapter 5). As in Chapter 5, two different proxy markers for parental SES were used: parental schooling in model 1 and total sibship size in model 2. Age was used in the analyses instead of their year of birth, but this is a good proxy measure as participants were recruited within two years (from December 2011 to January 2014).

*7.3.6.5. Association of birth order with HCC*

The association between birth order and HCC was estimated using logistic regression to compare HBsAg-positive HCC cases with HBsAg-positive population-based controls (first control group), and HBsAg-positive hospital-based controls (second control group). As the first control group should represent HBsAg positive population in The Western Gambia, the analysis used weights to account for stratification and non-attendance to the screening and the clinic, and adjusted confidence intervals for clustering by EA (see above). The probability of being recruited in the study was assumed to be 99% in the HCC cases and they were weighted accordingly (there were only 2 refusals and one death during the consultation). The ORs for the effect of birth order on HCC were adjusted for the same set of *a priori* confounders used to estimate the effect of birth order on the predictors of HCC (see above).

## 7.4. Results

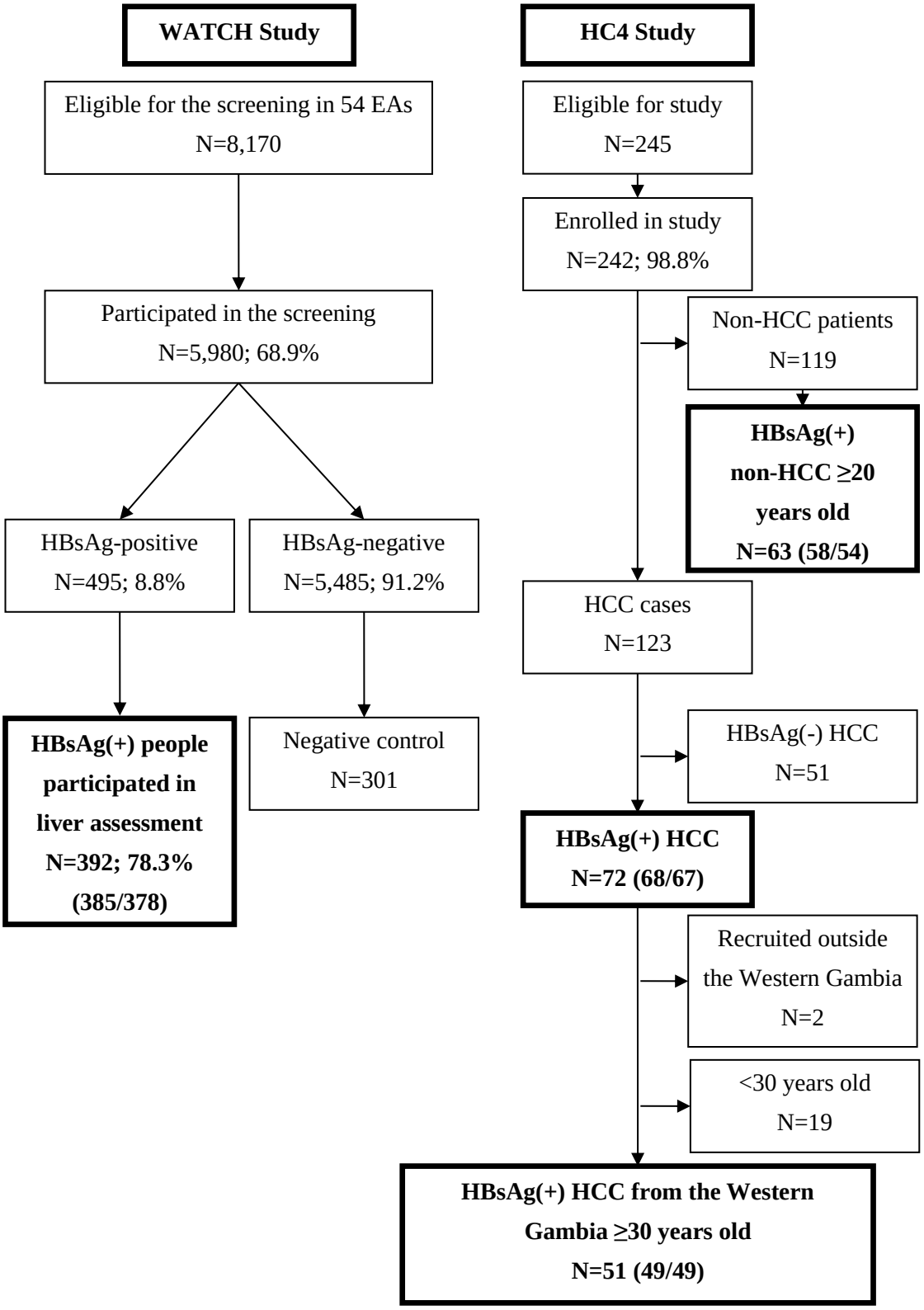
### 7.4.1. Attendance at screening

From December 2011 to January 2014, 27 urban and 27 rural EAs were screened, and 3,785 and 4,385 people ≥30 years old were registered as eligible for the screening, respectively (figure 7.6). The number registered varied from 33 in Banjul North (urban EA) to 424 in Basori (rural EA), with a median of 123 people/EA. The median number of adults attending the community sensitisation meeting was 30, ranging from 5 in Jalanbang to 110 in Basori (both are rural EAs). The number of days that the team of fieldworkers spent for the screening in each EA ranged from 2 to 8 days (median 3 days), and 13 and 41 EAs were screened during the rainy (from June to September) and dry season (from October to May), respectively.

Of 8,170 eligible people, 5,980 (68.9%, 95% CI: 65.1-72.6%) agreed to take part in the screening. The response rate varied from 48.9% in Tallinding (urban EA) to 95.1% in N'demban Jola (rural EA). The response rate was higher in women (75.5%, 95% CI: 71.9-79.0%) than in men (60.0%, 95% CI: 55.4-64.5%, p<0.001), and in the older age group (OR per 10-year increase in age: 1.2, 95% CI: 1.1-1.3, p<0.001) (table 7.3). There was evidence for an interaction between age and sex in relation to the screening attendance (p=0.04, test of homogeneity). In men, the response rate steadily increased from 63.0% (95% CI: 58.0-68.0%) in the age group 30-39 to 78.9% (95% CI: 75.0-82.9%) in ≥60 years old, respectively, whilst among women there was less of an increase (figure 7.7).

Areas where average household size was larger (OR 2.1, 95% CI: 1.6-2.7, p<0.001), and areas where the screening took place on weekends (OR 1.4: 1.0-1.9, p=0.05) were associated with higher participation (table 7.3). There was weak evidence that people in Jola community participated more than those in Mandinka community (OR 1.6, 95% CI: 1.0-2.5). Although the rural population had higher attendance than in urban population in the crude analysis (79.1%, 95% CI: 75.1-83.1% *versus* 66.3%, 61.7-71.0%), the association disappeared in the multivariable analysis because the rural population was older than the urban population (mean age: 46.7 ± 14.3 *versus* 44.1 ± 12.6 years, p=0.0001).

Figure 7.6 Flow chart of study participants in WATCH study and HC4 study



There are three case-control comparisons. First is between HBV-related HCC cases ≥30 years old recruited from the Western Gambia (n=51) and HBsAg-positive people from the community-based screening (n=392). Second is between all HBV-related HCC cases (n=72) and HBsAg-positive non-HCC patients ≥20 years old (n=63). Third is between HBsAg-negative HCC cases ≥30 years old recruited from the Western Gambia (n=43) and HBsAg-negative people from the community-based screening (n=301). Parenthesis presents the number of subjects who could report the number of full siblings/the number of half siblings.

Table 7.3 Attendance at screening, Western Gambia, 2011-2014

| | | Total eligible (8,170) | Percent attended | p-value | Odds ratio | | |
|---|---|---|---|---|---|---|---|
| | | | | | OR | 95%CI | p-value |
| 1. Individual-level variables[2] | | | | | | | |
| Sex | Male | 3,523 | 60% | <0.0001 | 1.0 | | <0.001 |
| | Female | 4,646 | 76% | | 2.1 | 1.8-2.4 | |
| Age group | 30-39 | 3,259 | 72% | <0.0001[1] | 1.0 | | <0.001[1] |
| | 40-49 | 1,822 | 77% | | 1.3 | 1.1-1.5 | |
| | 50-59 | 1,211 | 78% | | 1.4 | 1.2-1.7 | |
| | ≥60 | 1,347 | 81% | | 1.7 | 1.5-2.0 | |
| 2. Area-level variables | | | | | | | |
| 2-1. Community factors[3] | | | | | | | |
| Area | Urban | 3,785 | 66% | 0.0001 | 1.0 | | 0.9 |
| | Rural | 4,385 | 79% | | 1.0 | 0.7-1.4 | |
| Average household size | <8.5 | 4,098 | 64% | <0.0001 | 1.0 | | <0.001 |
| | ≥8.5 | 4,072 | 79% | | 2.1 | 1.6-2.7 | |
| Major ethnic group | Mandinka | 3,169 | 71% | 0.05 | 1.0 | | 0.09 |
| | Jola | 1,789 | 81% | | 1.6 | 1.0-2.5 | |
| | Mixture | 3,212 | 66% | | 1.2 | 0.9-1.6 | |
| 2-2. Time-related factors[4] | | | | | | | |
| Time since project started | 1st year | 4,162 | 68% | 0.5 | 1.0 | | 0.5 |
| | 2nd year | 4,008 | 70% | | 1.1 | 0.8-1.5 | |
| Season | Dry | 6,095 | 68% | 0.5 | 1.0 | | 0.6 |
| | Rainy | 2,075 | 72% | | 1.1 | 0.8-1.6 | |
| 2.3. Logistic factors[5] | | | | | | | |
| Number of days for screening in the area | <4 days | 4,169 | 68% | 0.7 | 1.0 | | 0.6 |
| | ≥4 days | 4,001 | 70% | | 0.9 | 0.7-1.3 | |
| Number of days in weekend in the area | 0-1 day | 5,799 | 67% | 0.02 | 1.0 | | 0.05 |
| | 2 days | 2,391 | 74% | | 1.4 | 1.0-1.9 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Involvement of village health worker | No | 5,856 | 68% | 0.005 | 1.0 | | 0.6 |
| | Yes | 2,314 | 79% | | 1.2 | 0.7-2.2 | |

Point estimates (percentages and ORs) were weighted for stratification. p-values and 95% CIs accounted for survey design (cluster sampling and stratification).

[1] Test for trend

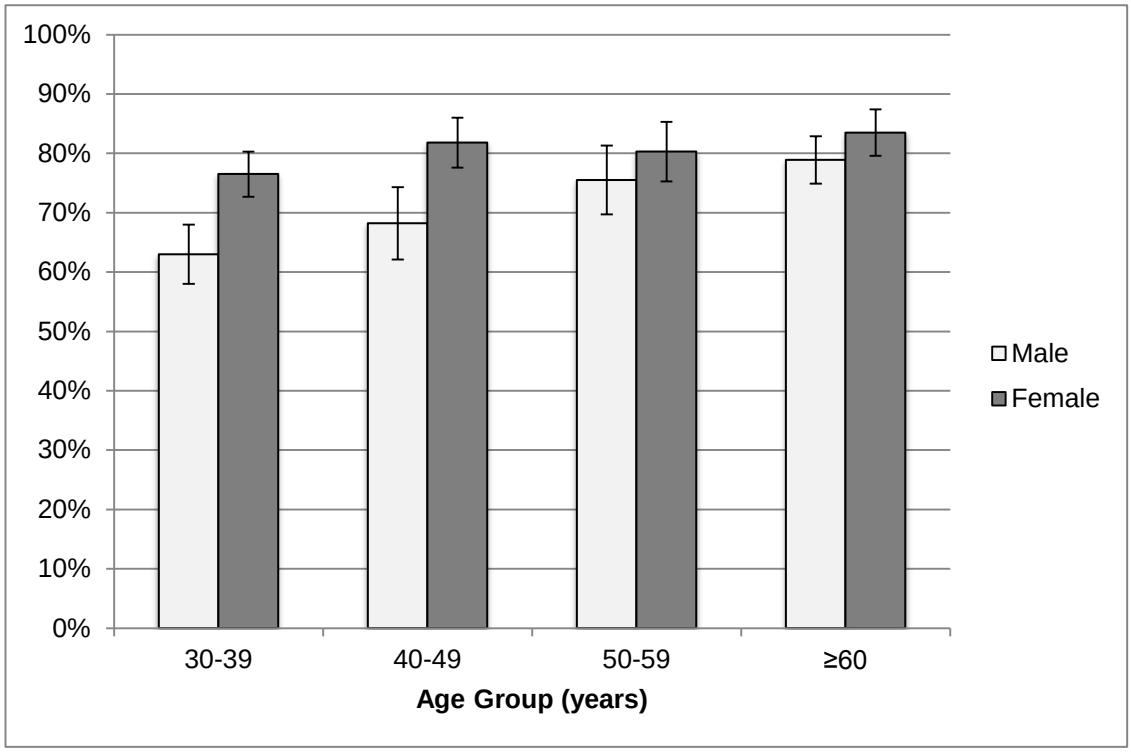
[2] Crude odds ratios are presented.

[3] OR from multivariable model which included sex, age, rural/urban area, household size and major ethnic group.

[4] OR from multivariable model which included rural/urban area, household size, major ethnic group, time since project started and season.

[5] OR from multivariable model which included rural/urban area, household size, major ethnic group, time since project started, season, number of days spent in the area, number of days in weekend spent in the area, and village health worker involvement.

Figure 7.7Attendance rate to the screening by age and sex



### 7.4.2. Reasons for non-attendance

A similar proportion of men could not attend due to absence (35.4%, 95% CI: 28.0-42.9%) or refusal (37.9%: 31.2-44.5%), but refusal (48.9%: 40.6-56.8%) was a more common reason for non attendance in women than absence (26.9%: 21.6-32.1%) (table 7.4). The most common reason for absence in men was work, and in women the most common reasons were travelling or social gathering. For both sexes, "no benefit" was the most common reason for refusal.

Table 7.4 Reasons for non-attendance by sex, Western Gambia, 2011-2014

| | Male (n=1,195) | | Female (n=994) | |
|---|---|---|---|---|
| | Number | Percentage adjusted for sample design | Number | Percentage adjusted for sample design |
| Absence | 440 | 35% | 274 | 27% |
| Work | 215 | 52% | 82 | 30% |
| Travelling/social gathering | 165 | 37% | 172 | 64% |
| Details unknown | 60 | 11% | 20 | 6% |
| Refusal | 443 | 38% | 480 | 49% |
| No benefit | 284 | 64% | 283 | 60% |
| Too busy | 35 | 10% | 30 | 8% |
| Feeling ill | 26 | 6% | 29 | 6% |
| Husband refused | N/A | N/A | 43 | 8% |
| Afraid of bleeding | 12 | 2% | 9 | 2% |
| No trust in MRC | 4 | 1% | 7 | 1% |
| Already tested before | 3 | 1% | 1 | 1% |
| Details unknown | 79 | 16% | 78 | 15% |
| Unknown | 312 | 27% | 240 | 24% |

7.4.3. HBsAg prevalence in the community-based screening

Of 5,980 people screened for HBsAg in the Western Gambia, 495 (8.8%, 95% CI: 7.9-9.7%) tested positive. The prevalence varied according to the EA from 1.9% (1/52) in Bundung (urban EA) to 18.2% (12/66) in Gunjur (rural EA). The prevalence in men (10.5%, 239/2328, 95% CI: 8.9-12.1%) was higher than that in women (7.6%, 256/3652, 95% CI: 6.5-8.7%, p=0.004) (table 7.5). In both sexes, the prevalence decreased with age: 16.1% (95% CI: 13.0-19.1%), 9.1% (6.1-12.1%), 6.4% (3.4-9.5%) and 4.0% (1.9-6.2%) in men and 9.5% (7.9-11.1%), 7.6% (5.5-9.7%), 3.9% (1.5-6.3%) and 4.4% (1.5-7.2%) in women, in the age group of 30-39, 40-49, 50-59 and ≥60 years old, respectively (figure 7.8). There was no evidence of an interaction between age and sex on HBsAg positivity (p=0.1). Figure 7.9 presents age- and sex-specific HBsAg prevalence derived from a model to which the age- and sex-specific HBsAg seroclearance rate and sex-specific mortality rate with end-stage liver disease in Keneba Manduar Cohort (Chapter 6) were applied. In women, except in the age group of 50-59 years, the reduction in prevalence is predicted by the model. In contrast, in men, the decay in HBsAg prevalence was much faster than the model prediction.

HBsAg prevalence also varied by ethnicity (low in Jola and Fula and high in minor ethnic groups (e.g. Manjago, Serere, or Balanta)) and birthplace (low in rural Gambia, high in foreigners and middle in urban Gambia).

