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Title

Maternal age at first childbirth and geographical variation in HBV prevalence in Cameroon:
Important role of mother-to-child transmission

Authors

Jude Moutchia,^{1,2} Richard Njouom,³ Eva Rumpler,¹ Camille Besombes,¹ Gaëtan Texier,^{4,5}
Mathurin Tejiokem,⁴ Pascal Crépey,² Arnaud Fontanet,^{1,6} Yusuke Shimakawa¹

Affiliations

¹ Unité d'Épidémiologie des Maladies Émergentes, Institut Pasteur, Paris, France.

² REPERES « Recherche en Pharmaco-Epidémiologie et Recours aux Soins » – EA 7449,
Université Rennes, Ecole des Hautes Etudes en Santé Publique, Rennes, France.

³ Department of Virology, Centre Pasteur du Cameroun, Yaoundé, Cameroon.

⁴ Department of Epidemiology and Public Health, Centre Pasteur du Cameroun, Yaoundé,
Cameroon.

⁵ Aix Marseille University, IRD, AP-HM, SSA, VITROME, IHU Méditerranée Infection, Marseille,
France.

⁶ PACRI unit, Conservatoire National des Arts et Métiers, Paris, France.

Corresponding authors

Yusuke Shimakawa

Unité d'Épidémiologie des Maladies Émergentes, Institut Pasteur, 25-28 rue du Dr Roux, 75015
Paris, France

Phone: +33 (0) 1 40 61 38 87

Fax: +33 (0) 1 45 68 88 76

Email: yusuke.shimakawa@gmail.com

Jude Moutchia

École des hautes études en santé publique, 20 avenue George Sand, 93210 Saint-Denis, France

Phone: +33 (0) 7 80 83 59 28

Email: msjude27@gmail.com

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Running title

Spatial variation of HBV in Cameroon

Summary

A nationwide population-based survey observed marked spatial variation in HBV prevalence in Cameroon. Maternal age at first childbirth, a proxy for HBV mother-to-child transmission, was significantly associated with HBsAg-positivity, suggesting a crucial role of MTCT in maintaining high HBV endemicity.

Abstract

Background

The prevalence of hepatitis B virus (HBV) infection varies geographically around the world. However, the underlying reasons for this variation are unknown. Using a nationally representative population-based sample from all 58 administrative divisions in Cameroon, we examined the association between median maternal age at first childbirth in a preceding generation, a proxy for the frequency of mother-to-child transmission (MTCT) of HBV in a region, and the risk of chronic HBV infection, defined as positive surface antigen (HBsAg), in the index generation.

Methods

We estimated a division-specific median maternal age at first childbirth using historical data from Demographic Health Surveys (DHS) in 1991/1998/2004/2011. We tested HBsAg in 2011 DHS participants. We used maps to display spatial variation and spatial models for the analysis.

Results

In 14,150 participants (median 27 years old, 51% females), the overall weighted prevalence of HBsAg was 11.9% (95%CI: 11.0–12.8), with a wide geographical variation across the divisions (range: 6.3-23.7%). After adjusting for confounding factors and spatial dependency, lower maternal age at first childbirth was significantly associated with positive HBsAg at the division level (β : 1.89 [95%CI: 1.26-2.52], $p < 0.001$), and at the individual level (OR: 1.20 [95%CI: 1.04-1.39], $p = 0.016$). A similar ecological correlation was observed across other African countries.

Conclusions

The significant association between the maternal age at first childbirth and HBsAg-positivity suggests a crucial role of MTCT in maintaining high HBV endemicity in some areas in Cameroon. This underlines an urgent need to effectively prevent MTCT in order to achieve WHO's global hepatitis elimination goals.

Introduction

Hepatitis B virus (HBV) infection imposes a major health burden worldwide. Approximately 257 million persons live with chronic HBV infection, and 887,000 HBV-related deaths occur annually [1]. In 2016, WHO launched an ambitious plan to eliminate HBV as a major public health threat globally by 2030.

HBV burden is not ubiquitously distributed across continents, and disproportionately affects sub-Saharan Africa and East Asia, where the adult prevalence of hepatitis B surface antigen (HBsAg), a marker of current infection, exceeds 8% and 5%, respectively [2, 3]. A wide variation in HBsAg prevalence is also recognized across countries within the same region, and even between villages within the same division [4, 5]. A classical mathematical model of HBV transmission dynamics, before the wide implementation of infant hepatitis B vaccination, explained that such a variation in HBsAg prevalence might be attributed to a feedback mechanism that relates to the rate of transmission, average age at infection, and age-dependent probability of developing chronic infection [6]. The risk of chronic HBV infection is inversely correlated with age at HBV infection: 80-90% in neonates perinatally infected by infectious mothers, 20-30% in children infected through horizontal transmission, and <5% in those infected during adulthood. Also, the average age at which individuals become infected with HBV is largely dependent on the prevalence of chronic HBV infection in the population: the higher the prevalence, the younger the age of infection [6]. In this loop of positive feedback, mother-to-child transmission (MTCT) could play a central role; compared to women who acquired chronic HBV infection through horizontal transmission, women who acquired chronic infection through MTCT are more likely to maintain persistent viral replication with positive hepatitis B e antigen (HBeAg) well into their childbearing years, thus are more prone to replicate MTCT [7-11].

However, the question of whether a high frequency of HBV MTCT could lead to a high HBsAg prevalence in a population has not been proven epidemiologically due to the difficulty in measuring the rate of MTCT in a given adult population. For example, at the time of sero-survey of an adult population (index generation), it is extremely difficult to ascertain whether their mothers (previous generation) carried HBsAg and HBeAg during their pregnancies. Alternatively, maternal age at first childbirth could be an indirect marker of HBV MTCT [12, 13]. HBeAg loss occurs spontaneously with age in people with chronic HBV infection; therefore, younger mothers, if chronically infected with HBV, have a higher likelihood of having HBeAg and a high viral load, which increases their baby's risk of perinatal infection [9, 14, 15]. Moreover, if the first child is infected through MTCT, this child is more likely to develop chronic HBV infection and is at greater risk of infecting subsequent siblings in the household through horizontal transmission, even when the mother has lost HBeAg and becomes less infectious during subsequent pregnancies [5, 16].

We therefore assessed a population-level association between the median age at first childbirth, a proxy for the frequency of HBV MTCT, and the prevalence of HBsAg using a nationally representative population-based sample in Cameroon. This country in Central Africa has a high HBsAg prevalence and a majority (>50-80%) of adults have been exposed to the virus, as indicated by a positive HBV core antibody (anti-HBc) [17, 18]. Hepatitis B vaccination starting at 6 weeks of age was integrated into the national infant immunization program in 2005. To further examine the above hypothesis, we also conducted an ecological analysis for a country-level association between the median age at first childbirth and the prevalence of HBsAg within the African continent.

Methods

Historical demographic data

We used publicly available data from a series of cross-sectional nationally representative household surveys (Demographic Health Surveys (DHSs)) conducted in 1991/1998/2001/2011 [19]. Secondary use of these data was approved by the University of Sheffield Research Ethics Committee (Ref: 033994).