There were positive associations between the number of full siblings and HBsAg (p=0.003), and between number of all siblings (full and half) and HBsAg (p=0.02). The prevalence of HBsAg increased with increasing birth order in full siblings: 6.7% (95% CI; 5.6-7.8%), 8.2%

(6.8-9.7%), 9.2% (6.8-11.7%), 9.6% (6.1-13.1%) and 12.0 % (8.3-15.7%) in 1$^{st}$, 2$^{nd}$, 3$^{rd}$, 4$^{th}$, and ≥5$^{th}$ birth order. The positive association remained after adjusting for age, ethnicity, birthplace and number of full siblings (OR per unit increase in birth order in full siblings: 1.1, 95% CI: 1.0-1.2, p=0.03). In contrast, there was no association when the number of siblings included both full- and half-siblings. HBsAg prevalence did not differ between urban and rural residence.

Table 7.5 Prevalence of hepatitis B surface antigen (HBsAg), Western Gambia, 2011-2014

| | | Total screened (5,980) | % HBsAg positive | p-value | Odds ratio | | |
|---|---|---|---|---|---|---|---|
| | | | | | OR | 95%CI | p-value |
| Sex[2] | Male | 2,328 | 10.5 | 0.004 | 1.0 | | 0.004 |
| | Female | 3,652 | 7.6 | | 0.7 | 0.6-0.9 | |
| Age group[3] | 30-39 | 2,397 | 11.9 | <0.0001[1] | 1.0 | | <0.001[1] |
| | 40-49 | 1,456 | 8.2 | | 0.7 | 0.5-0.9 | |
| | 50-59 | 988 | 5.1 | | 0.4 | 0.3-0.6 | |
| | ≥60 | 1,127 | 4.2 | | 0.3 | 0.2-0.5 | |
| Ethnicity[3] | Mandinka | 2,455 | 8.5 | 0.2 | 1.0 | | 0.04 |
| | Jola | 1,397 | 7.6 | | 0.9 | 0.6-1.3 | |
| | Fula | 763 | 7.7 | | 0.9 | 0.5-1.4 | |
| | Wolof | 469 | 9.7 | | 1.2 | 0.8-1.7 | |
| | Others | 891 | 11.3 | | 1.4 | 1.0-1.8 | |
| Birth place[3] | Urban Gambia | 1,250 | 10.0 | 0.006 | 1.0 | | 0.03 |
| | Rural Gambia | 4,037 | 7.5 | | 0.8 | 0.6-1.0 | |
| | Foreigners | 686 | 11.6 | | 1.3 | 0.9-1.7 | |
| | Senegal | 427 | 11.4 | | | | |
| | Bissau | 97 | 14.6 | | | | |
| | Conakry | 96 | 6.8 | | | | |
| | Others | 68 | 16.2 | | | | |
| Number of full siblings[3] | 1-2 | 886 | 5.9 | <0.001[1] | 1.0 | | 0.003[1] |
| | 3-4 | 1,333 | 8.3 | | 1.5 | 1.0-2.3 | |
| | 5-6 | 1,588 | 8.4 | | 1.4 | 1.0-2.0 | |
| | 7-8 | 1,223 | 10.0 | | 1.6 | 1.1-2.4 | |
| | ≥9 | 858 | 11.5 | | 1.9 | 1.2-2.9 | |
| Number of siblings | 0-5 | 1,022 | 5.3 | 0.002[1] | 1.0 | | 0.02[1] |
| | 6-8 | 1,508 | 9.0 | | 1.6 | 1.0-2.7 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| (full + half)[4] | 9-11 | 1,393 | 8.6 | | 1.5 | 1.0-2.4 | |
| | 12-14 | 866 | 10.4 | | 1.8 | 1.2-2.9 | |
| | ≥15 | 925 | 11.0 | | 1.8 | 1.2-2.9 | |
| Birth order in full siblings[5] | 1st | 1,935 | 6.7 | 0.001[1] | 1.0 | | 0.03[1] |
| | 2nd | 1,315 | 8.2 | | 1.2 | 0.9-1.6 | |
| | 3rd | 951 | 9.2 | | 1.2 | 0.9-1.8 | |
| | 4th | 630 | 9.6 | | 1.3 | 0.9-2.1 | |
| | ≥5th | 1,048 | 12.0 | | 1.6 | 1.0-2.5 | |
| Birth order in siblings (full + half)[6] | 1st | 1,136 | 7.3 | 0.04[1] | 1.0 | | 0.4[1] |
| | 2nd | 965 | 7.8 | | 1.1 | 0.8-1.5 | |
| | 3rd & 4th | 1,477 | 8.4 | | 1.1 | 0.8-1.5 | |
| | 5th & 6th | 930 | 9.8 | | 1.2 | 0.8-2.0 | |
| | ≥7th | 1,205 | 10.5 | | 1.2 | 0.7-1.8 | |
| Current residence[7] | Urban | 2,511 | 8.9 | 0.4 | 1.0 | | 0.5 |
| | Rural | 3,469 | 8.2 | | 1.1 | 0.9-1.3 | |

Point estimates (percentages and ORs) were weighted for stratification and non-attendance to the screening. p-values and 95% CIs accounted for survey design (cluster sampling and stratification) and non-attendance to the screening.

[1] Test for trend

[2] Crude odds ratio was presented.

[3] OR from multivariable model which included age, ethnicity, birthplace and number of full siblings.

[4] OR from multivariable model which included age, ethnicity, birthplace and number of siblings (full + half).

[5] OR from multivariable model which included age, ethnicity, birthplace, number of full siblings and birth order in full siblings.

[6] OR from multivariable model which included age, ethnicity, birthplace, number of siblings (full + half) and birth order in all siblings (full + half).

[7] OR from multivariable model which included sex, age, ethnicity, birthplace, number of full siblings, birth order in full siblings and current residence.

Figure 7.8 Prevalence of positive-HBsAg by age and sex

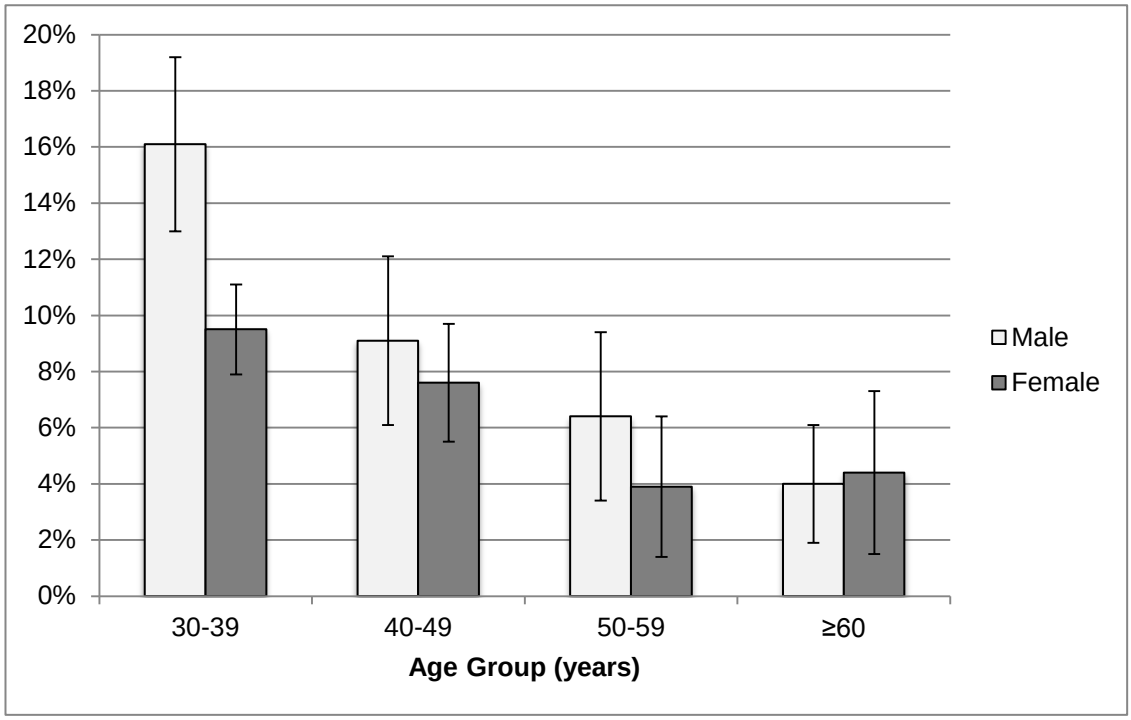
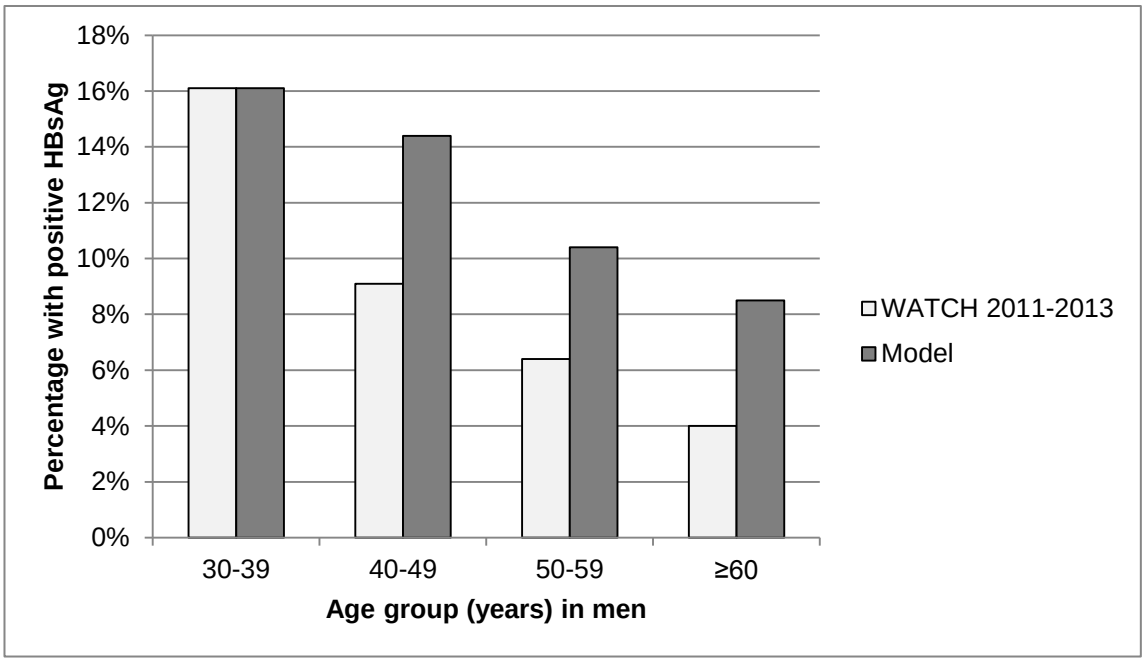
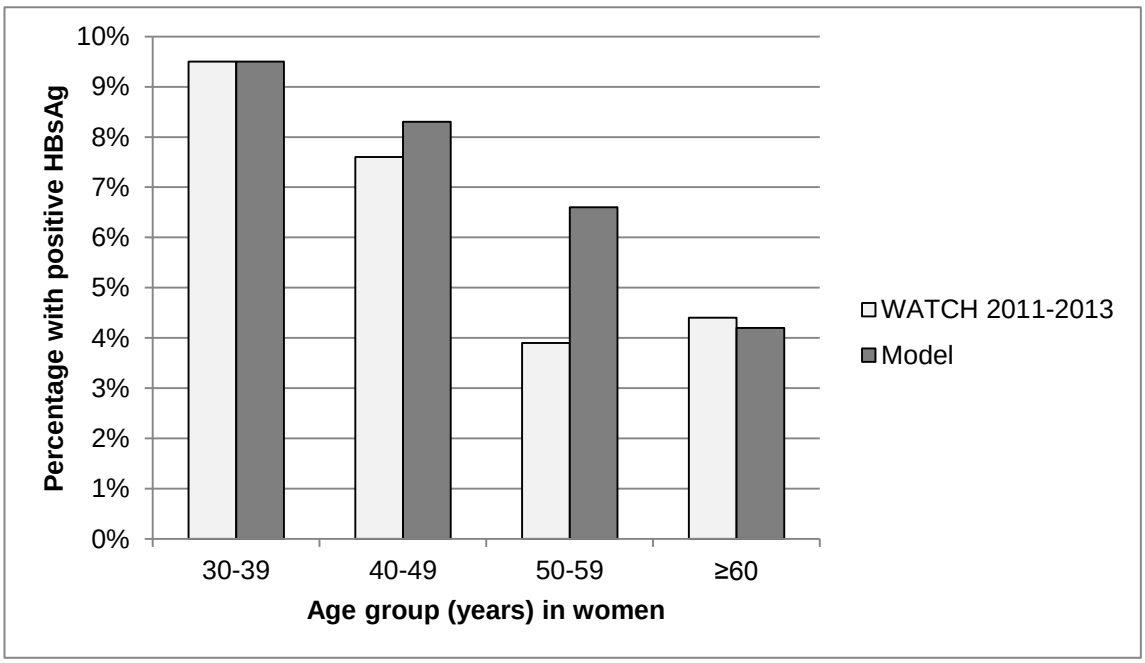


Figure 7.9 Age- and sex-specific prevalence of positive-HBsAg in the WATCH study and from a model applying the age- and sex-specific HBsAg seroclearance rate and sex-specific mortality rate with end-stage liver disease in Keneba Manduar Cohort (Chapter 6) to the HBsAg prevalence in previous age group



### 7.4.4. Proportion previously tested for HBsAg

From August to November 2013, as part of an ancillary study, residents in six enumeration areas (2 urban and 4 rural) were asked if they had previously been tested for HBV. Of 489 individuals questioned, 2 (0.4%, 0.0-1.6%) men aged 37 and 41 years reported that they had been tested before for HBV infection. Both men were negative for HBsAg on both occasions.

### 7.4.5. Attendance at liver clinic

Of 495 HBsAg-positive people, 392 (78.3%, 95% CI: 72.1-84.5%) visited the liver clinic. Participation was highest in the age group 50-59 years in both sexes although this was not statistically significant (table 7.6 and figure 7.10). None of the other factors examined were associated with clinic attendance.

Table 7.6 Attendance at the liver clinic at the MRC, Western Gambia, 2011-2014

| | | Total HBsAg positive (495) | Percent attended | p-value | Adjusted odds ratio | | |
|---|---|---|---|---|---|---|---|
| | | | | | OR | 95%CI | p-value |
| 1. Individual-level variables | | | | | | | |
| Sex[2] | Male | 239 | 80% | 0.6 | 1.0 | | 0.8 |
| | Female | 256 | 77% | | 0.9 | 0.5-1.7 | |
| Age group[2] | 30-39 | 284 | 77% | 0.8[1] | 1.0 | | 0.2[1] |
| | 40-49 | 117 | 78% | | 1.2 | 0.6-2.3 | |
| | 50-59 | 47 | 92% | | 3.7 | 0.9-15 | |
| | ≥60 | 45 | 78% | | 1.0 | 0.4-2.6 | |
| Ethnicity[3] | Mandinka | 207 | 82% | 0.4 | 1.0 | | 0.6 |
| | Jola | 100 | 83% | | 0.9 | 0.4-2.4 | |
| | Others | 187 | 75% | | 0.7 | 0.4-1.4 | |
| Birth place[2] | Urban Gambia | 120 | 79% | 0.1 | 1.0 | | 0.2 |
| | Rural Gambia | 308 | 82% | | 1.2 | 0.5-2.8 | |
| | Foreigners | 66 | 68% | | 0.6 | 0.2-1.3 | |
| Number of full siblings[2] | 1-2 | 55 | 81% | 0.9[1] | 1.0 | | 0.6[1] |
| | 3-4 | 103 | 71% | | 0.5 | 0.2-1.4 | |
| | 5-6 | 124 | 87% | | 1.3 | 0.5-3.4 | |
| | 7-8 | 115 | 76% | | 0.7 | 0.3-1.9 | |
| | ≥9 | 88 | 78% | | 0.9 | 0.3-2.6 | |
| Number of siblings (full + half)[3] | 0-5 | 60 | 82% | 0.5[1] | 1.0 | | 0.5[1] |
| | 6-8 | 121 | 74% | | 0.6 | 0.2-1.6 | |
| | 9-11 | 114 | 78% | | 0.8 | 0.3-2.2 | |
| | 12-14 | 83 | 86% | | 1.4 | 0.4-4.9 | |
| | ≥15 | 96 | 78% | | 0.9 | 0.3-2.4 | |
| Birth order in | 1st | 136 | 77% | 0.8[1] | 1.0 | | 0.5[1] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| full siblings[4] | 2nd | 105 | 78% | | 1.1 | 0.5-2.2 | |
| | 3rd | 77 | 81% | | 1.4 | 0.6-3.2 | |
| | 4th | 64 | 83% | | 1.5 | 0.4-5.4 | |
| | ≥5th | 103 | 77% | | 1.0 | 0.5-2.2 | |
| Birth order in siblings (full + half)[5] | 1st | 91 | 79% | 0.5[1] | 1.0 | | 0.6[1] |
| | 2nd | 70 | 78% | | 1.0 | 0.4-3.0 | |
| | 3rd & 4th | 120 | 79% | | 1.1 | 0.4-2.8 | |
| | 5th & 6th | 83 | 88% | | 1.9 | 0.7-5.6 | |
| | ≥7th | 110 | 72% | | 0.6 | 0.3-1.5 | |
| 2. Community factors[6] | | | | | | | |
| Current residence | Urban | 218 | 78% | 0.6 | 1.0 | | 0.9 |
| | Rural | 277 | 80% | | 1.0 | 0.5-1.8 | |
| 3. Time factors[7] | | | | | | | |
| Time since project started | 1st year | 250 | 80% | 0.5 | 1.0 | | 0.7 |
| | 2nd year | 245 | 77% | | 0.9 | 0.4-1.7 | |
| Season | Dry | 358 | 77% | 0.4 | 1.0 | | 0.5 |
| | Rainy | 137 | 83% | | 1.5 | 0.5-4.0 | |
| 4. Logistic factors[8] | | | | | | | |
| Staff experience (years) | <5 | 145 | 74% | 0.2[1] | 1.0 | | 0.1[1] |
| | 5-14 | 183 | 80% | | 1.5 | 0.9-2.4 | |
| | ≥15 | 167 | 82% | | 1.6 | 0.8-3.0 | |
| Involvement of village health worker | No | 347 | 78% | 0.2 | 1.0 | | 0.1 |
| | Yes | 148 | 85% | | 1.8 | 0.8-4.1 | |

Point estimates (percentages and ORs) were weighted for stratification and non-attendance to the screening. p-values and 95% CIs accounted for survey design (cluster sampling and stratification) and non-attendance to the screening.