HBV sero-survey in 2011

We also used data from a HBV sero-survey of the 2011 DHS participants. Cameroon is divided administratively into ten regions and 58 divisions. The 2011 DHS was conducted in all 58 divisions from January to August 2011 using a two-stage stratified probability sampling [20]. From each of 578 census clusters, a fixed number (25-30) of households were selected; a total of 14,214 households participated in the DHS interview (Supplementary Figure 1). All women aged 15-49 years and all men aged 15-59 years living in half of these households were further invited to participate in the sero-survey. Following an informed consent, capillary blood was collected as dried blood spots (DBS). DBS eluate was tested for HBsAg at the Centre Pasteur du Cameroun using a chemiluminescent microparticle immunoassay (ArchitectPlus, Abbott). The use of DBS was 99% sensitive and 100% specific to detect HBsAg compared to plasma sample [21]. The study was approved by the National Ethics Committee of Cameroon.

Study variables and data sources

The median age at first childbirth was computed at a cluster level (division), whilst HBsAg-positivity was considered both at an individual level (binary variable: positive/negative) and a division level (continuous variable: HBsAg prevalence). Since the age of the index participants in 2011 ranged from 15 to 59 years, and thus their birth years ranged from 1952 to 1996, we

computed the median age at first childbirth in women who were at their reproductive age (15-49 years) during this particular period (1952–1996) (Supplementary Figures 2-3). We first computed the division-level estimates from each of the historical DHS data (1991/1998/2004/2011) and then pooled these using random-effects inverse-variance models. As the maternal age at first birth may change over time, we stratified this period into two: 1952-1981 (corresponding to the years of birth of the older index cohort aged 30–59 years in 2011) and 1982-1996 (younger index cohort aged 15–29 years in 2011).

We identified the following as *a priori* confounders for the association between the median maternal age at first childbirth and the risk of chronic HBV infection: sibship size, wealth index, and age (Supplementary Figure 4). Sibship size was defined as the number of children born to the same biological mother as the respondent, irrespective of whether a sibling was alive or not at the time of the 2011 survey. Sibship size for each household in a division was aggregated to estimate the division-level mean sibship size. We used household wealth quintiles developed by the DHS program based on household characteristics in the 2011 survey. We categorized the age of participants in the 2011 DHS into three categories (15–29, 30–44, and 45–59 years) and also aggregated the ages to estimate the mean age of individuals in the division.

Statistical analyses

Descriptive analyses

HBsAg prevalence was weighted to take into account the stratified sampling and sampling probabilities. Due to a clustering of HBsAg-positivity within households (intraclass correlation coefficient (ICC): 0.39) and within divisions (ICC: 0.05), we used multilevel logistic regression to assess differences in weighted HBsAg prevalence across various participant groups.

Spatial variation

We used maps to display the spatial variation of HBsAg prevalence and relevant covariates. To avoid spatial variation fixed by the administrative boundaries, we generated smoothed interpolated maps using empirical Bayesian kriging. The division-level variables were assumed to follow a Gaussian distribution. We used Local Moran's I index to identify and map clusters of high/low HBsAg prevalence and local spatial outliers. Global spatial autocorrelation was assessed using the Global Moran's I index.

Division-level association between median maternal age at first childbirth and HBsAg prevalence

Using Pearson's correlation coefficient and bivariate linear regression analyses we assessed the division-level association between the median maternal age at first childbirth and the HBsAg prevalence. *A priori* confounders were included in a multiple linear regression model. Since there was evidence of global spatial autocorrelation (Moran's $I=0.34$), we adopted spatial models and selected the Eigenvector Spatial Filtered (ESF) model [22] as the best model based on the Moran's I value of the residuals, the AIC, and the R^2 . Since the association between the median maternal age at first childbirth and spatial variation of HBsAg-positivity might be modified by age of the index generation, we stratified regression analyses into two age groups (older versus younger index cohort as described above).

Individual-level association between median maternal age at first childbirth and HBsAg positivity.

We also assessed the association between the division-level median age at first childbirth and individual risks of HBsAg-positivity using multilevel logistic regression models accounting for spatial dependency [23]. We used ESF to account for spatial dependency and computed marginal odds ratios using generalized estimation equations with robust standard errors.

Country-level correlation between median maternal age at first childbirth and HBsAg prevalence in Africa.

Median age at first childbirth in each African country was obtained from the earliest available DHS. Country-level prevalence of HBsAg was obtained from two published global estimates [2, 3]. Pearson's correlation coefficient was used to assess the country-level correlation between the median maternal age at first childbirth and the prevalence of HBsAg in Africa.

The analyses were done using STATA, R and ArcGIS.

Results

HBsAg prevalence

Of 14,202 DBS collected in the 2011 DHS, 14,150 (99.6%) were tested for HBsAg. The median number of sero-survey participants was 2 (IQR: 1–3) per household and 185.5 (104–288) per division. The median age of sero-survey participants was 27 (IQR: 20–37) years, and there were 7,224 (51%) females (Table 1). The overall weighted prevalence of HBsAg in Cameroon was 11.9% (95%CI: 11.0–12.8). The prevalence was significantly higher in persons aged 15–29 years (12.3% [$p = 0.011$]), males (14.1% [$p < 0.001$]), and persons in the poorest wealth quintile (17.5% [$p = 0.023$]). There was also a marked geographical variation with the lowest prevalence observed in the North-West region (7.0%) and the highest in the Far-North region (17.7%). The mean (\pm SD) prevalence of HBsAg per division was $11.1 \pm 3.8\%$, with a remarkably wide range (6.3%-23.7%). The mean age at first childbirth per division from 1952 to 1996 was 19.0 ± 0.8 years (range: 17.4-21.1). The mean sibship size per division was 7.0 ± 0.5 (range: 5.8-8.8, Table 2).

Spatial variation of HBsAg prevalence and covariates

There was a cluster of divisions with the highest HBsAg prevalence in the northern part of Cameroon and clusters of divisions with the lowest HBsAg prevalence in the Adamawa plateau and western part of the country (Figure 1, Supplementary Figure 5). Median age at first childbirth followed a similar pattern; divisions with the lowest maternal age at first childbirth were clustered in the north and those with the highest maternal age were clustered in the west. Divisions in the northern and central parts had the highest mean sibship size while those in the west and east had the lowest. The highest mean wealth index was observed in the central and south-western parts.

Division-level association

A significant correlation was observed at the division level between the prevalence of HBsAg and the following covariates: median age at first childbirth ($r=0.56$, $p<0.001$), sibship size ($r=0.37$, $p=0.004$), mean wealth index ($r=0.44$, $p<0.001$), and mean age of the index cohort ($r=0.28$, $p=0.030$) (Supplementary Figure 6). There was also a significant correlation between the median age at first childbirth and the other covariates (Supplementary Figure 7).

On bivariate linear regression, the following division-level variables were all significantly associated with the division-level HBsAg prevalence (Table 3): median maternal age at first childbirth (β : -2.60 [95%CI: -3.72, -1.48]), mean sibship size (β : 2.64 [0.88, 4.40]), mean wealth index (β : -2.11 [-3.33, -0.91]), and mean age (β : -0.55 [-1.06, -0.03]). After adjusting for the mean sibship size, mean wealth index, mean age, and spatial dependency, on average, a unit increase in the division-level median age at first childbirth was significantly associated with a two-percent decrease in the division-level HBsAg prevalence (β : -1.89 [95%CI: -2.52, -1.26]). The division-level associations between the median age at first childbirth and the HBsAg prevalence remained

significant for the younger index cohort aged 15-29 years (β : -2.56 [-4.25, -0.87]) but not for the older cohort aged 30-59 years (β : -0.76 [-2.23, 0.72]) (Supplementary Figure 9).

Individual-level association

After adjusting for individual age, household wealth, division-level mean sibship size, and spatial dependency, a unit increase in the division-level median maternal age at first childbirth was associated with a 17% lower risk of HBsAg-positivity at the individual level (OR: 0.83 [95%CI: 0.72–0.96]). The significant associations were also observed in the index cohort aged 15-29 years (adjusted OR: 0.81 [0.68–0.96]) and in those aged 30-44 years (0.82 [0.70–0.96]); however, no association was observed in the oldest age group (45–59 years) (Table 4).

Country-level correlation in Africa

There was a significant negative correlation between country-level median maternal age at first childbirth and country-level HBsAg prevalence estimated by Schweitzer *et al* [2]. ($r=-0.45$, $p=0.003$), and country-level HBsAg prevalence estimated by the Polaris Observatory Collaborators [3] ($r=-0.40$, $p=0.032$) (Fig 2).

Discussion

Using nationally representative DHS data of adults in Cameroon born before the infant hepatitis B vaccination program, we found a high overall HBsAg prevalence (11.9%), an important spatial variation of HBsAg-positivity, and a statistically significant association between the median maternal age at first childbirth and HBsAg-positivity both at the division and individual level. These findings suggest an important role of MTCT in maintaining the high HBV endemicity in some areas in Cameroon. We also confirmed a significant country-level correlation between the maternal age at first childbirth and the HBsAg prevalence across Africa.

We assumed that a division with younger median age at first childbirth would have a higher frequency of MTCT compared to a division with an older median age. This is because HBeAg-positivity and/or high viral load, the major determinants of HBV MTCT, are lost over time in persons chronically infected with HBV [9, 14, 15]. In the natural history of chronic HBV infection, HBeAg is almost invariably present in children newly infected with HBV and is spontaneously lost over time at an annual rate of 6-7% [9, 10]. The prevalence of HBeAg in HBsAg-positive women is therefore estimated to decrease with age: 40.2%, 30.0%, and 22.7% in women in Central Africa aged 10-19, 20-29, and 30-39 years, respectively [14]. Indeed, lower maternal age has been identified as a risk factor for HBV MTCT [24].

The prevalence of HBeAg in HBsAg-positive pregnant women would have been a more direct marker of the frequency of MTCT. Although such nationwide data does not exist in Cameroon, previous cross-sectional studies in some regions in Cameroon supported our findings. In Yaoundé, capital city with a lower HBV prevalence (10.2% in our study), none of HBsAg-positive pregnant women were found to carry HBeAg [25]. In contrast, in the Far-North region, a zone with high HBV prevalence (17.7% in our study), 22.0% of HBsAg-positive pregnant women had HBeAg [26]. Moreover, in these HBsAg-positive pregnant women, the HBeAg prevalence was significantly higher in those aged <20 years (31.1%) than in those aged >28 years (14.3%, $p=0.01$) [26].

We adequately addressed potential confounders for the association between maternal age at first childbirth and HBsAg positivity. However, there is still a risk of residual confounding with unmeasured variables. Worldwide, there is a wide geographical variation in the distribution of predominant HBV genotypes, and this may have a role in defining local HBV epidemiology [27]. For example, in East Asia where genotypes B and C predominate, genotype C was found to be

associated with more frequent MTCT [28], and this might have contributed to a relatively high HBV prevalence in this region [29]. In Cameroon, there is a predominance of genotypes A and E, similar to other Central and West African countries [26, 30]. Whilst a relatively higher prevalence of genotype A has been documented in Central, West, South and East of Cameroon [30], a predominance of genotype E was reported in North where the prevalence of HBsAg is very high [26]. More studies are needed to elucidate the role of African genotypes, particularly its association with a specific mode of HBV transmission. Variation in host genetics such as the human leukocyte antigen (HLA) genes may also influence the natural history of HBV infection [31, 32], and this may need to be accounted in a future study. The risk of HBV MTCT in women co-infected with HIV may be different, especially when they receive antiretroviral therapy that is also active against HBV [33]. Nevertheless, the 2011 DHS participants were born in 1952-1996, before the wide availability of such an antiretroviral regimen.

Our findings provide two important implications for the control of hepatitis B. First, even in the absence of any preventive measure, delaying the age of marriage and childbearing may spontaneously result in a reduction in HBsAg prevalence. This confirms historical findings reported in South Africa in the pre-vaccination era [34]. Second, our study provides an important rationale for preventing HBV MTCT in Cameroon. The current hepatitis B vaccination schedule using pentavalent vaccine (6-10-14 weeks) effectively prevents horizontal transmission but not MTCT [33]. In 2020, the Government launched a national strategic plan for HBV and identified the introduction of birth dose hepatitis B vaccine as a priority. Moreover, to further eliminate the risk of MTCT, an additional strategy such as peripartum antiviral prophylaxis in HBV-infected women with a high viral load ($\geq 200,000$ IU/mL) or a positive HBeAg test should be considered as recently recommended by WHO [35-37].

The study has limitations. Since our exposure of interest (maternal age at first childbirth) was estimated at a cluster level, there is a possibility of ecological fallacy. This is particularly relevant for the country-level assessments across Africa. For the estimates within Cameroon, we tried to limit the bias by assessing the division-level effect on the individual-level outcome (HBsAg positivity). Evaluating the association between an individual's birth order and HBsAg positivity could also be informative to support our hypothesis; unfortunately, birth order was not systematically collected for the DHS respondents.

In conclusion, we found that maternal age at first childbirth, a proxy for the frequency of MTCT, is significantly associated with HBsAg-positivity. This suggests the crucial role of HBV MTCT in maintaining high HBV endemicity in some areas in Cameroon. We also found this correlation across the African countries. These findings underline an urgent need to scale up interventions to effectively prevent MTCT in Cameroon and other African countries.

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Potential conflict of Interests

YS received a research grant from Gilead. All other authors declare no competing interests.