[1] Test for trend

[2] OR from multivariable model which included sex, age, ethnic group, birthplace and number of full siblings.

[3] OR from multivariable model which included sex, age, ethnic group, birthplace and number of siblings (full + half).

[4] OR from multivariable model which included sex, age, ethnic group, birthplace, number of full siblings and birth order in full siblings..

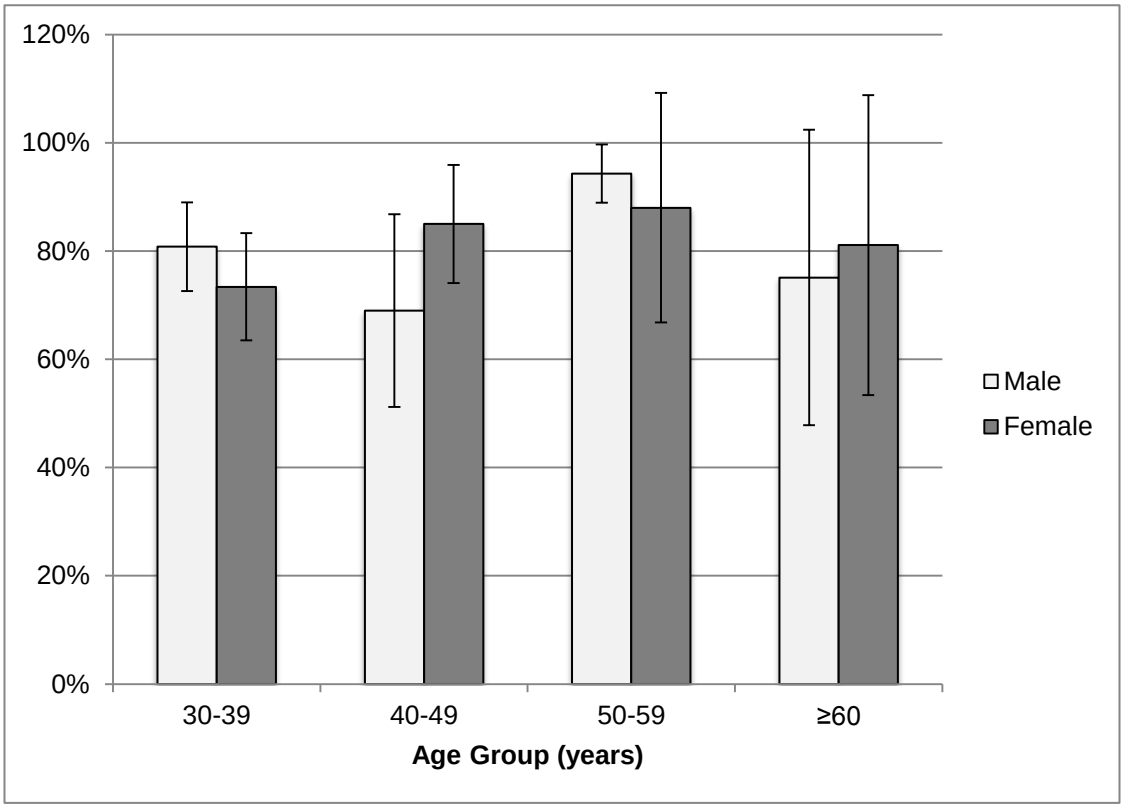
[5] OR from multivariable model which included sex, age, ethnic group, birthplace, number of siblings (full + half) and birth order in all siblings (full + half)..

[6] OR from multivariable model which included sex, age, ethnic group, birthplace, number of full siblings, birth order in full siblings and rural/urban area.

[7] OR from multivariable model which included rural/urban area, time since project started and season.

[8] OR from multivariable model which included rural/urban area, time since project started, season, experience of fieldworkers who performed a post-test counselling, and village health worker involvement.

Figure 7.10 Attendance rate to the liver clinic in HBsAg-positive people by age and sex



### 7.4.6. Characteristics of population-based HBsAg-positive controls

At the enrolment, none of the HBsAg-positive participants from the screening met the HCC criteria. Of 392 HBsAg-positive controls, seven did not report the number of siblings. The characteristics of 385 HBsAg-positive controls are presented by birth order (full-siblings) in table 7.7. The number of full-siblings was highly correlated with the birth order in the full-siblings ($p<0.0001$). The remaining characteristics did not differ by birth order.

Table 7.7 Characteristics of HBsAg-positive controls from the community-based screening by birth order in full siblings, Western Gambia, 2011-2014

| | Birth order in full siblings (n=385) | | | | | P |
|---|---|---|---|---|---|---|
| | 1st (105) | 2nd (83) | 3rd (64) | 4th (53) | 5th (80) | |
| Male sex | 52 (54%) | 37 (45%) | 36 (60%) | 24 (43%) | 33 (42%) | 0.3 |
| Median age (range) | 38 (30, 75) | 39 (30, 80) | 36 (30, 80) | 37 (30, 65) | 37 (30, 88) | 0.8[1] |
| Age group | | | | | | 0.7 |
| 30 – 39 | 58 (62%) | 44 (54%) | 39 (66%) | 32 (68%) | 47 (60%) | |
| 40 – 49 | 24 (21%) | 23 (25%) | 14 (14%) | 11 (17%) | 19 (25%) | |
| ≥50 | 23 (17%) | 16 (21%) | 11 (20%) | 10 (15%) | 14 (15%) | |
| Ethnic group | | | | | | 0.07 |
| Mandinka | 46 (49%) | 37 (37%) | 28 (41%) | 22 (25%) | 33 (38%) | |
| Jola | 32 (21%) | 15 (11%) | 12 (20%) | 14 (33%) | 12 (7%) | |
| Others | 27 (30%) | 31 (52%) | 24 (39%) | 17 (42%) | 35 (55%) | |
| Birth place | | | | | | 1.0 |
| Urban | 23 (37%) | 19 (35%) | 16 (41%) | 11 (39%) | 24 (43%) | |
| Rural | 70 (48%) | 52 (55%) | 40 (46%) | 37 (47%) | 50 (46%) | |
| Foreigners | 12 (15%) | 12 (10%) | 8 (13%) | 5 (14%) | 6 (11%) | |
| Parental schooling | | | | | | 0.1 |
| None | 44 (42%) | 35 (41%) | 27 (35%) | 29 (62%) | 38 (40%) | |
| One parent only | 27 (22%) | 13 (9%) | 15 (26%) | 12 (25%) | 17 (20%) | |
| Both parents | 34 (36%) | 35 (50%) | 22 (39%) | 12 (13%) | 25 (40%) | |
| Number of full siblings | | | | | | <0.0001 |
| 1-2 | 31 (29%) | 15 (20%) | 0 | 0 | 0 | |
| 3-4 | 26 (26%) | 17 (23%) | 21 (27%) | 13 (21%) | 0 | |
| 5-6 | 17 (17%) | 24 (27%) | 18 (34%) | 27 (59%) | 17 (23%) | |
| 7-8 | 17 (14%) | 20 (23%) | 18 (31%) | 3 (2%) | 32 (39%) | |

| | | | | | | |
|---|---|---|---|---|---|---|
| ≥9 | 14 (14%) | 7 (7%) | 7 (8%) | 10 (18%) | 31 (38%) | |
| Current address | | | | | | 0.3 |
| Urban | 38 (78%) | 36 (82%) | 30 (86%) | 21 (81%) | 40 (87%) | |
| Rural | 67 (22%) | 47 (18%) | 34 (14%) | 32 (19%) | 40 (13%) | |
| Education level | | | | | | 0.6 |
| None | 29 (24%) | 22 (25%) | 10 (14%) | 15 (29%) | 21 (23%) | |
| Koranic only | 21 (18%) | 25 (29%) | 20 (30%) | 14 (21%) | 25 (28%) | |
| Primary school or above | 54 (58%) | 34 (46%) | 32 (56%) | 24 (50%) | 33 (50%) | |
| Family history of HCC | 5 (5%) | 5 (11%) | 1 (2%) | 0 | 5 (10%) | 0.2 |
| Ever drunk alcohol | 7 (13%) | 5 (7%) | 6 (16%) | 4 (8%) | 4 (4%) | 0.3 |
| Ever smoked cigarettes | 40 (36%) | 24 (24%) | 20 (29%) | 17 (34%) | 17 (23%) | 0.6 |
| Obesity (BMI≥30) | 4 (3%) | 8 (13%) | 4 (7%) | 5 (10%) | 11 (18%) | 0.09 |
| Abnormal ALT (≥40 IU/L) | 11 (15%) | 10 (15%) | 9 (13%) | 4 (2%) | 8 (12%) | 0.3 |
| HBeAg positive | 2 (1%) | 1 (3%) | 3 (5%) | 2 (1%) | 2 (3%) | 0.5 |
| High HBV DNA (>2,000 IU/ml) | 10 (6%) | 8 (20%) | 3 (8%) | 6 (15%) | 7 (12%) | 0.4 |
| HIV positive | 1 (0.4%) | 1 (0.7%) | 0 | 1 (0.7%) | 2 (0.9%) | 0.9 |
| HCV positive | 0 | 0 | 2 (1%) | 0 | 0 | 0.7 |
| HDV positive | 1 (7%) | 1 (2%) | 0 | 0 | 1 (1%) | 0.4 |
| Successful LSM[2] | | | | | | 0.3 |
| Failure | 0 | 3 (6%) | 1 (2%) | 2 (5%) | 1 (2%) | |
| Unreliable | 12 (11%) | 4 (2%) | 6 (11%) | 8 (7%) | 9 (13%) | |
| Successful | 93 (89%) | 75 (92%) | 56 (87%) | 42 (88%) | 69 (85%) | |

Point estimates (percentages) were weighted for stratification and non-attendance to the screening and clinic. p-values accounted for survey design (cluster sampling and stratification) and non-attendance to the screening and clinic.

[1] Kruskal-Wallis test

[2] Unreliable measurement defined as success rate of <60% and/or ratio of IQR and LSM exceeding 30%.

7.4.7. Factors associated with active chronic hepatitis B diseases

Four HBsAg-positive controls from the community screening (1.2%, 95% CI: 0.0-2.6%) were immunotolerant, four (0.3%: 0.0-0.6%) were HBeAg-positive CHB disease, two (0.5%: 0.0-1.3%) were HBeAg-positive unclassified, nine (3.4%: 0.8-5.9%) were HBeAg-negative CHB disease, 324 (81.8%: 75.1-88.5%) were inactive carriers and 49 (12.8%: 7.1-18.5%) were HBeAg-negative unclassified. Factors associated with active chronic hepatitis B disease (13/392, 3.7%, 95% CI: 1.2-6.2%) are presented in table 7.8. Younger age group, higher parental education levels, a family history of liver cancer, HBeAg positivity and higher viral load were all associated with active chronic hepatitis B disease. Active chronic hepatitis B disease was not associated with birth order based on full-siblings or all siblings (full + half) (table 7.9).

7.4.8. Factors associated with significant liver fibrosis or above

Of 392, transient elastography was unavailable for seven. The measurement with transient elastography was classified as a failure in seven individuals (2.7%, 95% CI: 0.7-4.8%) and 40 (9.5%: 5.2-13.7%) had unreliable measurements. The prevalence of significant liver fibrosis (LSM ≥7.5 kPa) was 13.2% (36/338, 95% CI: 6.5-19.8%). Male sex, higher parental education levels, higher education levels of the participants, and positive anti-HDV were all associated with significant liver fibrosis (table 7.8). Fewer full siblings, positive HBeAg, higher viral load and early birth order were marginally ($0.05 < p < 0.1$) associated with significant liver fibrosis (table 7.8 and 7.9). The prevalence of significant liver fibrosis was 17.4% (95% CI: 2.5-32.3%), 16.6% (6.4-26.9%), 13.6% (0.6-26.6%), 9.5% (0.0-22.4%), and 7.8% (0.0-16.6%), in $1^{st}$, $2^{nd}$, $3^{rd}$, $4^{th}$, and $\geq 5^{th}$ birth order in full-siblings respectively ($p = 0.06$) (table 7.9). After adjusting for age, ethnicity, birthplace and number of full-siblings, there was no association between birth order in

full-siblings and significant liver fibrosis, whereas there was a significant association after adjusting for age, ethnicity, birth place and parental education levels ($p = 0.04$). Birth order was not associated with degree of liver fibrosis when defined using all siblings (full + half).

### 7.4.9. Factors associated with requiring antiviral treatment

The proportion of individuals who required antiviral treatment was 4.1% (16/392, 95% CI: 1.5-6.7%). This was more common for men, younger ages, higher parental education levels, family history of liver cancer, ever smoked cigarettes, positive HBeAg and high viral load (table 7.8). There was no association with birth order (table 7.9).

Table 7.8 Prevalence of active CHB disease, condition requiring the antiviral therapy and significant liver fibrosis or above in HBsAg-positive people from the community-based screening, Western Gambia, 2011-2014

| | | Total examined | | | | | Successful liver stiffness measurement[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total (392) | Active CHB disease | | Requiring antiviral therapy | | Total (338) | Significant liver fibrosis or above | |
| | | | % | P | % | P | | % | P |
| Sex | Male | 188 | 6 | 0.1 | 7 | 0.05 | 174 | 20 | 0.02 |
| | Female | 204 | 2 | | 1 | | 164 | 5 | |
| Age group | 30 – 39 | 221 | 6 | 0.03[1] | 6 | 0.02[1] | 194 | 17 | 0.2[1] |
| | 40 – 49 | 94 | 0 | | 1 | | 83 | 6 | |
| | ≥50 | 77 | 1 | | 1 | | 61 | 10 | |
| Ethnic group | Mandinka | 170 | 5 | 0.2 | 5 | 0.4 | 146 | 12 | 0.9 |
| | Jola | 86 | 1 | | 1 | | 75 | 13 | |
| | Others | 136 | 3 | | 4 | | 117 | 14 | |
| Birth place | Urban | 94 | 5 | 0.6 | 4 | 0.7 | 79 | 15 | 0.4 |
| | Rural | 254 | 2 | | 3 | | 220 | 10 | |
| | Foreigners | 44 | 6 | | 7 | | 39 | 20 | |
| Parental schooling | None | 177 | 1 | <0.001[1] | 1 | <0.001[1] | 144 | 8 | 0.04[1] |
| | One parent only | 86 | 1 | | 1 | | 78 | 15 | |
| | Both parents | 129 | 9 | | 10 | | 116 | 18 | |
| Number of full siblings | 1-2 | 46 | 7 | 0.5[1] | 6 | 0.7[1] | 40 | 24 | 0.06[1] |
| | 3-4 | 77 | 7 | | 7 | | 70 | 18 | |
| | 5-6 | 103 | 1 | | 2 | | 88 | 13 | |
| | 7-8 | 90 | 2 | | 3 | | 74 | 6 | |
| | ≥9 | 69 | 5 | | 5 | | 60 | 10 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Number of siblings (full + half) | 0-5 | 48 | 9 | 0.6[1] | 8 | 0.3[1] | 41 | 12 | 0.1[1] |
| | 6-8 | 91 | 2 | | 7 | | 78 | 24 | |
| | 9-11 | 91 | 4 | | 1 | | 78 | 9 | |
| | 12-14 | 70 | 4 | | 4 | | 61 | 5 | |
| | ≥15 | 78 | 3 | | 3 | | 67 | 12 | |
| Current address | Urban | 168 | 4 | 0.9 | 4 | 0.7 | 144 | 14 | 0.3 |
| | Rural | 224 | 4 | | 5 | | 194 | 10 | |
| Education level | None | 100 | 1 | 0.2[1] | 2 | 0.06[1] | 82 | 4 | 0.01[1] |
| | Koranic only | 107 | 5 | | 3 | | 97 | 6 | |
| | Primary school or above | 179 | 4 | | 6 | | 156 | 21 | |
| Family history of liver cancer | No | 376 | 3 | 0.006 | 3 | 0.008 | 323 | 13 | 0.7 |
| | Yes | 16 | 19 | | 19 | | 15 | 18 | |
| Ever drunk alcohol | No | 359 | 3 | 0.6 | 4 | 0.5 | 309 | 12 | 0.2 |
| | Yes | 28 | 7 | | 8 | | 27 | 21 | |
| Ever smoked cigarettes | No | 262 | 3 | 0.4 | 2 | 0.02 | 220 | 11 | 0.1 |
| | Yes | 125 | 5 | | 9 | | 116 | 19 | |
| Obesity (BMI≥30) | No | 352 | 4 | 0.1 | 5 | 0.3 | 316 | 14 | 0.3 |
| | Yes | 32 | 1 | | 0 | | 17 | 0 | |
| HBeAg | Negative | 286 | 3 | 0.03 | 4 | 0.0001 | 246 | 11 | 0.06 |
| | Positive | 10 | 16 | | 36 | | 7 | 44 | |
| HBV DNA (IU/ml) | Undetectable | 126 | 1 | <0.001[1] | 0 | <0.001[1] | 100 | 9 | 0.09[1] |
| | 50-200 | 71 | 0 | | 1 | | 67 | 8 | |
| | 200-2,000 | 82 | 0 | | 1 | | 74 | 10 | |
| | 2,000-100,000 | 22 | 17 | | 29 | | 18 | 30 | |
| | >100,000 | 13 | 61 | | 55 | | 11 | 26 | |
| HIV | Negative | 256 | 4 | 0.7 | 6 | 0.3 | 219 | 12 | 0.4 |
| | Positive | 5 | 0 | | 18 | | 3 | 28 | |
| HCV | Negative | 355 | 4 | 0.8 | 4 | 0.8 | 307 | 13 | 0.7 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Positive | 2 | 0 | | 0 | | 1 | 0 | |
| HDV | Negative | 151 | 8 | 0.7 | 9 | 0.7 | 126 | 8 | <0.001 |
| | Positive | 3 | 0 | | 0 | | 2 | 87 | |