References

1. World Health Organization. Global Hepatitis Report. Available at: <http://apps.who.int/iris/bitstream/10665/255016/1/9789241565455-eng.pdf?ua=1>. Accessed 8 March 2017.
2. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *Lancet* **2015**; 386(10003): 1546-55.
3. Razavi-Shearer D, Gamkrelidze I, Nguyen MH, et al. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. *Lancet Gastroenterol Hepatol* **2018**; 3(6): 383-403.
4. McNaughton AL, Lourenço J, Bester PA, et al. Hepatitis B virus seroepidemiology data for Africa: Modelling intervention strategies based on a systematic review and meta-analysis. *PLOS Medicine* **2020**; 17(4): e1003068.
5. Whittle H, Inskip H, Bradley AK, et al. The pattern of childhood hepatitis B infection in two Gambian villages. *The Journal of infectious diseases* **1990**; 161(6): 1112-5.
6. Medley GF, Lindop NA, Edmunds WJ, Nokes DJ. Hepatitis-B virus endemicity: heterogeneity, catastrophic dynamics and control. *Nature medicine* **2001**; 7(5): 619-24.
7. Kirk GD, Bah E, Montesano R. Molecular epidemiology of human liver cancer: insights into etiology, pathogenesis and prevention from The Gambia, West Africa. *Carcinogenesis* **2006**; 27(10): 2070-82.
8. Tseng YR, Wu JF, Ni YH, et al. Long-term effect of maternal HBeAg on delayed HBeAg seroconversion in offspring with chronic hepatitis B infection. *Liver international : official journal of the International Association for the Study of the Liver* **2011**; 31(9): 1373-80.
9. Shimakawa Y, Lemoine M, Njai HF, et al. Natural history of chronic HBV infection in West Africa: A longitudinal population-based study from The Gambia. *Gut* **2016**; 65(12): 2007-16.
10. Yang Y, Huang A, Zhao Y. Spontaneous loss of chronic HBV infection markers in treatment-naïve children: a systematic review and pooled meta-analyses. *Expert review of anti-infective therapy* **2020**.
11. Shimakawa Y, Yan HJ, Tsuchiya N, Bottomley C, Hall AJ. Association of early age at establishment of chronic hepatitis B infection with persistent viral replication, liver cirrhosis and hepatocellular carcinoma: a systematic review. *PLoS One* **2013**; 8(7): e69430.
12. Shimakawa Y, Lemoine M, Bottomley C, et al. Birth order and risk of hepatocellular carcinoma in chronic carriers of hepatitis B virus: a case-control study in The Gambia. *Liver international : official journal of the International Association for the Study of the Liver* **2015**; 35(10): 2318-26.
13. Lin HH, Kao JH, Chang TC, Hsu HY, Chen DS. Secular trend of age-specific prevalence of hepatitis B surface and e antigenemia in pregnant women in Taiwan. *Journal of medical virology* **2003**; 69(4): 466-70.

14. Ott JJ, Stevens GA, Wiersma ST. The risk of perinatal hepatitis B virus transmission: hepatitis B e antigen (HBeAg) prevalence estimates for all world regions. *BMC Infect Dis* **2012**; 12: 131-.
15. Liu J, Zhang S, Liu M, Wang Q, Shen H, Zhang Y. Maternal pre-pregnancy infection with hepatitis B virus and the risk of preterm birth: a population-based cohort study. *Lancet Glob Health* **2017**; 5(6): e624-e32.
16. Whittle HC, McLauchlan K, Bradley AK, et al. Hepatitis B virus infection in two Gambian villages. *Lancet* **1983**; 321(8335): 1203-6.
17. Bigna JJ, Amougou MA, Asangbeh SL, et al. Seroprevalence of hepatitis B virus infection in Cameroon: a systematic review and meta-analysis. *BMJ open* **2017**; 7(6): e015298-e.
18. Magoro T, Gachara G, Mavhandu L, et al. Serologic and genotypic characterization of hepatitis B virus in HIV-1 infected patients from South West and Littoral Regions of Cameroon. *Viol J* **2016**; 13(1): 178.
19. The D. H. S. Program. Demographic and Health Survey (DHS). Available at: <https://dhsprogram.com/What-We-Do/Survey-Types/DHS.cfm>. Accessed 23 March.
20. The D. H. S. Program. Sampling and household listing manual. Available at: https://dhsprogram.com/pubs/pdf/DHSM4/DHS6_Sampling_Manual_Sept2012_D_HSM4.pdf. Accessed 19 March 2012.
21. Besombes C, Njouom R, Paireau J, et al. The epidemiology of hepatitis delta virus infection in Cameroon. *Gut* **2020**; 69(7): 1294-300.
22. Park YM, Kim Y. A spatially filtered multilevel model to account for spatial dependency: Application to self-rated health status in South Korea. *Int J Health Geogr* **2014**; 13(1): 6-.
23. Chaix B, Merlo J, Chauvin P. Comparison of a spatial approach with the multilevel approach for investigating place effects on health: The example of healthcare utilisation in France. *J Epidemiol Community Health* **2005**; 59(6): 517-26.
24. Zou H, Chen Y, Duan Z, Zhang H, Pan C. Virologic factors associated with failure to passive-active immunoprophylaxis in infants born to HBsAg-positive mothers. *J Viral Hepat* **2012**; 19(2): e18-25.
25. Kfutwah AKW, Tejiokem MC, Njouom R. A low proportion of HBeAg among HBsAg-positive pregnant women with known HIV status could suggest low perinatal transmission of HBV in Cameroon. *Viol J* **2012**; 9: 62-.
26. Ducancelle A, Abgueguen P, Birguel J, et al. High endemicity and low molecular diversity of hepatitis B virus infections in pregnant women in a rural district of north cameroon. *PLoS ONE* **2013**; 8(11).
27. Kramvis A. The clinical implications of hepatitis B virus genotypes and HBeAg in pediatrics. *Reviews in medical virology* **2016**; 26(4): 285-303.
28. Wen WH, Chen HL, Ni YH, et al. Secular trend of the viral genotype distribution in children with chronic hepatitis B virus infection after universal infant immunization. *Hepatology* **2011**; 53(2): 429-36.

29. Rajoriya N, Combet C, Zoulim F, Janssen HLA. How viral genetic variants and genotypes influence disease and treatment outcome of chronic hepatitis B. Time for an individualised approach? *Journal of hepatology* **2017**; 67(6): 1281-97.
30. Tufon KA, Meriki HD, Anong DN, Mbunkah HA, Nkuo-Akenji T. Genetic diversity, viraemic and aminotransferases levels in chronic infected hepatitis B patients from Cameroon. *BMC Res Notes* **2016**; 9(1): 117.
31. Matsuura K, Isogawa M, Tanaka Y. Host genetic variants influencing the clinical course of hepatitis B virus infection. *Journal of medical virology* **2016**; 88(3): 371-9.
32. Yengo CK, Torimiro J, Kowo M, et al. Variation of HLA class I (-A and -C) genes in individuals infected with hepatitis B or hepatitis C virus in Cameroon. *Heliyon* **2020**; 6(10): e05232.
33. Keane E, Funk AL, Shimakawa Y. Systematic review with meta-analysis: the risk of mother-to-child transmission of hepatitis B virus infection in sub-Saharan Africa. *Aliment Pharmacol Ther* **2016**; 44(10): 1005-17.
34. Song E, Kew MC, Hwang LY, Beasley RP. Epidemiology of hepatitis B virus infection in South African Chinese. *American journal of epidemiology* **1988**; 128(4): 828-38.
35. World Health Organization. Prevention of mother-to-child transmission of Hepatitis B virus: Guidelines on antiviral prophylaxis in pregnancy. Available at: <https://apps.who.int/iris/bitstream/handle/10665/333391/9789240002708-eng.pdf?sequence=1&isAllowed=y>. Accessed 23 September 2020.
36. Boucheron P, Lu Y, Yoshida K, et al. Accuracy of HBeAg to identify pregnant women at risk of transmitting hepatitis B virus to their neonates: a systematic review and meta-analysis. *Lancet Infect Dis* **2021**; 21(1): 85-96.
37. Funk AL, Lu Y, Yoshida K, et al. Efficacy and safety of antiviral prophylaxis during pregnancy to prevent mother-to-child transmission of hepatitis B virus: a systematic review and meta-analysis. *Lancet Infect Dis* **2021**; 21(1): 70-84.