Point estimates (percentages) were weighted for stratification and non-attendance to the screening and clinic. p-values accounted for survey design (cluster sampling and stratification) and non-attendance to the screening and clinic.

[1] Test for trend

[2] Excluding participants with failure or unreliable measurements with transient elastography.

Table 7.9 Birth order and predictors of HCC (active CHB disease, significant liver fibrosis or above, and condition requiring the antiviral therapy) in HBsAg-positive people from the community-based screening, Western Gambia, 2011-2014

| Variables | | Prevalence with outcome | Crude OR (95% CI) | P[1] | Model 1 (parental schooling as proxy for parental SES) | | Model 2 (total sibship size as proxy for parental SES) | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Adjusted OR (95% CI) | P[1] | Adjusted OR (95% CI) | P[1] |
| **1. Active CHB disease (n=13)** | | | | | | | | |
| Birth order in full siblings[3] | 1st | 3/105 (1%) | 1.0 | 1.0 | 1.0 | 0.8 | 1.0 | 0.3 |
| | 2nd | 4/83 (10%) | 8.9 (1.8-43.2) | | 13.6 (1.4-135.7) | | 24.3 (2.8-212.1) | |
| | 3rd | 1/64 (1%) | 0.3 (0.0-3.5) | | 0.3 (0.0-7.2) | | 0.1 (0.0-5.9) | |
| | 4th | 2/53 (1%) | 1.0 (0.1-6.4) | | 3.5 (0.2-51.5) | | 0.9 (0.1-16.2) | |
| | ≥5th | 2/80 (5%) | 4.0 (0.6-26.4) | | 6.5 (0.5-92.3) | | 18.8 (0.7-478.6) | |
| | Not reported | 7 | | | | | | |
| Birth order in siblings (full + | 1st | 2/70 (1%) | 1.0 | 0.4 | 1.0 | 0.5 | 1.0 | 0.3 |
| | 2nd | 2/56 (6%) | 4.9 (0.6-41.0) | | 8.6 (0.3-279.4) | | 28.8 (1.2-700.1) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| half)[4] | 3rd & 4th | 4/97 (4%) | 3.1 (0.4-24.5) | | 6.0 (0.2-206.9) | | 12.5 (0.4-367.2) | |
| | 5th & 6th | 0/71 (0%) | N/A | | N/A | | N/A | |
| | ≥7th | 4/84 (8%) | 6.7 (1.3-35.9) | | 8.3 (0.3-196.6) | | 49.7 (0.8-3229) | |
| | Not reported | 14 | | | | | | |
| **2. Significant liver fibrosis or above (n=36)**[2] | | | | | | | | |
| Birth order in full siblings[3] | 1st | 11/92 (17%) | 1.0 | 0.06 | 1.0 | 0.04 | 1.0 | 0.4 |
| | 2nd | 7/74 (17%) | 0.9 (0.2-3.9) | | 1.0 (0.3-3.9) | | 1.2 (0.3-4.6) | |
| | 3rd | 7/56 (14%) | 0.7 (0.2-2.5) | | 0.7 (0.2-2.5) | | 1.0 (0.3-3.9) | |
| | 4th | 6/42 (10%) | 0.5 (0.2-1.3) | | 0.5 (0.2-1.6) | | 0.5 (0.1-2.1) | |
| | ≥5th | 4/68 (8%) | 0.4 (0.1-1.5) | | 0.4 (0.1-1.4) | | 0.7 (0.2-2.6) | |
| | Not reported | 6 | | | | | | |
| Birth order in siblings (full + half)[4] | 1st | 7/61 (18%) | 1.0 | 0.2 | 1.0 | 0.1 | 1.0 | 0.3 |
| | 2nd | 6/51 (19%) | 1.1 (0.2-5.5) | | 1.2 (0.3-5.0) | | 1.3 (0.4-4.9) | |
| | 3rd & 4th | 9/80 (10%) | 0.5 (0.1-2.0) | | 0.4 (0.1-1.8) | | 0.5 (0.1-1.7) | |
| | 5th & 6th | 7/59 (13%) | 0.7 (0.2-2.6) | | 0.8 (0.2-2.9) | | 0.8 (0.2-3.0) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ≥7th | 5/74 (8%) | 0.4 (0.1-2.4) | | 0.3 (0.1-1.9) | | 0.5 (0.1-3.6) | |
| | Not reported | 13 | | | | | | |
| **3. Requiring antiviral therapy (n=16)** | | | | | | | | |
| Birth order in full siblings[3] | 1st | 6/105 (4%) | 1.0 | 0.7 | 1.0 | 0.8 | 1.0 | 0.9 |
| | 2nd | 4/83 (9%) | 2.4 (0.4-14.0) | | 2.2 (0.2-27.1) | | 3.9 (0.4-35.8) | |
| | 3rd | 1/64 (1%) | 0.1 (0.0-1.1) | | 0.1 (0.0-1.1) | | 0.1 (0.0-1.8) | |
| | 4th | 2/53 (1%) | 0.3 (0.0-2.0) | | 0.6 (0.1-6.2) | | 0.4 (0.0-5.8) | |
| | ≥5th | 2/80 (5%) | 1.2 (0.2-8.9) | | 1.5 (0.1-20.1) | | 2.3 (0.2-34.5) | |
| | Not reported | 7 | | | | | | |
| Birth order in siblings (full + half)[4] | 1st | 4/70 (2%) | 1.0 | 0.3 | 1.0 | 0.4 | 1.0 | 0.1 |
| | 2nd | 2/56 (4%) | 1.8 (0.2-16.4) | | 2.2 (0.1-39.0) | | 4.2 (0.3-58.4) | |
| | 3rd & 4th | 3/97 (3%) | 1.5 (0.2-11.7) | | 1.7 (0.1-25.5) | | 2.6 (0.2-33.4) | |
| | 5th & 6th | 3/71 (3%) | 1.5 (0.2-12.1) | | 3.2 (0.2-57.7) | | 5.4 (0.3-93.6) | |
| | ≥7th | 3/84 (7%) | 3.2 (0.6-16.5) | | 3.0 (0.3-25.7) | | 13.9 (0.8-243.0) | |
| | Not reported | 14 | | | | | | |

Point estimates (percentages and ORs) were weighted for stratification and non-attendance to the screening and clinic. p-values and 95% CIs accounted for survey design (cluster sampling and stratification) and non-attendance to the screening and clinic.

[1] Test for trend

[2] Excluding participants with failure or unreliable measurements with transient elastography.

[3] OR from multivariable model which included age, ethnicity, birthplace, birth order in full siblings and parental education (model 1) or number of full siblings (model 2).

[4] OR from multivariable model which included age, ethnicity, birthplace, birth order in siblings (full + half) and parental education (model 1) or number of siblings (full + half) (model 2).

7.4.10. Characteristics of HBV-related HCC cases and hospital-based HBsAg-positive controls

Two-hundred forty-two patients were enrolled in the HC4 study from June 2012 to November 2013 (figure 7.6). There were 72 HBsAg-positive HCC cases. The basis of diagnosis was histopathological confirmation (n=16), clinically confirmed (17) and clinically suspected (39). There was no imbalance between the groups in either the distribution of birth order or the distribution of potential confounders (confounders of the association of birth order with HCC) (table 7.2). The three groups were therefore pooled for the subsequent analyses. The first case-control analysis which compared HCC cases with HBsAg-positive population-based controls from the community-screening excluded two cases who were recruited outside the Western Gambia (one in Farafenni and one in Bansang Hospital) and 19 who were <30 years old at recruitment.

Of 119 patients who did not have HCC, 63 were HBsAg-positive and aged ≥20 years, and were used as hospital-based controls (figure 7.6). Their final diagnoses were: liver cirrhosis (39, 61.9%), chronic HBsAg carriers without HCC and cirrhosis (21, 33.3%) and other malignancy (3, 4.8%: one metastatic liver cancer, one gastric cancer and one lymphoma, all were clinically diagnosed using ultrasound and/or CT scan).

7.4.11. HCC cases compared to population-based controls

In the first-case control analysis male sex, older age group, ethnic groups other than Mandinka and Jola, rural born, lower parental education levels, lower education levels of the study participants, positive HBeAg, high HBV DNA levels, positive HIV, positive HCV and lower birth order in full-siblings to be significantly associated with HCC (table 7.10 and 7.11). Odds

ratios decreased with increasing birth order in full-siblings in the crude analysis: 1.00 (reference), 0.79 (95% CI: 0.36-1.74), 0.65 (0.27-1.58), 0.62 (0.23-1.72) and 0.23 (0.07-0.74) in $1^{st}$, $2^{nd}$, $3^{rd}$, $4^{th}$, and $\geq 5^{th}$ birth order, respectively ($p = 0.009$). After taking account of age, sex, ethnic group, birthplace and parental schooling (model 1), there was good evidence for an inverse association between birth order (full-siblings) and HCC (OR per unit increase in birth order: 0.72. 95% CI: 0.55- 0.95). There was also an inverse association based on the model that used the number of full-siblings as a proxy for SES (OR per unit increase in birth order: 0.70, 95% CI: 0.53-0.93). Although there was a similar inverse association when birth order was defined to include full and half siblings, this did not reach statistical significance.

7.4.12. HCC cases compared to hospital-based controls

In the analysis that used HBsAg-positive hospital-based controls older age group, rural born, lower education levels of the study participants, no family history of liver cancer, positive HBeAg and high viral load were all significantly associated with HBV-related HCC cases (table 7.10). There was a non-significant inverse association between birth order (full-siblings) and HCC in the crude analysis (table 7.11). After adjusting for confounding factors, there was a statistically significant association between birth order (full-siblings) and HCC: the OR per unit increase in birth order was 0.77 (95% CI: 0.59-1.00) in model 1 and 0.73 (95% CI: 0.54-0.98) in model 2.

Table 7.10 A comparison of HBV-related hepatocellular carcinoma cases with HBsAg-positive population controls and HBsAg-positive hospital controls

| | | First case-control comparison | | | | | | Second case-control comparison | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HBsAg(+) HCC cases (N=51)[1] | | HBsAg(+) population controls (N=392)[2] | | Crude OR (95% CI) | P[3] | HBsAg(+) HCC cases (N=72) | | HBsAg(+) hospital controls (N=63)[4] | | Crude OR (95% CI) | P[5] |
| | | No. | % | No. | % | | | No. | % | No. | % | | |
| Sex | Male | 40 | 78 | 188 | 50 | 1.0 | 0.0002 | 54 | 76 | 50 | 81 | 1.0 | 0.6 |
| | Female | 11 | 22 | 204 | 50 | 0.3 (0.1-0.6) | | 17 | 24 | 12 | 19 | 1.3 (0.6-3.0) | |
| Median age (range) | | 43 (30, 78) | | 38 (30, 88) | | | 0.005[5] | 40 (21, 78) | | 35 (20, 84) | | | 0.1[5] |
| Age group | 20 – 29 | 0 | 0 | 0 | 0 | | 0.004[6] | 18 | 25 | 24 | 39 | 1.0 | 0.04[6] |
| | 30 – 39 | 16 | 31 | 221 | 61 | 1.0 | | 17 | 24 | 16 | 26 | 1.4 (0.6-3.5) | |
| | 40 – 49 | 22 | 43 | 94 | 21 | 3.9 (1.9-8.2) | | 23 | 33 | 12 | 19 | 2.6 (1.0-6.5) | |
| | 50 – 59 | 8 | 16 | 40 | 10 | 3.0 (1.1-7.9) | | 8 | 11 | 6 | 10 | 1.8 (0.5-6.0) | |
| | ≥60 | 5 | 10 | 37 | 8 | 2.5 (0.8-7.6) | | 5 | 7 | 4 | 6 | 1.7 (0.4-7.1) | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ethnic group | Mandinka | 11 | 23 | 170 | 40 | 1.0 | 0.006 | 17 | 25 | 15 | 26 | 1.0 | 0.3 |
| | Jola | 4 | 8 | 86 | 17 | 0.9 (0.2-3.0) | | 5 | 7 | 9 | 15 | 0.5 (0.1-1.8) | |
| | Others | 34 | 69 | 136 | 44 | 2.8 (1.3-6.2) | | 47 | 68 | 35 | 59 | 1.2 (0.5-2.7) | |
| Birth place | Urban | 5 | 10 | 94 | 39 | 1.0 | 0.0003 | 8 | 12 | 20 | 34 | 1.0 | 0.01 |
| | Rural | 39 | 80 | 254 | 49 | 6.2 (2.3-16.8) | | 54 | 78 | 34 | 58 | 4.0 (1.6-10.0) | |
| | Foreigners | 5 | 10 | 44 | 12 | 3.1 (0.8-11.7) | | 7 | 10 | 5 | 8 | 3.5 (0.9-14.3) | |
| Parental schooling | None | 31 | 60 | 177 | 43 | 1.0 | 0.01[6] | 40 | 56 | 24 | 38 | 1.0 | 0.2[6] |
| | One parent only | 11 | 21 | 86 | 20 | 0.8 (0.4-1.6) | | 14 | 19 | 24 | 38 | 0.4 (0.2-0.8) | |
| | Both parents | 10 | 19 | 129 | 37 | 0.4 (0.2-0.8) | | 18 | 25 | 15 | 24 | 0.7 (0.3-1.7) | |
| Number of full siblings | 1-2 | 7 | 14 | 46 | 11 | 1.0 | 0.7[6] | 9 | 13 | 5 | 9 | 1.0 | 0.7[6] |
| | 3-4 | 11 | 23 | 77 | 19 | 0.9 (0.3-2.7) | | 14 | 21 | 14 | 24 | 0.6 (0.1-2.1) | |
| | 5-6 | 10 | 20 | 103 | 29 | 0.5 (0.2-1.6) | | 18 | 26 | 23 | 39 | 0.4 (0.1-1.5) | |
| | 7-8 | 12 | 25 | 90 | 23 | 0.8 (0.3-2.3) | | 16 | 24 | 8 | 14 | 1.1 (0.3-4.4) | |
| | ≥9 | 9 | 18 | 69 | 18 | 0.8 (0.3-2.4) | | 11 | 16 | 8 | 14 | 0.8 (0.2-3.2) | |
| Number of siblings (full + half) | 0-5 | 6 | 13 | 48 | 11 | 1.0 | 0.5[6] | 8 | 13 | 10 | 19 | 1.0 | 0.4[6] |
| | 6-8 | 16 | 35 | 91 | 25 | 1.2 (0.4-3.5) | | 19 | 30 | 15 | 28 | 1.6 (0.5-5.0) | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 9-11 | 8 | 17 | 91 | 23 | 0.7 (0.2-2.1) | | 15 | 24 | 13 | 25 | 1.4 (0.4-4.7) | |
| | 12-14 | 5 | 11 | 70 | 20 | 0.5 (0.1-1.7) | | 5 | 8 | 5 | 9 | 1.3 (0.3-5.9) | |
| | ≥15 | 11 | 24 | 78 | 21 | 1.0 (0.3-2.9) | | 16 | 25 | 10 | 19 | 2.0 (0.6-6.8) | |
| Current address | Urban | 39 | 80 | 168 | 83 | 1.0 | 0.7 | 53 | 77 | 49 | 83 | 1.0 | 0.4 |
| | Rural | 10 | 20 | 224 | 17 | 1.2 (0.5-3.2) | | 16 | 23 | 10 | 17 | 1.5 (0.6-3.6) | |
| Education level | None | 17 | 37 | 100 | 23 | 1.0 | 0.04[6] | 24 | 36 | 11 | 18 | 1.0 | 0.04[6] |
| | Koranic only | 12 | 26 | 107 | 25 | 0.7 (0.3-1.5) | | 16 | 24 | 18 | 30 | 0.4 (0.2-1.1) | |
| | Primary school or above | 17 | 37 | 179 | 52 | 0.5 (0.2-1.0) | | 26 | 40 | 31 | 52 | 0.4 (0.2-0.9) | |
| Family history of liver cancer | No | 51 | 100 | 376 | 94 | 1.0 | 0.5 | 72 | 100 | 58 | 92 | 1.0 | 0.02 |
| | Yes | 0 | 0 | 16 | 6 | N/A | | 0 | 0 | 5 | 8 | N/A | |
| Ever drunk alcohol | No | 45 | 96 | 359 | 90 | 1.0 | 0.2 | 64 | 97 | 54 | 92 | 1.0 | 0.2 |
| | Yes | 2 | 4 | 28 | 10 | 0.4 (0.1-1.7) | | 2 | 3 | 5 | 8 | 0.3 (0.1-1.8) | |
| Ever smoked cigarettes | No | 26 | 55 | 262 | 69 | 1.0 | 0.07 | 40 | 61 | 35 | 59 | 1.0 | 0.9 |
| | Yes | 21 | 45 | 125 | 31 | 1.8 (0.9-3.6) | | 26 | 39 | 24 | 41 | 0.9 (0.5-1.9) | |
| HBeAg | Negative | 18 | 60 | 286 | 97 | 1.0 | <0.001 | 20 | 50 | 22 | 79 | 1.0 | 0.02 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive | 12 | 40 | 10 | 3 | 25.8 (7.3-91.6) | | 20 | 50 | 6 | 21 | 3.7 (1.2-11.0) | |
| HBV DNA (IU/ml) | Undetectable | 2 | 5 | 126 | 43 | 1.0 | <0.001[6] | 2 | 4 | 7 | 23 | 1.0 | 0.03[6] |
| | 50-200 | 4 | 11 | 71 | 20 | 4.3 (0.8-24.6) | | 5 | 11 | 4 | 13 | 4.4 (0.6-33.9) | |
| | 200-2,000 | 7 | 20 | 82 | 26 | 5.8 (1.1-30.1) | | 8 | 17 | 6 | 19 | 4.7 (0.7-31.0) | |
| | 2,000-100,000 | 7 | 20 | 22 | 6 | 23.4 (4.0-137.1) | | 10 | 22 | 3 | 10 | 11.7 (1.5-89.1) | |
| | >100,000 | 15 | 43 | 13 | 5 | 60.7 (12.0-307.8) | | 21 | 46 | 11 | 35 | 6.7 (1.2-37.8) | |
| HIV | Negative | 26 | 93 | 256 | 99 | 1.0 | 0.0001 | 33 | 94 | 24 | 92 | 1.0 | 0.8 |
| | Positive | 2 | 7 | 5 | 1 | 13.9 (2.5-76.7) | | 2 | 6 | 2 | 8 | 0.7 (0.1-5.5) | |
| HCV | Negative | 46 | 98 | 355 | 99 | 1.0 | 0.007 | 64 | 98 | 51 | 93 | 1.0 | 0.1 |
| | Positive | 1 | 2 | 2 | 1 | 13.5 (1.2-156.2) | | 1 | 2 | 4 | 7 | 0.2 (0.0-1.8) | |
| HDV | Negative | 26 | 96 | 151 | 97 | 1.0 | 0.8 | 30 | 94 | 22 | 92 | 1.0 | 0.8 |
| | Positive | 1 | 4 | 3 | 3 | 1.4 (0.1-16.2) | | 2 | 6 | 2 | 8 | 0.7 (0.1-5.6) | |