Tables

Table 1. Characteristics of participants in the 2011 HBsAg sero-survey (N=14,150)

| Variables | Total, n (%) | HBsAg positive, n (weighted %) | *p value |
|------------------------------------|--------------|--------------------------------|----------|
| Individuals (N= 14,150) | | | |
| Age group | | | 0.010 |
| 15-29 years | 8,051 (56.9) | 972 (12.3) | |
| 30-44 years | 4,386 (31.0) | 504 (12.2) | |
| 45-59 years | 1,713 (12.1) | 145 (9.1) | |
| Gender | | | <0.001 |
| Female | 7,224 (51.1) | 664 (9.7) | |
| Male | 6,926 (49.0) | 957 (14.1) | |
| Education | | | 0.021** |
| No education | 598 (8.6) | 246 (13.4) | |
| Primary | 2,355 (34.0) | 586 (12.4) | |
| Secondary | 3,441 (49.7) | 704 (11.2) | |
| Higher | 532 (7.7) | 85 (10.8) | |
| Occupation (missing = 2447) | | | 0.301 |
| Not working/Student/Retired | 911 (7.8) | 121 (13.2) | |
| Farmers/Hunters/Fishers | 4,599 (39.3) | 580 (13.3) | |
| Military/Drivers | 507 (4.3) | 57 (12.1) | |
| Craftsmen | 2,097 (17.9) | 236 (10.8) | |
| Higher functions | 525 (4.5) | 60 (12.1) | |
| Intermediate professions | 3,064 (26.2) | 335 (11.5) | |
| DHS Wealth Index Quintiles | | | 0.043** |
| Poorest | 2,092 (14.8) | 363 (17.5) | |
| Poorer | 2,827 (20.0) | 345 (12.8) | |
| Middle | 2,979 (21.1) | 293 (11.3) | |
| Richer | 3,054 (21.6) | 301 (9.5) | |
| Richest | 3,198 (22.6) | 319 (10.3) | |
| Marital status | | | 0.123 |
| Never in union | 5,136 (36.3) | 627 (12.2) | |
| Married/living with partner | 8,014 (56.6) | 889 (11.8) | |
| Divorced/separated/widowed | 1,000 (7.1) | 105 (11.1) | |
| Type of residence | | | 0.595 |
| Rural | 7,136 (50.4) | 907 (13.3) | |
| Urban | 7,014 (49.6) | 714 (10.7) | |
| Region | | | <0.001 |
| Adamawa | 1,117 (7.9) | 89 (8.3) | |
| Centre | 2,376 (16.8) | 158 (11.1) | |
| East | 936 (6.6) | 102 (10.9) | |
| Far-North | 1,379 (9.8) | 267 (17.7) | |
| Littoral | 2,176 (15.4) | 91 (9.5) | |
| North | 1,537 (10.9) | 265 (17.5) | |
| North-West | 1,377 (9.7) | 100 (7.0) | |
| West | 1,196 (8.5) | 110 (9.2) | |
| South | 922 (6.5) | 97 (10.7) | |
| South-West | 1,134 (8.0) | 122 (11.1) | |
| Ethnicity | | | <0.001 |
| Arab-choa/peulh/haoussa/kanuri | 1,293 (9.2) | 121 (10.7) | |

| | | | |
|---|---------------|--------------|-------|
| Biu-mandara | 1,478 (10.5) | 278 (18.3) | |
| Adamaoua-oubangui | 1,522 (10.8) | 255 (18.1) | |
| Bantoede-southwest | 212 (1.5) | 13 (5.9) | |
| Grassfields | 1,990 (14.1) | 179 (8.7) | |
| Bamilike/Bamoun | 3,032 (21.5) | 278 (9.4) | |
| Cetier/ngoe/oroko | 673 (4.8) | 55 (8.6) | |
| Beti/bassa/mbam | 2,996 (21.3) | 344 (11.6) | |
| Kako/meka/pygmie | 563 (4.0) | 56 (9.8) | |
| Stranger/other | 328 (2.3) | 33 (10.9) | |
| Injections in past 12 months (<i>missing = 11</i>) | | | 0.867 |
| 0 | 8,367 (59.2) | 989 (12.3) | |
| 1-2 | 2,772 (19.6) | 310 (11.5) | |
| 3-9 | 2,356 (16.7) | 250 (11.1) | |
| >9 | 644 (4.6) | 72 (11.0) | |
| Number of sexual partners in lifetime | | | 0.020 |
| 0 | 2,070 (14.9) | 243 (11.3) | |
| 1-2 | 4,568 (32.9) | 513 (11.9) | |
| 3-9 | 4,922 (35.5) | 585 (12.4) | |
| >9 | 2,313 (16.7) | 259 (11.8) | |
| Place of circumcision (Male only = 6,926) | | | 0.908 |
| Health facility/Home of Health worker | 3,244 (51.5) | 425 (13.1) | |
| Ritual site | 487 (7.7) | 81 (17.8) | |
| Home/other home | 2,572 (40.8) | 335 (13.7) | |
| Anti-HCV antibody | | | 0.012 |
| Negative | 13,991 (98.9) | 1,616 (12.0) | |
| Positive | 159 (1.1) | 5 (4.4) | |
| HIV | | | 0.841 |
| Negative | 13,501 (95.4) | 1,553 (11.9) | |
| Positive | 649 (4.6) | 68 (11.6) | |

**p* value for association between the variable and HBsAg positivity using weighted univariate multilevel logistic regression to account for clustering of HBV within households and within divisions.

***p* value for linear trend

Table 2. Description of covariates at division level

| Variable | N = 58 divisions | |
|--|------------------|-----------|
| | Mean (SD) | Range |
| Prevalence of HBsAg per division in 2011 (%) | | |
| Persons aged 15 – 59 years (all index cases) | 11.1 (3.8) | 6.3-23.7 |
| Persons aged 15 – 29 years (younger index cohort) | 11.8 (5.6) | 2.5-28.5 |
| Persons aged 30 – 59 years (older index cohort) | 10.7 (5.3) | 3.2-30.3 |
| Median age at first childbirth per division (years) | | |
| Time period 1952 to 1996 | 19.0 (0.8) | 17.4-21.1 |
| Time period 1952 to 1981 | 18.9 (0.9) | 17.1-21.1 |
| Time period 1982 to 1996 | 19.0 (0.7) | 17.5-20.7 |
| Mean sibship size per division | 7.0 (0.5) | 5.8-8.8 |
| Mean wealth index per division* | 2.8 (0.8) | 1.3-4.6 |
| Mean age per division (years) | 27.2 (2.0) | 23.1-31.5 |

* Wealth index ranges from 1 to 5; 1 is the poorest and 5 is the richest wealth quintile.

Table 3. Spatial association between the division-level median maternal age at first childbirth and the division-level HBsAg prevalence, stratified by the age group (N=58 divisions of Cameroon)

| Variable | Univariate OLS | | Multiple OLS | | Multiple *ESF | |
|--|-----------------------|----------------|-----------------------|----------------|-----------------------|----------------|
| | β (95% CI) | <i>p</i> value | $a\beta$ (95% CI) | <i>p</i> value | $a\beta$ (95% CI) | <i>p</i> value |
| Median age at first childbirth | -2.60 (-3.72 – -1.48) | <0.001 | -1.89 (-3.38 – -0.40) | 0.013 | -1.89 (-2.52 – -1.26) | <0.001 |
| Mean sibship size | 2.64 (0.88 – 4.40) | 0.004 | 0.83 (-1.06 – 2.73) | 0.381 | 0.83 (0.03 – 1.64) | 0.042 |
| Mean wealth index | -2.11 (-3.33 – -0.91) | <0.001 | -0.90 (-2.33 – 0.53) | 0.211 | -0.91 (-1.51 – -0.30) | 0.004 |
| Mean age | -0.55 (-1.06 – -0.03) | 0.039 | 0.03 (-0.52 – 0.58) | 0.913 | 0.02 (-0.20 – 0.26) | 0.796 |
| Model parameters | | | | | | |
| Eigenvector selection | - | - | - | - | 9 vectors selected | - |
| Moran's I of residuals | - | - | 0.597 | <0.001 | -0.17 | 0.527 |
| AIC | - | - | 309.56 | - | 216.58 | - |
| R ² | - | - | 0.319 | - | 0.899 | - |
| Stratified analysis: Persons aged 15 – 29 years (younger index cohort with birth period 1982 to 1996) | | | | | | |
| Median age at first childbirth | -3.32 (-5.17 – -1.47) | 0.001 | -2.56 (-4.81 – -0.31) | 0.026 | -2.56 (-4.25 – -0.87) | 0.004 |
| Mean sibship size | 3.26 (0.65 – 5.87) | 0.015 | 1.42 (-1.44 – 4.29) | 0.325 | 1.42 (-0.73 – 3.57) | 0.190 |
| Mean wealth index | -2.13 (-3.99 – -0.28) | 0.025 | -0.55 (-2.62 – 1.53) | 0.600 | -0.54 (-2.10 – 1.01) | 0.484 |
| Model parameters | | | | | | |
| Eigenvector selection | - | - | - | - | 6 vectors selected | - |
| Moran's I of residuals | - | - | 0.300 | <0.001 | -0.12 | 0.509 |
| AIC | - | - | 352.99 | - | 325.04 | - |
| R ² | - | - | 0.213 | - | 0.609 | - |
| Stratified analysis: Persons aged 30 – 59 years (older index cohort with birth period 1952 to 1981) | | | | | | |
| Median age at first childbirth | -1.35 (-2.96 – 0.27) | 0.099 | -0.76 (-2.41 – 0.89) | 0.360 | -0.76 (-2.23 – 0.72) | 0.308 |
| Mean sibship size | 1.85 (-0.71 – 4.41) | 0.152 | 0.34 (-2.39 – 3.07) | 0.802 | 0.34 (-2.11 – 2.79) | 0.779 |
| Mean wealth index | -2.40 (-4.12 – -0.68) | 0.007 | -2.06 (-4.00 – -0.12) | 0.038 | -0.54 (-3.81 – -0.31) | 0.015 |
| Model parameters | | | | | | |
| Eigenvector selection | - | - | - | - | 4 vectors selected | - |
| Moran's I of residuals | - | - | 0.131 | 0.022 | -0.11 | 0.521 |
| AIC | - | - | 345.18 | - | 336.38 | - |

| | | | | | | |
|----------------|---|---|-------|---|-------|---|
| R ² | - | - | 0.144 | - | 0.366 | - |
|----------------|---|---|-------|---|-------|---|

OLS: Ordinary least squares regression

ESF: Eigenvector spatial filtering regression *ESF chosen as best spatial model (see Supplementary Figure 8 for performance of the ESF model)

β : Linear regression coefficient for a unit increase in the covariate (median age at first childbirth in years, mean sibship size, mean wealth index, and mean age in years)

a β : adjusted linear regression coefficient, adjusted for the other variables on the table

Table 4. Association between the division-level median maternal age at first childbirth and the individual-level HBsAg positivity, stratified by the age group (N=14,150)*

| Variable | Univariate multilevel logistic regression | | Multiple multilevel logistic regression | | Multiple multilevel logistic regression with ESF | |
|--|---|---------|---|---------|--|---------|
| | OR (95% CI) | p value | aOR (95% CI) | p value | aOR (95% CI) | p value |
| Individual level | | | | | | |
| Age group | | 0.001 | | 0.001 | | 0.001 |
| 15-29 years | Ref. | | Ref. | | Ref. | |
| 30-44 years | 0.95 (0.83 – 1.07) | | 0.93 (0.82 – 1.06) | | 0.93 (0.82 – 1.05) | |
| 45-59 years | 0.67 (0.54 – 0.84) | | 0.65 (0.52 – 0.81) | | 0.64 (0.52 – 0.80) | |
| Household level | | | | | | |
| DHS Wealth Index Quintiles | | <0.001 | | 0.008 | | 0.004 |
| Poorest | Ref. | | Ref. | | Ref. | |
| Poorer | 0.66 (0.51 – 0.86) | | 0.76 (0.58 – 0.99) | | 0.81 (0.63 – 1.03) | |
| Middle | 0.52 (0.39 – 0.70) | | 0.61 (0.45 – 0.84) | | 0.64 (0.50 – 0.83) | |
| Richer | 0.52 (0.40 – 0.68) | | 0.65 (0.49 – 0.86) | | 0.67 (0.52 – 0.85) | |
| Richest | 0.53 (0.40 – 0.69) | | 0.71 (0.52 – 0.97) | | 0.69 (0.52 – 0.91) | |
| Division level | | | | | | |
| Mean sibship size | 1.55 (1.23 – 1.96) | <0.001 | 1.17 (0.93 – 1.47) | 0.180 | 1.12 (0.88 – 1.44) | 0.346 |
| Median age at first childbirth | 0.76 (0.67 – 0.86) | <0.001 | 0.86 (0.76 – 0.97) | 0.013 | 0.83 (0.72 – 0.96) | 0.016 |
| Model parameters | | | | | | |
| Eigenvector selection | - | - | - | - | 4 vectors selected | - |
| Deviance | - | - | 9922.51 | - | 9871.42 | - |
| Stratified analysis: Persons aged 15–29 years (N=8,051) | | | | | | |
| Median age at first childbirth | 0.70 (0.61 – 0.81) | <0.001 | 0.82 (0.71 – 0.94) | 0.006 | 0.81 (0.68 – 0.96)** | 0.013 |
| Stratified analysis: Persons aged 30–44 years (N=4,386) | | | | | | |
| Median age at first childbirth | 0.84 (0.73 – 0.96) | 0.013 | 0.88 (0.77 – 1.00) | 0.061 | 0.82 (0.70 – 0.96)** | 0.014 |
| Stratified analysis: Persons aged 45–59 years (N=1,713) | | | | | | |
| Median age at first childbirth | 0.83 (0.68 – 1.03) | 0.088 | 1.05 (0.84 – 1.32) | 0.643 | 1.01 (0.79 – 1.28)** | 0.944 |

* Marginal (population average) odds ratios reported

**Adjusted for age group, DHS Wealth Index, Mean sibship size

Figure legends

Figure 1. Spatial distribution of HBsAg positivity and covariates in Cameroon

Figure 2. Ecological correlation between country-level median maternal age at first childbirth and country-level HBsAg prevalence in Africa. A: HBsAg prevalence estimated by Schweitzer *et al.* **B:** HBsAg prevalence estimated by The Polaris Observatory Collaborators.