P-value was obtained using chi-squared test unless indicated.

[1] Excluding patients recruited outside the Western Gambia (n=2) and <30 years old (n=19).

[2] Point estimates (percentages) were weighted for stratification and non-attendance to the screening and clinic.

[3] P-values accounted for survey design (cluster sampling and stratification) and non-attendance to the screening and clinic.

[4] Excluding patients <20 years old (n=6).

[5] Wilcoxon rank-sum test

[6] Test for trend

Table 7.11 Association between birth order and HCC in two case-control comparisons

| Variables | | HCC cases | Controls | Crude OR (95% CI) | P[1] | Model 1 (parental schooling as proxy for parental SES) | | Model 2 (total sibship size as proxy for parental SES) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Adjusted OR (95% CI) | P[1] | Adjusted OR (95% CI) | P[1] |
| **1. HBsAg(+) HCC cases vs. HBsAg(+) population controls[2]** | | | | | | | | | |
| Birth order in full siblings[3] | 1st | 18 (37%) | 105 (24%) | 1.0 | 0.009 | 1.0 | 0.02 | 1.0 | 0.01 |
| | 2nd | 12 (25%) | 83 (20%) | 0.8 (0.4-1.7) | | 0.6 (0.2-1.3) | | 0.5 (0.2-1.4) | |
| | 3rd | 9 (18%) | 64 (19%) | 0.7 (0.3-1.6) | | 0.6 (0.2-1.7) | | 0.5 (0.2-1.6) | |
| | 4th | 6 (12%) | 53 (13%) | 0.6 (0.2-1.7) | | 0.6 (0.2-1.9) | | 0.6 (0.2-2.1) | |
| | ≥5th | 4 (8%) | 80 (24%) | 0.2 (0.1-0.7) | | 0.2 (0.1-0.7) | | 0.1 (0.0-0.6) | |
| | Not reported | 2 | 7 | | | | | | |
| Birth order in siblings (full + half)[4] | 1st | 12 (25%) | 70 (16%) | 1.0 | 0.2 | 1.0 | 0.3 | 1.0 | 0.6 |
| | 2nd | 7 (14%) | 56 (15%) | 0.7 (0.2-1.8) | | 0.4 (0.1-1.4) | | 0.6 (0.1-2.4) | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3rd & 4th | 13 (27%) | 97 (25%) | 0.7 (0.3-1.6) | | 0.5 (0.2-1.4) | | 0.8 (0.2-2.9) | |
| | 5th & 6th | 8 (16%) | 71 (20%) | 0.5 (0.2-1.5) | | 0.3 (0.1-1.1) | | 0.5 (0.1-1.9) | |
| | ≥7th | 9 (18%) | 84 (24%) | 0.5 (0.2-1.3) | | 0.7 (0.2-2.1) | | 1.0 (0.3-3.4) | |
| | Not reported | 2 | 14 | | | | | | |
| **2. HBsAg(+) HCC cases vs. HBsAg(+) hospital controls** | | | | | | | | | |
| Birth order in full siblings[3] | 1st | 25 (37%) | 12 (21%) | 1.0 | 0.1 | 1.0 | 0.05 | 1.0 | 0.04 |
| | 2nd | 12 (18%) | 10 (17%) | 0.6 (0.2-1.7) | | 0.3 (0.1-1.0) | | 0.4 (0.1-1.3) | |
| | 3rd | 9 (13%) | 15 (26%) | 0.3 (0.1-0.8) | | 0.2 (0.1-0.7) | | 0.2 (0.1-0.7) | |
| | 4th | 11 (16%) | 8 (14%) | 0.7 (0.2-2.1) | | 0.6 (0.2-2.2) | | 0.7 (0.2-3.0) | |
| | ≥5th | 11 (16%) | 13 (22%) | 0.4 (0.1-1.2) | | 0.3 (0.1-0.9) | | 0.2 (0.1-0.9) | |
| | Not reported | 4 | 5 | | | | | | |
| Birth order in siblings (full + half)[4] | 1st | 15 (22%) | 5 (9%) | 1.0 | 0.1 | 1.0 | 0.2 | 1.0 | 0.1 |
| | 2nd | 10 (15%) | 7 (13%) | 0.5 (0.1-1.9) | | 0.3 (0.1-1.5) | | 0.3 (0.1-1.8) | |
| | 3rd & 4th | 13 (19%) | 15 (28%) | 0.3 (0.1-1.0) | | 0.3 (0.1-1.0) | | 0.2 (0.0-0.8) | |
| | 5th & 6th | 14 (21%) | 13 (24%) | 0.4 (0.1-1.3) | | 0.3 (0.1-1.2) | | 0.2 (0.1-1.2) | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ≥7th | 15 (22%) | 14 (26%) | 0.4 (0.1-1.2) | | 0.3 (0.1-1.4) | | 0.2 (0.0-1.0) | |
| | Not reported | 5 | 9 | | | | | | |

[1] Test for trend

[2] Point estimates (percentages and ORs) were weighted for stratification and non-attendance to the screening and clinic. P-values and 95% CIs accounted for survey design (cluster sampling and stratification) and non-attendance to the screening and clinic.

[3] OR from multivariable model which included age, ethnicity, birthplace, birth order in full siblings and parental education (model 1) or number of full siblings (model 2).

[4] OR from multivariable model which included age, ethnicity, birthplace, birth order in siblings (full + half) and parental education (model 1) or number of siblings (full + half) (model 2).

## 7.5. Discussion

7.5.1. Attendance at screening and liver clinic

The WATCH study is the first community-based screening programme for HBV infection in Africa, it aimed to identify chronic carriers who might benefit from antiviral therapy. The proportion who attended the screening was significantly higher in women than men. This is not surprising because women generally seek medical attention more frequently than men and are more likely to participate in screening programmes.[293–295] For example, among the African Americans uptake of colorectal cancer screening (endoscopy or faecal occult blood test) is higher in women than in men.[296,297] A qualitative study found: i) distrust in health care system, ii) fear of being seen by a doctor, and iii) the sexual connotation of having a colonoscopy, to be related with negative perceptions towards the colorectal cancer screening in African American men.[298] In PROLIFICA, extensive qualitative work has not been undertaken. Therefore, it is difficult to understand the reasons for non-attendance. Nevertheless, lower attendance in men, particularly young men, can be partly explained by work-related absence during the screening campaign. This was also reflected in the finding that areas being screened during weekends had higher attendance rate although there was no evidence for an interaction between sex and weekend screening in relation to the screening attendance ($p=0.4$, test of homogeneity).

The positive relationship between the areas with larger household size and higher uptake might be explained by better sharing of information in villages where people live in large traditional extended families. In this programme, information was disseminated by the alkalo (head of village, often from a founding family of the settlement), who invited inhabitants to a community sensitisation meeting. Although all members of the community were supposed to be invited to

the meeting, it was usually only village elders close to the alkalo and their family who attended. The alkalo and village elders themselves are also the head of large compound. This mode of communication might have favoured the areas with extended families. Moreover, a sense of collectivism within large family and within traditional communities might also have a favourable effect on screening participation, as demonstrated in a study of colorectal cancer screening in African Americans.[299]

The most common reason for non participation in the screening was "no benefit", which may be related with the lack of knowledge on HBV infection. A recent questionnaire survey in Nigeria found that only 10% of pregnant women attending an antenatal care at primary care facility had certain level of knowledge on HBV infection.[300] Despite a prevailing belief in The Gambia that MRC steals the blood of study participants and sells it in Europe,[301] only 1% said they did not trust the MRC. Attendance at the liver clinic in people notified to be infected with HBV was relatively good, and was similar in men and women and in different age groups. This suggests that pre- and post-test counselling at the screening site was effective.

There are two lessons to be learn from this study. First attendance can be improved by opening the screening site during weekend. Second, alternative methods of communication, such as using the radio, should be considered in settlements where family sizes are small.

### 7.5.2. HBsAg prevalence in the community-based screening

There was a clear trend of decreasing HBsAg prevalence with age, and the prevalence was lower in women. These findings are not unique to The Gambia.[2,302] Higher prevalence in men

can be explained by: i) higher risk of CHB infection in men than women despite similar risk of HBV exposure in both sexes,[62,303] and ii) lower rate of spontaneous HBsAg seroclearance in men[77,90] (Chapter 6). Possible explanations for the reduction with age are: i) spontaneous HBsAg loss over time, ii) higher mortality rate in people who continue to carry HBsAg, iii) low sensitivity of point-of-care test to identify chronic HBV carriers with low viral replication and/or low HBsAg levels, and iv) birth cohort effects. A birth cohort effect is unlikely, as the HBsAg prevalence in adults in The Gambia reported in previous studies is higher than the prevalence observed in this study (table 7.12). Nevertheless, most of the previous studies targeted people outside the Western Gambia or people recruited in hospitals, and thus care should be taken for this comparison.

As shown in Figure 7.9, spontaneous HBsAg loss and death related with CHB infection alone could not explain the reduction in HBsAg-positivity with increasing age, especially amongst men. Another explanation could be the low sensitivity of point-of-care tests (Determine) in people with low level of HBsAg or viral replication.[304] Older people are more likely to be inactive carrier state than in the immune tolerant phase (Chapter 6), and the former is associated with relatively low HBsAg levels and low HBV DNA levels.

It was not surprising to observe differences in HBsAg prevalence by ethnic group and birthplace.[305] For example, Keneba and Manduar, two adjacent villages situated 8km apart, have very different HBsAg prevalence in adults (9.9-13.9% *versus* 26.6-34.5%, respectively).[42,280] Although the exact mechanism of such a difference is unknown, it might be explained by the replication-infection cycle in a community;[160] the number of infectious inhabitants (especially

mothers and children) determines the average age at HBV infection in a community, and the latter defines the persistence of the infection in individuals who later become a source of the infection in the community. The cycle is maintained because there is limited mixing between villages and might explain the regional or ethnic variation in HBsAg prevalence in The Gambia.

Table 7.12 Prevalence of HBsAg in Gambian adults (aged 15 year or more) without hepatitis B vaccination in previous studies

| Author, year | Year of survey | Area | Target | No tested | % Anti-HBc(+) | Test used | % HBsAg(+) | Test used | % HBeAg(+) in HBsAg(+) people | Test used |
|---|---|---|---|---|---|---|---|---|---|---|
| **1. Keneba Manduar** | | | | | | | | | | |
| Whittle, 1983 | 1980 | Keneba | Mothers | 111 | N/R | | 11 (9.9%) | RPHA | 0 (0%) | RIA |
| | | Manduar | | 58 | | | 20 (34.5%) | | 4 (20.0%) | |
| Whittle, 1990 | 1984 | Keneba | 15-19 years old | 47 | N/R | | 8 (17.0%) | RPHA | 1 (12.5%) | EIA |
| | | Manduar | | 30 | | | 10 (33.3%) | | 5 (50.0%) | |
| Van der Sande, 2006 | 2003 | Keneba | ≥15 years old | 632 | N/R | | 88 (13.9%) | IC | 6 (6.8%) | EIA |
| | | Manduar | | 372 | | | 99 (26.6%) | | 9 (9.1%) | |
| | | Keneba & Manduar | 15-24 | 200 | | | 73 (36.5%) | | N/R | |
| | | | 25-34 | 207 | | | 50 (24.2%) | | | |
| | | | 35-49 | 146 | | | 22 (15.1%) | | | |
| | | | 50-69 | 194 | | | 33 (17.0%) | | | |
| | | | ≥70 | 115 | | | 9 (7.8%) | | | |
| **2. GHIS** | | | | | | | | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Vall Mayans, 1990 | 1988 | Villages (LRR) | Parents | 839 | 94.3% (mothers) 89.9% (fathers) | RIA | 115 (13.7%) | RPHA | 7/103 (6.8%) | RIA |
| Inskip, 1991 | 1986 | Essau (NBR) and Brikama (WR) | Fathers | 242 | 226 (93.4%) | RIA | 25 (10.3%) | RPHA | 2 (8.0%) | RIA |
| | | | Mothers | 290 | 250 (86.2%) | | 39 (13.4%) | | 4 (10.3%) | |
| Chotard, 1992 | 1986 | Essau (NBR), Brikama,(WR), Dankunku & Kudang (CRR), and Badjakunda & Yorobawal (URR) | Mothers | 1,000 | N/R | RIA | 131 (13%, overall) Ranging from 8% in Essau to 18% in Dankunku & Kudan | RPHA | 18 (13.7%, overall) | RIA |
| Van der Sande, 2007 | 2004 | N/R | 15 years old | 424 | 226 (53.3%) | EIA | 56 (13.2%) | IC | N/R | |
| Peto, 2014 | 2007-8 | Villages in CRR & URR | 17-22 years old | 475 | 267 (56.2%) | EIA | 59 (12.4%) | IC | 17/65 (26.2%) | EIA |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **3. Case-control study of HCC** | | | | | | | | | | |
| Ryder, 1992 | 1981-2 | Neighbourhood controls | 15-49 | 38 | N/R | | 9 (24%) | RPHA | 0 (0%) | RIA |
| | | | 50-72 | 29 | | | 4 (14%) | | 0 (0%) | |
| Kirk, 2004 | 1997-2001 | Hospital controls from RVTH, MRC and Bansang Hospital | Overall | 402 | N/R | | 64 (15.9%) | RPHA | 2 (3.1%) | RIA |
| | | | Men | 286 | | | 52 (18.2%) | | 2 (3.8%) | |
| | | | Women | 116 | | | 12 (10.3%) | | 0 (0%) | |
| | | | 17-29, male | 55 | | | 17 (30.9%) | | 0 (0%) | |
| | | | 30-39, male | 49 | | | 9 (18.4%) | | 2 (22.2%) | |
| | | | 40-49, male | 54 | | | 9 (16.7%) | | 0 (0%) | |
| | | | 50-59, male | 59 | | | 10 (17.0%) | | 0 (0%) | |
| | | | 60-80, male | 69 | | | 7 (10.1%) | | 0 (0%) | |
| | | | 19-29, female | 27 | | | 4 (14.8%) | | 0 (0%) | |
| | | | 30-39, female | 27 | | | 3 (11.1%) | | 0 (0%) | |
| | | | 40-49, female | 24 | | | 2 (8.3%) | | 0 (0%) | |
| | | | 50-59, female | 19 | | | 1 (5.3%) | | 0 (0%) | |
| | | | 60-75, female | 19 | | | 2 (10.5%) | | 0 (0%) | |

Abbreviations: GHIS, Gambia Hepatitis Intervention Study; RPHA, reverse passive haemagglutination; IC, immunochromatography; EIA, enzyme immunoassay; WR, Western Region; LRR, Lower River Region; NBR, North Bank Region; CRR, Central River Region; URR, Upper River Region; N/R, not reported.

7.5.3. Effect of birth order on HBsAg prevalence

The prevalence of HBsAg increased with increasing birth order when the latter was defined by the number of full-siblings. This suggests that birth order is acting as a proxy for number of infectious older siblings and reflects the probability of early horizontal transmission, because early age at HBV infection increases the risk of chronic HBV infection,[68,69] and in The Gambia infectious older siblings were the main source of HBV infection during childhood before the widespread use of HBV vaccines.[42,60,65] The association between birth order and HBsAg positivity was linear, and not J- or U-shape that would have been observed if the perinatal mother-to-infant transmission also played a substantial role. This supports two previous observations which suggested that mother-to-infant transmission might not be important in maintaining the prevalence of CHB infection in The Gambia: the prevalence of HBeAg among HBsAg-positive mothers in The Gambia (6.8-13.7%)[55,114] is lower than that in Asia (40%);[306] and in Keneba and Manduar the risk of the youngest child in each household being sero-positive for HBsAg was strongly associated with the number of HBsAg-positive older siblings after adjusting for maternal HBsAg status in the pre-vaccination era.[60] The latter is in contrast with a cohort study in Taiwan, which demonstrated that the association between having an HBeAg-positive mother and the incidence of postnatal HBV infection in the second and third year of life remained significant after adjusting for number of HBsAg-positive older siblings, whilst the association between the number of positive older siblings and the incidence of infection disappeared after controlling for maternal sero-status.[9]

When birth order was defined to include half-siblings, the association between birth order and HBsAg was not statistically significant. The Gambia is patrilineal and polygamous society.

Co-wives may live in the same compound, but usually in separate dwellings within the compound, and children sleep in the mother's room.[42] This implies that children spend more time with full-siblings sharing the biological mother than half-siblings, and thus birth order counting half-siblings might have been less accurate to predict the probability of early horizontal transmission than birth order in full-siblings. This is in line with a study in Ghana which revealed that the number of HBsAg-positive individuals in the same household, rather than within the large domestic compound, were the main source of HBV infection for children.[52]

### 7.5.4. Effect of birth order on HCC and its predictors

Both case-control comparisons found an inverse association between birth order in full-siblings and HBV-related HCC. The results are in agreement with the findings from other studies in this thesis that perinatal mother-to-infant transmission might increase the risk of HCC and its predictors beyond its effect of increasing the risk of CHB infection (Chapter4-6). The direction of the association was opposite to the one observed between birth order and HBsAg prevalence. This agrees with the results in the Keneba and Manduar cohort study (Chapter 6); while the proportion of chronic carriers attributable to the mother-to-infant transmission is low (13.9%), the proportion of high-risk chronic carriers (who require the antiviral therapy) due to this mode of transmission is high (71.4%). The risk of CHB infection, historically a common event in The Gambia, is largely determined by the age at infection during childhood as indicated by the linear positive association with birth order. Of course, the risk of CHB infection should be even higher with perinatal maternal transmission,[68,69] however, this mode is uncommon in The Gambia and therefore birth order mainly affects the probability of early horizontal transmission. In contrast,

the risk of HBV-related HCC, a rare event compared to CHB infection, is largely determined by the mode of transmission (perinatal maternal *versus* postnatal horizontal) rather than the timing of postnatal transmission (early *versus* late childhood) since the lowest risk of HBV-related HCC was observed in individuals with high birth order ($\geq 5^{th}$ birth order).

The inverse associations between birth order and HCC in both case-control comparisons did not show a clear dose response. First and $\geq 5^{th}$ birth order were associated with the highest and lowest risk of HCC respectively, however, the birth order between these (i.e., from $2^{nd}$ to $4^{th}$) had a similar risk. This could be explained by a maternal age at first birth and rate of spontaneous HBeAg loss in Gambian women. In The Gambia, average age at first birth is estimated at 21.4 years old (taken from a data in Senegal in 2010).[307] According to the observation in Keneba and Manduar Cohort (Chapter 6), about half of HBsAg-positive women still have HBeAg at the age of 13, a beginning of reproductive age in The Gambia. Then, there is a steep decrease in proportion of HBeAg-positive women until the age of 20, by that time only 30% of carrier women are also positive for HBeAg (Figure 7.11). The rate of HBeAg loss becomes slower after the age of 20, and after the age of 30, more than 90% of women had already cleared HBeAg. This may explain the limited change in HCC risk between $2^{nd}$ and $4^{th}$ birth order.

The results are not consistent with the previous case-control studies in Greece where a positive association between birth order and HBV-related HCC was demonstrated in people with CHB infection.[187,194] The discrepancy might be related to: i) a higher frequency of mother-to-infant transmission in The Gambia than in Greece, as suggested by higher HBeAg prevalence in

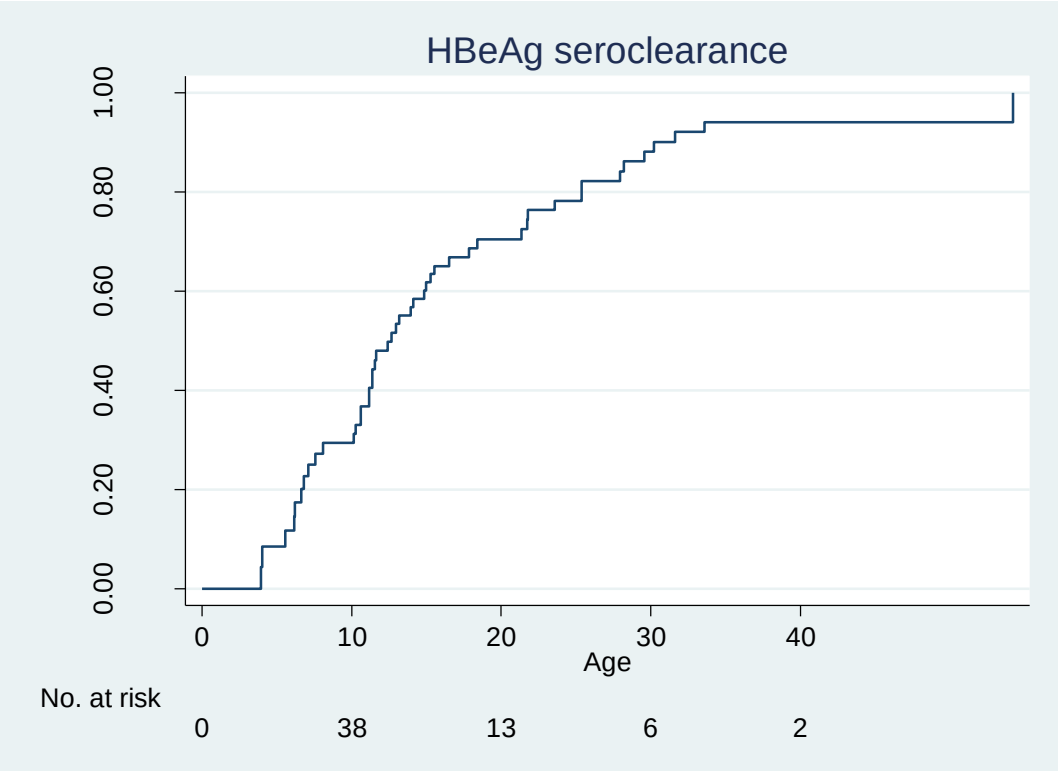
HBsAg-positive women of child-bearing age in The Gambia (6.8-13.7%)[55,114] than in Greece (2.7%);[270] ii) most of Greek mothers might have already lost HBeAg when they start having children (median age of first birth is 21.4 years in The Gambia and 31.2 years in Greece)[307] and iii) selection bias due to differential participation between cases and controls according to their birth order. The latter was virtually eliminated for the current hospital-based case-control comparison, as the participation rate for the study was 98.8%. In addition, although the participation was low in the population-based controls (68.9% attended the screening and 78.0% of screening positive came for the liver assessment), attendance to the liver clinic was independent of birth order (table 7.6) and non-attendance weights were applied to each of the participants in the analysis such that their birth order distribution would be representative of the general population.

Birth order was not associated with HCC when its definition included half siblings. For HCC risk, birth order acts as a proxy for maternal age at birth and therefore reflects the probability of perinatal maternal transmission. Including half-siblings in the definition of birth order is likely to have reduced the reliability of birth order as a proxy for maternal age.

Amongst the predictors of HCC, only significant liver fibrosis was positively related with full-sibling birth order. Birth order was not associated with active CHB diseases or antiviral therapy. This may have been because of a lack of statistical power.

The risk factors for HCC and its predictors identified in this study (male sex, older age, ethnic group, birthplace, SES, positive HBeAg, high HBV DNA levels, and co-infection with HIV) have previously been reported.[308]

Figure 7.11 Proportion of women with CHB infection in Keneba and Manduar Cohort who cleared HBeAg as a function of age



### 7.5.5. Study limitations

Birth order was self-reported and participants who answered "I don't remember" were excluded from the analysis. This might be a source of bias because those who cannot recall are more likely to be from larger families and have higher birth rank.[216] However, the numbers excluded were small for both cases and controls; 3.9% (2/51) and 1.8% (7/391) in the first case-control comparison using population-based controls and 5.6% (4/72) and 7.9% (5/63) in the second case-control comparison using hospital controls, respectively.

Maternal age at birth would be a closer proxy marker than birth order for the probability of perinatal maternal transmission. Indeed, an attempt was made to estimate the maternal age at birth by including a series of questions in the questionnaire (Appendix 7). Unfortunately maternal age at birth could only be estimated in 40.3% (158/392) of the population-based controls and in 28.1% (68/242) of hospital-based participants, because of recall error. Even in those who could answer the question, the accuracy of the information was questionable. Consequently, the effect of maternal age at birth was not evaluated.

Although most of variables associated with HCC in the analysis using population-based controls were also found to be associated with HCC in another analysis using hospital-based controls, there were few exceptions. For example, male sex was a risk factor in the former analysis but not in the latter. Concern related with the use of the community control is that not all of them may afford to come to a hospital when they develop HCC. Thus, it is unclear whether gender is a genuine risk factor for HCC or the factor influencing one's affordability to end up in a tertiary care hospital in The Gambia.

7.5.6. Public health implications

The study confirmed that the major route of HBV infection in The Gambia is a postnatal sibling-to-sibling transmission. Therefore, even delayed administration of the first dose of hepatitis B vaccine (i.e. 1-2 weeks after the birth) should reduce the prevalence of CHB infection in The Gambia, as has been shown in vaccine trials.[173,238] However, the study also implied that perinatal maternal transmission might be a risk factor for HCC. Consequently, the proportion of HBV-related HCC prevented by the delayed provision of the first vaccine would be smaller than the proportion of CHB infection prevented. In Taiwan, the reduction in incidence of paediatric HCC (64%) was not as dramatic as the reduction in the prevalence of CHB infection in children (93%) after the introduction of HBV vaccination programme in which the first dose was given within the first week of the birth.[309] Interruption of perinatal mother-to-infant transmission of HBV needs to be considered in The Gambia.

## 7.6. Summary

A comparison of birth order in HBsAg-positive and –negative people from the community-based screening demonstrated that higher birth order is associated with higher risk of HBsAg-positivity. This is consistent with previous data from The Gambia which suggest that postnatal sibling-to-sibling transmission is the major mode of HBV transmission and early age at infection through infectious older siblings is an important risk factor for chronic HBV infection. In this context, the role of perinatal maternal transmission seems to be small in The Gambia.

In contrast, there was a significant inverse association between birth order and HCC in people with chronic HBV infection. This was confirmed in two different case-control comparisons, which suggest that early birth order - a proxy for young maternal age and high viral replication in mothers - may increase the risk of HCC. Provision of birth dose of HBV vaccine within 24 hours of birth needs to be considered in sub-Saharan Africa where the perinatal mother-to-infant transmission might increase the risk of HCC.

# Chapter 8. Discussion

## 8.1. Key results

The hypothesis of this thesis is that early age at hepatitis B virus (HBV) infection increases the risk of hepatocellular carcinoma (HCC) and its predictors in The Gambia. In order to separate the effect of the early age at infection on the risk of chronic HBV (CHB) infection, which is already established,[68,69] the analyses have been restricted to HBsAg positive individuals. The systematic review of Chapter 3 showed that early age at infection may increases the sequelae of CHB infection, but it did not include any studies from Africa.

Data are presented from four studies in The Gambia, including two historical studies. The hepatitis B e antigeaemia study (Chapter 4) showed that having an hepatitis B e antigen (HBeAg)-positive mother was a risk factor for HBeAg positivity in children after adjusting for number of HBeAg-positive older siblings. In a historical sero-survey in Gambian children, the risk of the youngest child being sero-positive for hepatitis B surface antigen (HBsAg) was strongly associated with the number of HBsAg-positive older siblings after taking account for maternal HBsAg status.[60] Together these findings suggest that the chance of CHB infection is largely determined by sibling-to-sibling transmission during early childhood whilst the risk of persistent viral replication (hepatitis B e antigenaemia) in those who already established chronic carrier state might be influenced by perinatal maternal transmission.

In Chapter 6, a community-based open cohort in rural villages in The Gambia was used to assess the effect of maternal HBsAg on HCC and its predictors. Having an HBsAg-positive mother was significantly associated with delayed HBeAg sero-clearance, higher viral load and alanine transaminase (ALT) levels over time, active CHB disease, advanced liver fibrosis and requiring antiviral therapy. Three end-stage liver disease cases had mothers with known HBV sero-status, and in all of them the mother was HBsAg-positive. The proportion of chronic HBV carriers attributable to maternal transmission was only 14%, whilst 71% of carriers requiring antiviral therapy according to the international treatment guidelines were due to maternal transmission.

The association between birth order and HBV-related HCC was examined using data from a historical case-control study (Chapter 5) and data from the ongoing PROLIFICA project (Chapter 7). In both studies the risk of HCC was highest for first birth order, although the association was not statistically significant in the historic case control study. Data from the community-based screening in PROLIFICA suggested a positive association between birth order and HBsAg positivity in general population in the Western Gambia.

These findings are consistent with the hypothesis that early age at HBV infection through perinatal maternal transmission increases the risk of HCC and its predictors in people with CHB infection.

## 8.2. Replication-infection cycle

There were striking differences in the epidemiology of CHB infection between Asia and sub-Saharan Africa (sSA) in the pre-vaccination era, particularly with regard to the frequency of perinatal mother-to-infant transmission, the patterns of viral replication decay and the HCC incidence (Chapter 1). These differences probably reflect different "HBV replication-infection cycles".[160] In Asia, the persistence of viral replication is often observed beyond reproductive ages, leading to a higher prevalence of HBeAg-positive mothers. The infants born to these mothers have a markedly increased risk of developing chronic infection, and tend to suffer from a prolonged period of viral replication, which may lead to a higher incidence of cirrhosis and HCC. On the other hand, most African mothers with CHB infection do not have active viral replication, and transmission of HBV is therefore usually postponed until early childhood. Delayed age at HBV infection is associated with a lower chance of developing CHB infection, and may also reduce the duration of active viral replication. In this infection cycle loop, the critical time period for the epidemiology of CHB infection is the age at HBV infection (or the mode of transmission). This thesis provided the evidence to support this hypothesis at individual levels in The Gambia.