Figure 1. Spatial distribution of HBsAg positivity and covariates in Cameroon

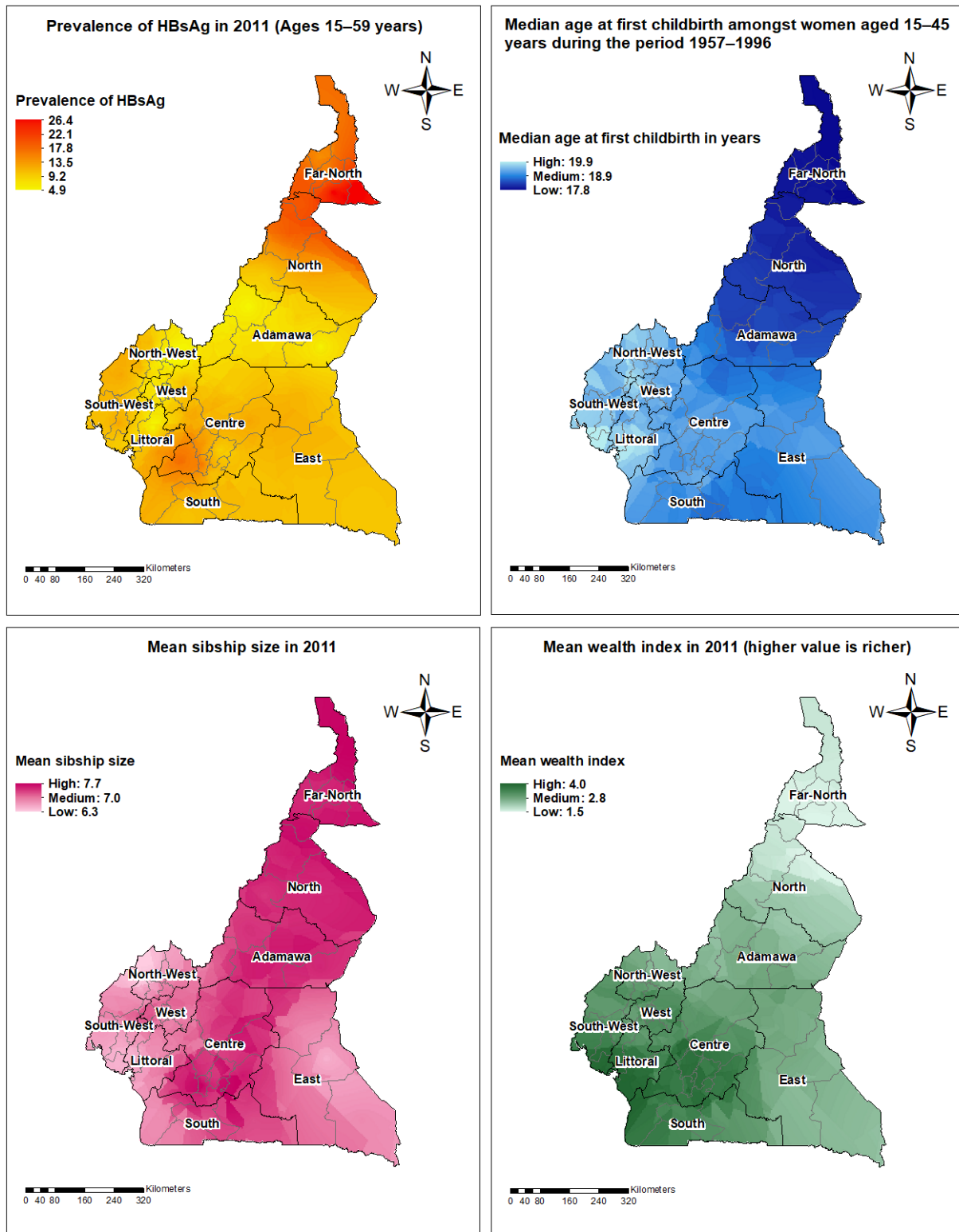
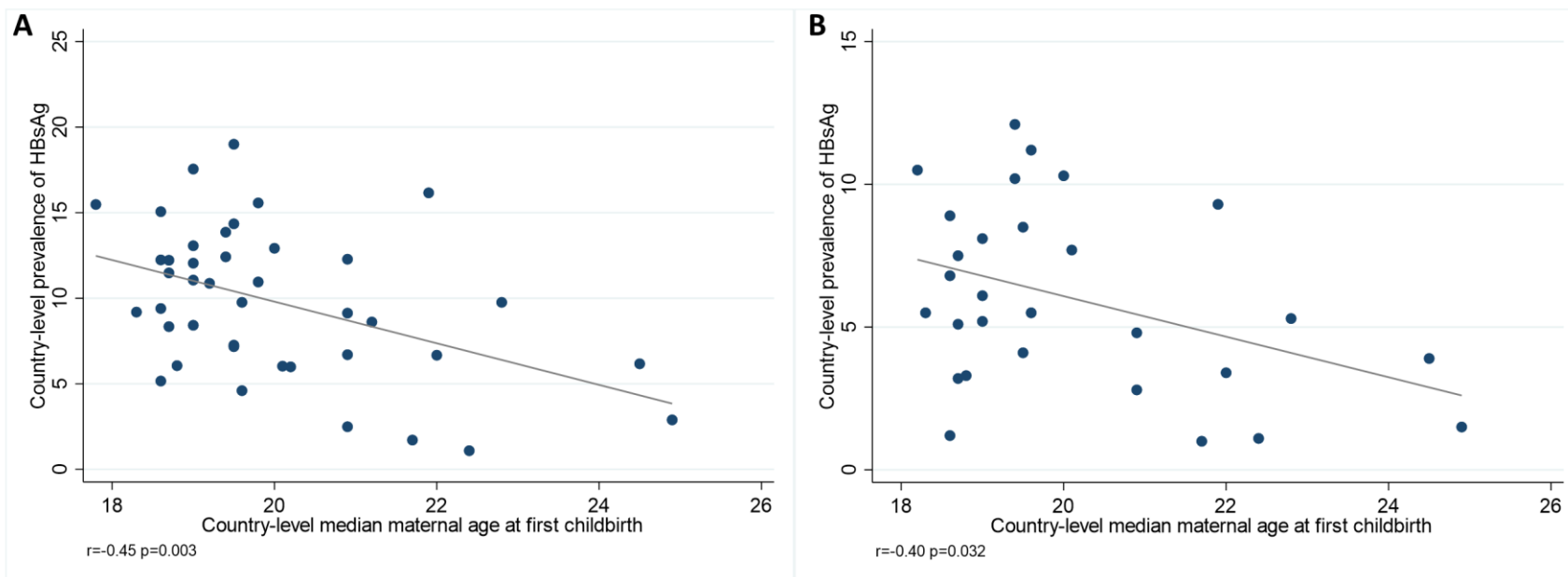


Figure 2. Ecological correlation between country-level median maternal age at first childbirth and country-level HBsAg prevalence in Africa.