## 8.3. Limitations in the interpretation

There are several limitations related to the use of proxy measures for age at HBV infection (or mode of transmission). First, it is unclear whether the risk of HCC and its predictors differs between people who established the CHB infection during early childhood (1-3 years old) and those established during late childhood at school entry (6-8 years old). In the HBeAg study

(Chapter 4), risk of hepatitis B e antigenaemia in children still tended to increase with increasing number of HBeAg-positive older siblings after adjusting for maternal HBV status. Although the association was not statistically significant, this suggests that the timing of postnatal infection during childhood might affect the persistence of HBeAg. However, the results of birth order studies (Chapter 5 and 7) did not support this; no increment in HCC risk was observed in people with higher birth order (≥5), who should have higher chance of having had early postnatal transmission, compared to those with intermediate birth order (3 or 4).

Second, having an HBeAg-positive (and particularly HBsAg-positive) mother does not always indicate in sSA that the chronic infection was perinatally established. As discussed in Chapter 1, the risk of perinatal transmission from an HBeAg-positive mother is low in sSA;[10] accordingly without immunoprophylaxis the prevalence of HBsAg by the age of 9 months in children born to HBeAg-positive mother is 20-25% in sSA,[13,58,59] compared with >90% in Taiwan.[37,38] The incidence of HBV infection increases after 9 months in sSA, and being born to an HBsAg (or HBeAg) positive mother is an important risk factor for postnatal HBV infection.[13,17,61] Many chronic carriers with an HBsAg (or HBeAg)-positive mother in sSA might therefore have been infected postnatally rather than perinatally. The thesis could not determine whether the higher risk of HCC and its predictors is specifically associated with perinatal maternal transmission or generally associated with maternal transmission irrespective of the timing of the infection.

## 8.4. Public health implications

The results presented in this thesis suggest that the burden of HBV-related chronic liver disease in The Gambia might be reduced by interrupting maternal transmission. This can be achieved by providing the first dose of HBV vaccine as soon as after the birth.[116] There is also good evidence that the use of hepatitis B immunoglobulin (HBIG) as an adjunct to hepatitis B vaccine further reduces the HBV infection in children born to HBeAg-positive mothers.[112] However, the supply, safety and cost of HBIG prohibit its use in many areas including sSA.[116]

Despite the WHO recommendation of a timely birth dose within 24 hours,[116] only a few African countries provide monovalent HBV vaccine at birth.[117] It is important to explore the cost-effectiveness of a timely birth dose in light of the findings from this thesis since previous modelling studies[103,310] have assumed that the risk of HCC is not affected by age at infection once a CHB infection has become established. In addition, it needs to be determined what would be the most effective way to deliver the timely birth dose: universal timely birth dose or maternal HBV screening and subsequent selective birth dose to those born to infected mothers.

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# Appendix 1. Search strategy

(1) Electronic Searches

Following electronic databases were searched using the OvidSP search platform:

- MEDLINE (1946 to January (week 2) 2012);
- EMBASE (1974 to January (week 2) 2012);
- CNKI (1979 to September (week 2) 2012);
- SinoMed ( 1979 to September (week 2) 2012).

(1-1) MEDLINE

#1 explode “Hepatitis B”/ all subheadings or explode “Hepatitis B virus”/ all subheadings

#2 hepatitis b or hepatitis-b or (type b adj1 hepatitis) or hbv or hep b or hbsag or hbs-ag or hbs antigen* or hbs-antigen* or hepatitis b surface antigen* or hepatitis-b surface antigen*

#3 “Alanine Transaminase”/ all subheadings

#4 alanine transaminase* or alanine aminotransferase* or glutamic pyruvate transaminase* or ALT or ALAT or GPT or SGPT

#5 “Hepatitis B e Antigens”/ all subheadings

#6 hbeag or hbe-ag or hbe antigen* or hbe-antigen* or (hepatitis b adj3 e antigen*) or (hepatitis-b adj3 e antigen*) or ((type b adj1 hepatitis) adj3 e antigen*) or (hbv adj3 e antigen*)

#7 “DNA, viral”/ all subheadings or “Virus replication”/ all subheadings

#8 dna or replication* or viral titre or viral titer or viral load

#9 “Liver Cirrhosis”/ all subheadings

#10 cirrho* or ((liver or hepat*) adj3 fibro*)

#11 “Carcinoma, Hepatocellular”/ all subheadings

#12 ((liver or hepat*) adj3 (cancer* or carcinom* or neoplasm* or malign* or tumo?r*)) or hcc or hepatoma

#13 "Age Factors"/ all subheadings

#14 age adj5 infect*

#15 "Infectious Disease Transmission, Vertical"/ all subheadings

#16 perinatal or peri-natal or vertical or MTCT or mother-to-child or mother to child or mother-child or mother child or mother-to-infant or mother to infant or mother-infant or mother infant or maternal-to-child or maternal to child or maternal-child or maternal child or maternal-to-infant or maternal to infant or maternal-infant or maternal infant or adult-to-child or adult to child or adult-child or adult child or adult-to-infant or adult to infant or adult-infant or adult infant or maternal-f?etal or maternal f?etal or f?etomaternal

#17 postnatal or post-natal or horizontal or child-to-child or child to child or child-child or child child or sibling-to-sibling or sibling to sibling or

sibling-sibling or sibling sibling or between children or between siblings or between family or within household

#18 "Birth Order"/ all subheadings

#19 birth adj3 (order* or link*)

#20 (serolog* or seropositiv* or serostatus) adj5 (famil* or parent* or father* or paternal or mother* or maternal or sibling* or brother* or sister*)

#21 (hepatitis b or hepatitis-b or (type b adj1 hepatitis) or hbv or hep b or hbsag or hbs-ag or hbs antigen* or hbs-antigen* or hepatitis b surface antigen* or hepatitis-b surface antigen*) adj5 (famil* or parent* or father* or paternal or mother* or maternal or sibling* or brother* or sister*)

#22 #1 or #2

#23 #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12

#24 #13 or #14

#25 #15 or #16 or #17

#26 #18 or #19

#27 #20 or #21

#28 #22 and #23 and #24

#29 #22 and #23 and #25

#30 #22 and #23 and #26

#31 #22 and #23 and #27

#32 #28 or #29 or #30 or #31

(1-2) EMBASE

#1 “hepatitis B”/ all subheadings or “hepatitis b B virus”/ all subheadings

#2 hepatitis b or hepatitis-b or (type b adj1 hepatitis) or hbv or hep b or hbsag or hbs-ag or hbs antigen* or hbs-antigen* or hepatitis b surface antigen* or hepatitis-b surface antigen*

#3 “alanine aminotransferase”/ all subheadings

#4 alanine transaminase* or alanine aminotransferase* or glutamic pyruvate transaminase* or ALT or ALAT or GPT or SGPT

#5 “hepatitis B(e) antigen”/ all subheadings

#6 hbeag or hbe-ag or hbe antigen* or hbe-antigen* or (hepatitis b adj3 e antigen*) or (hepatitis-b adj3 e antigen*) or ((type b adj1 hepatitis) adj3 e antigen*) or (hbv adj3 e antigen*)

#7 “virus DNA”/ all subheadings or “virus replication”/ all subheadings

#8 dna or replication* or viral titre or viral titer or viral load

#9 “liver cirrhosis”/ or “decompensated liver cirrhosis”/ all subheadings

#10 cirrho* or ((liver or hepat*) adj3 fibro*)

#11 “liver cancer”/ or “ascites hepatoma”/ or “liver carcinoma”/ or “liver cell carcinoma”/ all subheadings

#12 (liver or hepat*) adj3 (cancer* or carcinom* or neoplasm* or malign* or tumo?r*) or hcc or hepatoma

#13 “age”/ all subheadings

#14 age adj5 infect*

#15 “vertical transmission”/ all subheadings

#16 perinatal or peri-natal or vertical or MTCT or mother-to-child or mother to child or mother-child or mother child or mother-to-infant or mother to infant or mother-infant or mother infant or maternal-to-child or maternal to child or maternal-child or maternal child or maternal-to-infant or maternal to infant or maternal-infant or maternal infant or adult-to-child or adult to child or adult-child or adult child or adult-to-infant or adult to infant or adult-infant or adult infant or maternal-f?etal or maternal f?etal or f?etomaternal

#17 postnatal or post-natal or horizontal or child-to-child or child to child or child-child or child child or sibling-to-sibling or sibling to sibling or sibling-sibling or sibling sibling or between children or between siblings or between family or within household

#18 “birth order”/ all subheadings

#19 birth adj3 (order* or link*)

#20 (serolog* or seropositiv* or serostatus) adj5 (famil* or parent* or father* or paternal or mother* or maternal or sibling* or brother* or sister*)

#21 (hepatitis b or hepatitis-b or (type b adj1 hepatitis) or hbv or hep b or hbsag or hbs-ag or hbs antigen* or hbs-antigen* or hepatitis b surface antigen* or hepatitis-b surface antigen*) adj5 (famil* or parent* or father* or paternal or mother* or maternal or sibling* or brother* or sister*)

#22 #1 or #2

#23 #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12

#24 #13 or #14

#25 #15 or #16 or #17

#26 #18 or #19

#27 #20 or #21

#28 #22 and #23 and #24

#29 #22 and #23 and #25

#30 #22 and #23 and #26

#31 #22 and #23 and #27

#32 #28 or #29 or #30 or #31

(1-3) CNKI

(关键词=乙肝 or 乙型肝炎 or HBV or HBsAgor 乙肝表面抗原) AND (关键词=ALT or 丙氨酸氨基转移酶 or HBeAg or 乙肝核心抗原 or 肝癌 or 肝细胞癌 or 肝硬化 or 肝纤维化 or HBVDNAor HBV-DNA) AND (关键词=年龄 or 出生顺序分析 or 垂直传播 or 水平传播 or 家庭)

(1-4) SinoMed

1.主题词:肝炎，乙型，慢性/全部树/全部副主题词 -限定:-

2.全部字段:乙肝 -限定:-

3.全部字段:乙型肝炎 -限定:-

4.全部字段:HBV -限定:-

5.全部字段:hbsag -限定:-

6.主题词:丙氨酸转氨酶/全部树/全部副主题词 -限定:-

7.全部字段:ALT -限定:-

8.全部字段:丙氨酸氨基转移酶 -限定:-

9.主题词:肝炎 e 抗原，乙型/全部树/全部副主题词 -限定:-

10.全部字段:HBeAg -限定:-

11.全部字段:乙肝 e 抗原 -限定:-

12.主题词:病毒复制/全部树/全部副主题词 -限定:-

13.全部字段:DNA -限定:-

14.全部字段:复制 -限定:-

15.全部字段:病毒滴度 -限定:-

16.全部字段:病毒载量 -限定:-

17.主题词:肝硬化/全部树/全部副主题词 -限定:-

18.全部字段:肝纤维化 -限定:-

19.主题词:肝肿瘤/全部树/全部副主题词 -限定:-

20.主题词:年龄因素/全部树/全部副主题词 -限定:-

21.主题词:疾病传播，垂直/全部树/全部副主题词 -限定:-

22.全部字段:母婴传播 -限定:-

23.主题词:疾病传播，水平/全部树/全部副主题词 -限定:-

24.全部字段:水平传播 -限定:-

25.主题词:出生顺序/全部树/全部副主题词 -限定:-

26.全部字段:出生顺序分析 -限定:-

27.主题词:家庭/全部树/全部副主题词 -限定:-

28.#5 or #4 or #3 or #2 or #1 -限定:-

29.#19 or #18 or #17 or #16 or #15 or #14 or #13 or #12 or #11 or #10 or #9 or #8 or #7 or #6 -限定:-

30.#24 or #23 or #22 or #21 -限定:-

31.#29 and #28 and #20 -限定:-

32.#26 or #25 -限定:-

33.#30 and #29 and #28 -限定:-

34.#32 and #29 and #28 -限定:-

35.#29 and #28 and #27 -限定:-

36.#35 or #34 or #33 or #31 -限定:-

(2) Additional Searches

Additional studies were sought manually by checking reference lists of all included papers. None of the searches were restricted by date, language or publication status

# Appendix 2. Protocol for the systematic review

1. Objectives

To review the association between the age at HBV infection and risk of high HBV DNA level, persistent viral replication, elevation of serum alanine transaminase level, advanced fribrosis and/or HCC in persons with chronic hepatitis B infection

2. Criteria for Considering Studies for this Review

2-1. Types of Studies

Any studies whether interventional or observational, reported in any language, irrespective of publication status. Studies must have a reference group to make a comparison. The only exception is a case series using the Greenwood-Yule method or its related approaches to examine the effect of birth order, because these methods compare the observed birth order distribution of affected individuals with the expected distribution and do not require control group.[192] In a case-control design, a control group which only consists of people chronically infected with HBV must be reported.

2-2. Types of Participants

Participants of any age who were found to have CHB infection at some stage of study are considered. Chronic hepatitis B is defined as a condition proven by serum HBsAg positivity on two occasions at least 6 months apart. However, because new HBV infections in adults are not so common in highly endemic areas where the vast majority of HBsAg positive people acquire

the infection perinatally or during childhood, HBsAg positivity on only one occasion in an adult living in highly prevalent communities is assumed to reflect chronic carriage of HBsAg.[97]

2-3. Exposure of interest

The age at the time of infection with HBV is estimated either by:

- Direct measurement through frequent follow-up examination of an uninfected cohort to determine the time point at which a person seroconverted to positive HBsAg
- HBV serological profile of the mother of the participant
- Person's birth order

2-4. Outcome of Interest

- Levels of serum alanine transaminase (ALT)
- Presence of serum hepatitis B e antigen (HBeAg)
- Quantitative/qualitative serum HBV DNA
- Liver fibrosis determined by either liver histology or non-invasive tests
- Cirrhosis
- HCC

3. Data Collection

3-1. Study Selection

First, the title and abstract of all papers identified by the electronic searches will be screened by two independent reviewers, by applying the inclusion criteria. Second, papers detected through

screening process will be retrieved and reviewed to assess the eligibility. When there is any doubt whether a paper can be included, clarification will be sought from the author of the paper. Disagreements will be resolved by discussion with a third author. Only studies which fulfilled all inclusion criteria will be included in the review.

3-2. Data Extraction

Data extraction will be carried out, by using a standardised pre-piloted data extraction sheet. The information included in the sheet is summarised in Appendix 3.

4. Assessment of Risk of Bias

The included studies will be evaluated for the risk of bias by modified framework that was introduced by Altman[240] (Appendix 4).

5. Data Analysis

For the study of maternal HBV serological profile, the odds ratio (OR) of having a worse outcome (high HBV DNA load (>2,000 IU/ml), positive HBeAg, elevated serum ALT (>40 U/L), advanced liver fibrosis (Metavir F≥2, or equivalent value in non-invasive tests), cirrhosis or HCC) in participants who have seropositive mother compared with those with seronegative mother will be calculated. For the birth order study, the odds of outcomes in each rank of birth will be compared with the odds in the first-born child as the reference. Measure of effect, its 95% confidence interval and p-value will be all reconstructed from the information reported in each included article. The results of multivariable analyses reported in the original paper will be presented without any modification in this review. STATA version 11 will be used for all

analyses. This protocol was made in accordance with checklists presented in the PRISMA guideline.[241]

# Appendix 3. Information included in the Standardised Data Extraction Sheet

- First author
- Country or region of study
- Year of study
- Study design
- Selection of participants
- Characteristics of participants studied (age, sex, whether asymptomatic, whether cirrhotic, prevalence of HBsAg, definition of cases and controls in case-control study, participation rate, drop-out rate in cohort study)
- Exposures of interest:
  - If age at infection was directly measured, the interval between each follow-up, test used for detect seroconversion
  - If serological profile of participant's mother was assessed, what serological test was performed and when the sample was taken (before the birth of study participant or current serostatus)
  - If birth order was examined, how the information was collected and the definition that the authors used
- Outcomes of interest:
  - Definition of high HBV DNA level, which method was used to measure this
  - Method used to measure HBeAg
  - Definition of elevated ALT level, which method was used to measure this
  - Definition of advanced fibrosis, which criteria was used when liver histology was performed, and which method was used when non-invasive test was carried out

- Case definition of cirrhosis
- Case definition of HCC

- Follow-up of participants (in a cohort study)
- Univariable analyses
- Multivariable analyses and confounding factors adjusted for

# Appendix 4. Framework for assessing the risk of bias in individual studies

Adapted from the framework presented by Altman (Egger M, Smith GD, Altman D, eds. *Systematic Reviews in Health Care: Meta-analysis in Context.* Wiley-Blackwell; 2001).

| Study feature | Qualities sought | | | | |
|---|---|---|---|---|---|
| 1. Sample of patients | Eligibility criteria defined | Good | Poor | N/R | N/A |
| | Sample selection explained (setting, locations and periods of recruitment) | Good | Poor | N/R | N/A |
| | Clinical and demographic characteristics fully described | Good | Poor | N/R | N/A |
| | Representative (unbiased selection of controls) | Good | Poor | N/R | N/A |
| | Assembled at a common stage in the course of their disease | Good | Poor | N/R | N/A |
| | Completeness | Good | Poor | N/R | N/A |
| 2. Follow up of patients | Sufficiently long | Good | Poor | N/R | N/A |
| 3. Outcome | Fully defined | Good | Poor | N/R | N/A |
| | Known for all or a high proportion of patients | >80% | 60-79% | <60% | N/R |
| | Outcome assessor blinded to exposure | Good | Poor | N/R | N/A |

| | | | | | |
|---|---|---|---|---|---|
| | status | | | | |
| 4. Prognostic variable | Fully defined, including details of method of measurement (maternal HBsAg study: when maternal serology was investigated?) | Good | Poor | N/R | N/A |
| | Available for all or a high proportion of patients | >80% | 60-79% | <60% | N/R |
| | Exposure assessor blinded to outcome status | Good | Poor | N/R | N/A |
| 5. Analysis | Continuous predictor variable analysed appropriately | Good | Poor | N/R | N/A |
| | Appropriate control for confounding factors | Good | Poor | N/R | N/A |
| | Appropriate statistical method | Good | Poor | N/R | N/A |
| 6. Treatment subsequent to inclusion in cohort | Fully described | Good | Poor | N/R | N/A |
| | Treatment standardized or randomised | Good | Poor | N/R | N/A |

Abbreviations: N/A, not applicable; N/R, not reported.

# Appendix 5. Description of risk of bias in included studies

Appendix 5-1. Description of Risk of Bias in Case-Control Studies, Cross-Sectional Studies and Case Series

| First Author, Year, (Reference No.) | 1. Sample of Patients | | | 2. Outcome | | | 3. Exposure | | | 4. Analysis | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Eligibility Criteria | Sample Selection | Representativeness in Controls | Fully Defined | Blinded to Exposure Status | Known for All Subjects? | Fully Defined | Blinded to Outcome Status | Known for All Subjects? | Confounding Factors Adjusted for | Appropriate Analysis |
| Beasley, 1982 [242] | N/R | N/R | N/R | N/R | N/R | N/R | N/R | N/R | N/R | None | good |
| Chang , 1989 [243] | good | poor[b] | poor[d] | poor[e] | N/A | 100% | good | N/R | 65% | matched on age | poor[e] |
| Hsu, 1988 [244] | poor[a] | poor[c] | N/A | good | N/R | 100% | poor[g] | N/R | 67% | None | good |
| Wheeley, 1989 [247] | poor[a] | poor[c] | N/A | good | N/R | 100% | good | N/A | 90% | None | poor[m] |
| Habu, 1991 [248] | poor[a] | poor[c] | N/A | good | N/R | 100% | poor[h] | N/R | 100%[k] | stratified by age group | good |
| Tai, 1999 | good | good | N/A | good | N/R | 90% | poor[i] | N/R | 52% | None | poor[m] |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| [249] | | | | | | | | | | | |
| Hopkirk, 2000 [250] | N/R | poor[c] | N/A | poor[h] | N/R | 89% | poor[g] | N/R | 49% | adjusted for age | good |
| Soderstrom ,2002 [251] | good | poor[c] | N/A | good | N/R | 100% | poor[g] | N/R | 75% | None | good |
| Hsieh, 1992 [16] | good | good | good | good | Good | 100% | good | N/R | 100% | adjusted for age, sex, smoking, anti-HCV, HBsAg and sibship size | poor[n] |
| Kuper, 2000 [194] | good | poor[b] | good | poor[e] | N/A | 100% | good | N/R | 99% for cases and 97% for controls | matched on age and sex, adjusted for age, sex, schooling, smoking, alcohol, anti-HCV, HBsAg and sibship size | poor[l, n] |
| Tai, 2002 [252] | good | good | N/A | good | N/R | 95% | good | N/R | 100% | stratified by relationship with index case | good |
| Cai, 2003 [253] | good | poor[c] | N/A | good | N/R | 100% | poor[j] | N/A | 100% | None | poor[o] |
| Cao, 2005 [254] | good | poor[c] | N/A | poor[f] | N/R | 100% | poor[j] | N/A | 100% | None | poor[o] |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Song, 2009 [255] | good | good | N/A | good | N/R | 96% | poor[j] | N/A | 100% | None | poor[o] |

Abbreviations: HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; N/A, not applicable; N/R, not reported.

[a] Definition of HBsAg carrier was not presented.

[b] The way of selecting control subjects was unclear.

[c] Setting of sample selection was unclear.

[d] Hospital-based cases were compared with population-based controls.

[e] Cut-off value of alpha fetoprotein (AFP) for hepatocellular carcinoma (HCC) diagnosis was not presented.

[f] Diagnostic criteria for HCC were not presented.

[g] When maternal sero-status was examined was unclear.

[h] Method of testing hepatitis B virus (HBV) marker was not presented.

[i] When maternal sero-status was examined was not presented in the original paper, however this was described in a subsequent paper of the same study [252].

[j] Birth order was not defined.

[k] Maternal sero-status was known in all the participants because having a mother alive was one of the eligibility criteria.

[l] Matched design, but no matched analysis.

[m] Clustering effect of being born to the same mother was not taken account.

[n] Two different control groups were combined.

[o] Both the Greenwood-Yule and Haldane-Smith method are prone to bias due to change in population dynamics.

Appendix S5-2. Description of Risk of Bias in Cohort Studies

| First Author, Year, (Reference No.) | 1. Sample of Patients | | | 2. Follow up | 3. Outcome | | | 4. Exposure | | | 5. Analysis | | 6. Treatment After Inclusion in Cohort |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Eligibility Criteria | Sample Selection | Assembled at a Common Stage | Sufficiently Long | Fully Defined | Blinded to Exposure Status | Known for all Subjects? | Fully Defined | Blinded to Outcome Status | Known for all Patients? | Confounding Factors Adjusted for | Appropriate Analysis | Fully Described |
| McMahon,2001 [81] | good | poor[b] | Good | good | Good | N/R | 100% | poor[e] | N/A | 100% | N/R | poor[i] | poor[j] |
| Kojima, 1985 [245] | poor[a] | poor[b] | Good | good | poor[c] | N/R | 100% | poor[f] | N/R | 100%[h] | N/R | poor[i] | good (no treatment) |
| Kojima, 1985 [246] | poor[a] | poor[b] | Good | good | poor[d] | N/R | 100% | poor[f] | N/R | 72% | N/R | poor[i] | good (steroid) |
| Chang, 1989 [78] | good | poor[b] | Good | good | Good | N/R | 100% | poor[g] | N/R | 95% | Stratified by age | poor[i] | good (no treatment) |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | group | | |
| Tseng, 2011 [83] | good | poor[b] | Good | good | good | N/R | 100% | good | N/R | 95% | adjusted for HBV genotype and maternal HBV marker | Good | good (no treatment) |

Abbreviations: HBV, hepatitis B virus; N/A, not applicable; N/R, not reported.

[a] Definition of hepatitis B surface antigen (HBsAg) carrier was not presented.

[b] Setting of sample selection was unclear.

[c] Method of measuring serum alanine transaminase (ALT) was not presented.

[d] Method of testing HBV marker was not presented.

[e] How often blood sample was obtained to determine timing of HBV infection was not reported.

[f] When maternal sero-status was examined was not presented in this paper, but this was confirmed by the communication with the author.

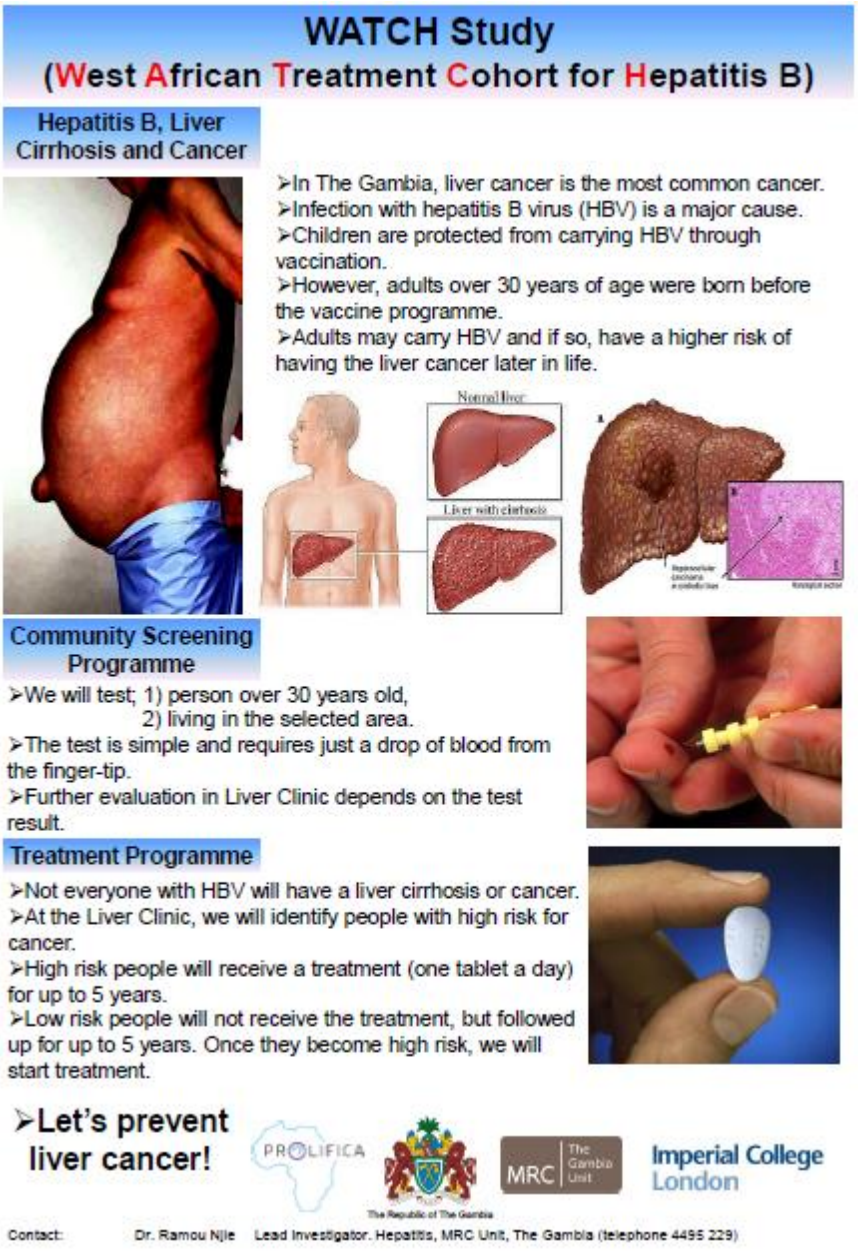
[g] When maternal sero-status was examined was unclear.

[h] Maternal sero-status was known in all the participants because having a mother tested was one of the eligibility criteria.

[i] Person-years at risk were not taken account in the analysis.

[j] For the sub-study of patients with known age at HBV infection, number of those who were treated was not reported.

# Appendix 6. Poster used for the community sensitisation



# Appendix 7. Questionnaire for birth order

**Community-Based Screening Questionnaire**

(Version 3.0., drafted on 19th Feb 2012)

**(1) Registered Information**

1. Enter: Screening IDNO [ S | G |___|___|___|___|___]
2. Enter: Compound number [___|___|___]
3. Enter: Name of head of compound _____
4. Enter: Year of birth [ 1 | 9 |___|___]
5. Enter: Age [___|___] years old
6. Enter: Phone numbers [___|___|___|___|___|___|___]

(If more than one phone number) [___|___|___|___|___|___|___]

[___|___|___|___|___|___|___]

7. Enter: Date of interview Day [___|___] Month [___|___] Year [___|___]
8. Enter: Code of interviewer [___|___]
9. Enter: Enumeration area code [___|___|___]
10. Enter: First name _____
11. Enter: Family name _____
12. Enter: Sex 1. Male 2. Female

**(2) Demographic Questionnaire**

13. In which **district** were you born? _____

8. I don't know

14. To which ethnic group do you belong?

1. Mandinka 2. Fula 3. Wolof

4. Jola 5. Serahule 6. Aku

7. Bambara 8. Manjago 9. Serere

10. Others _____

88. I don't know

15. What is the name of your true father?

(first name) _____ (family name) _____

8. I don't know.

16. What is the name of your true mother?

(first name) _____ (family name) _____

8. I don't know,

17. Is your true mother still alive?

1. Yes 2. No 8. I don't know

If yes or "I don't know", go to question 18. If no, skip to question 20.

18. How old is your true mother?

[___|___] years old

8. I don't know

19. Is she living in the same compound as you now?

1. Yes 2. No 8. I don't know

If yes, enter Screening IDNO of the mother (if she was allocated).

[ S | G |___|___|___|___|___]

Now, skip to question 22.

20. When did your true mother die? [___|___] years ago

8. I don't know

21. How old was your true mother, when she died? [___|___] years old

8. I don't know

22. For the following questions please include both living and deceased sisters and brothers. Multiple pregnancies are counted as independent births (twins: 2; triplets: 3; etc). I will ask you the numbers of brothers and sisters from the same father and same mother, then I will ask you the numbers from the same father and different mother, and finally I will ask you the numbers from the same mother and different father.

22-1. How many brothers do you have by the same mother and father?

Number [___|___]

88. I don't know

22-2. How many of these brothers are older than you?

Number [___|___]

88. I don't know

22-3. Are there any brothers by the same mother and father who are living in the same compound as you now?

1. Yes 2. No 8. I don't know

If yes, what is the name of these brothers? (up to 10 persons)

(first name) _____ (family name) _____

8. I don't know

22-4. How many sisters do you have by the same mother and father?

Number [___|___]

88. I don't know

22-5. How many of these sisters are older than you?

Number [___|___]

88. I don't know

22-6. Are there any sisters by the same mother and father who are living in the same compound as you now?

1. Yes 2. No 8. I don't know

If yes, what is the name of these brothers? (up to 10 persons)

(first name) _____ (family name) _____

8. I don't know

22-7. How many brothers do you have by the same father but different mother?

Number [___|___]

88. I don't know

22-8. How many of these brothers are older than you?

Number [___|___]

88. I don't know

22-9. Are there any brothers by the same father but different mother who are living in the same compound as you now?

1. Yes 2. No 8. I don't know

If yes, what is the name of these brothers? (up to 10 persons)

(first name) _____ (family name) _____

_

8. I don't know

22-10. How many sisters do you have by the same father but different mother?

Number [___|___]

88. I don't know

22-11. How many of these sisters are older than you?

Number [___|___]

88. I don't know

22-12. Are there any sisters by the same father but different mother who are living in the same compound as you now?

1. Yes 2. No 8. I don't know

If yes, what is the name of these brothers? (up to 10 persons)

(first name) _____ (family name) _____

_

8. I don't know

22-13. How many brothers do you have by the same mother but different father?

Number [___|___]

88. I don't know

22-14. How many of these brothers are older than you?

Number [___|___]

88. I don't know

22-15. Are there any brothers by the same mother but different father who are living in the

same compound as you now?

1. Yes 2. No 8. I don't know

If yes, what is the name of these brothers? (up to 10 persons)

(first name) _____ (family name) _____

_

8. I don't know

22-16. How many sisters do you have by the same mother but different father?

Number [___|___]

88. I don't know

22-17. How many of these sisters are older than you?

Number [___|___]

88. I don't know

22-18. Are there any sisters by the same mother but different father who are living in the same compound as you now?

1. Yes 2. No 8. I don't know

If yes, what is the name of these brothers? (up to 10 persons)

(first name) _____ (family name) _____

_

8. I don't know

**(4) Point-of-care Test Result**

23. Enter: code of examiner _____

24. Enter: test result

    1. Positive

    2. Negative

**(5) Appointment at the Clinic for Enrolment**

All participants tested positive for the test have an appointment at the clinic for enrolment. Some participants tested negative will be later selected randomly as healthy controls and will have an appointment at the clinic.

25. Are you pregnant now? (this question only applies to female participants aged <55 years)

    1. Yes  2. No  8. I don't know

    If yes, go to question 26. If no or "I don't know", skip to question 27.

26. How many months pregnant are you?

    [___|___] months

    Thank you, we will call you after your delivery to invite to the Liver Clinic.

27. Enter: date of appointment  Day [___|___] Month [___|___] Year [___|___]

28. Enter: time of appointment  [___|___] o'clock

29. Enter: name of clinic

1. The Liver Clinic in Medical Research Council (MRC), in Fajara
2. The Liver Clinic in Royal Victoria Teaching Hospital (RVTH), in Banjul
3. The Liver Clinic in Serrekunda Hospital, in Kanifing
4. The Clinic in Sibanor

5. The MRC Clinic in Keneba