A: HBsAg prevalence estimated by Schweitzer *et al.*

B: HBsAg prevalence estimated by The Polaris Observatory Collaborators.

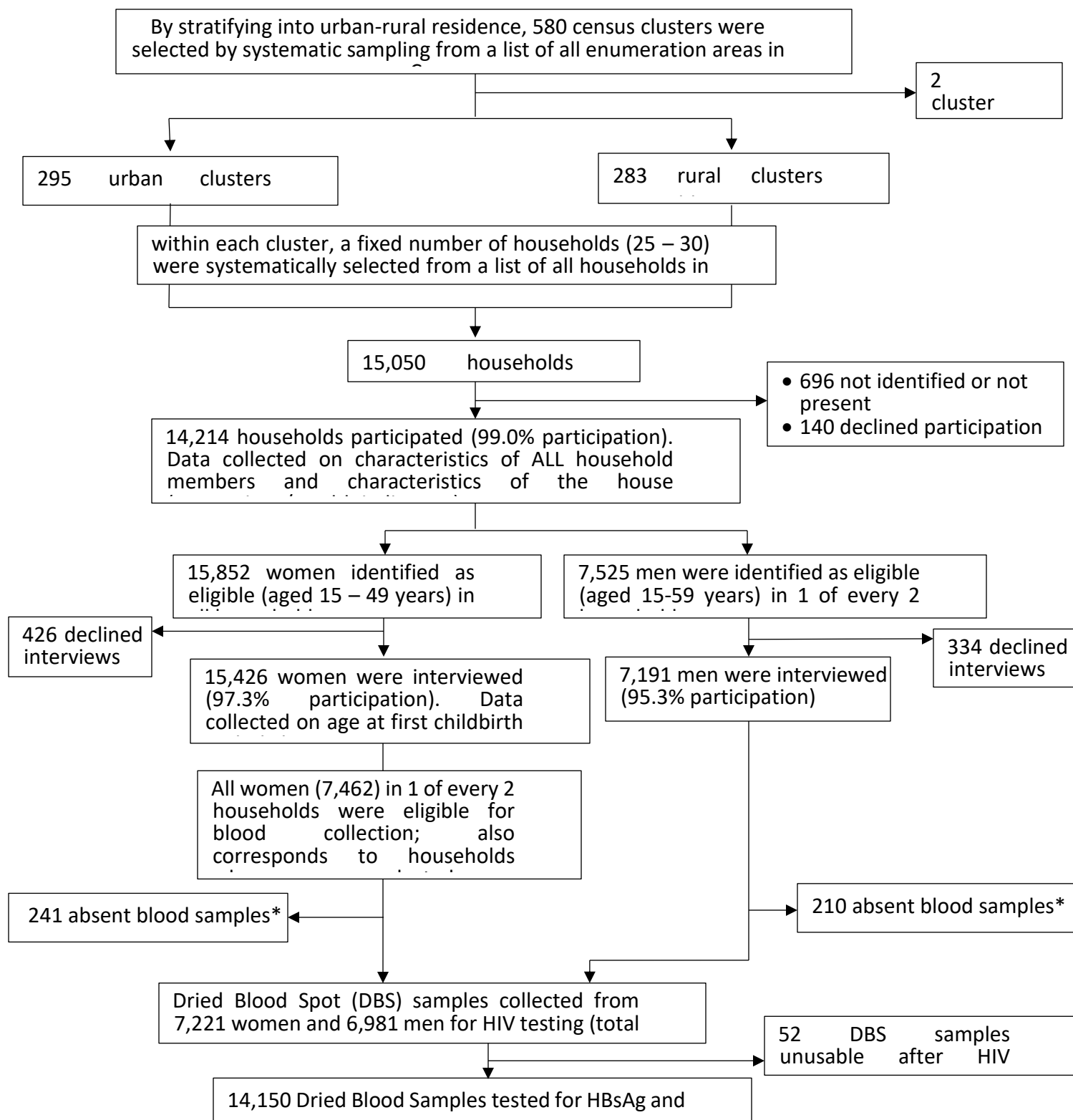


Supplementary material

Table of Contents

| | |
|--|----|
| Supplementary Figure 1: Flowchart of participants in Cameroon DHS 2011 | 2 |
| Supplementary Figure 2: Computation of median maternal age at first childbirth from the DHS datasets | 3 |
| Supplementary Figure 3: Computation of median maternal age at first childbirth for Fako division (as an example)..... | 5 |
| Supplementary Figure 4: Directed Acyclic Graph | 6 |
| Supplementary Figure 5: Clusters and Spatial Outliers of HBsAg in Cameroon based on Local Moran's I values. | 7 |
| Supplementary Figure 6: Ecological correlations between the prevalence of HBsAg and the covariates at division level (N=58 divisions of Cameroon)..... | 8 |
| Supplementary Figure 7: Ecological correlations between the median maternal age at first childbirth and the other covariates at division level (N=58 divisions of Cameroon)..... | 9 |
| Supplementary Figure 8: Performance of the ESF model in adjusting for spatial autocorrelation | 10 |
| Supplementary Figure 9: Scatter plot with linear model fitted showing interaction between the current age and the association between the division-level median maternal age at first childbirth and the division-level prevalence of HBsAg..... | 11 |

Supplementary Figure 1: Flowchart of participants in Cameroon DHS 2011



*includes persons who denied giving blood, absent at time of blood collection, missing samples, and other reasons such as labelling inconsistencies and insufficient samples

Supplementary Figure 2: Computation of median maternal age at first childbirth from the DHS datasets

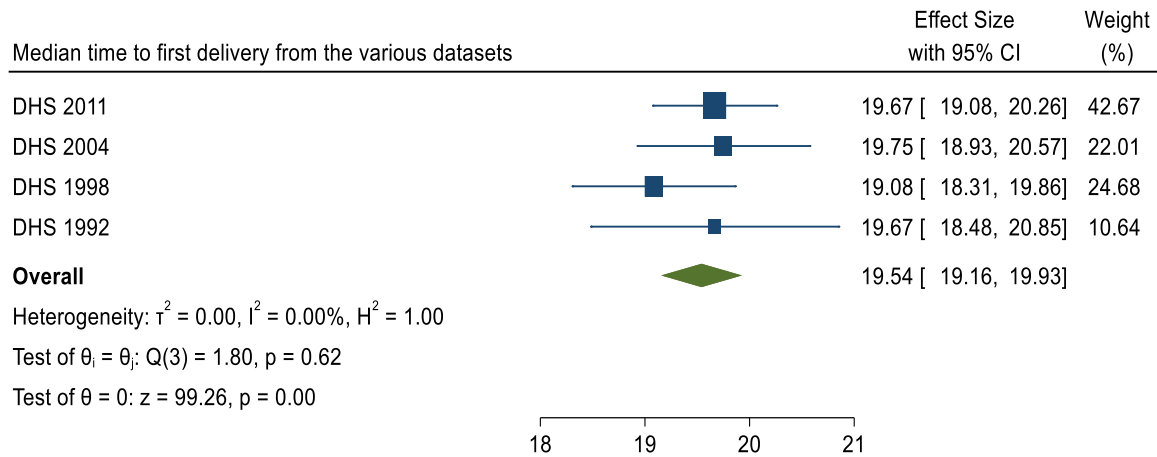
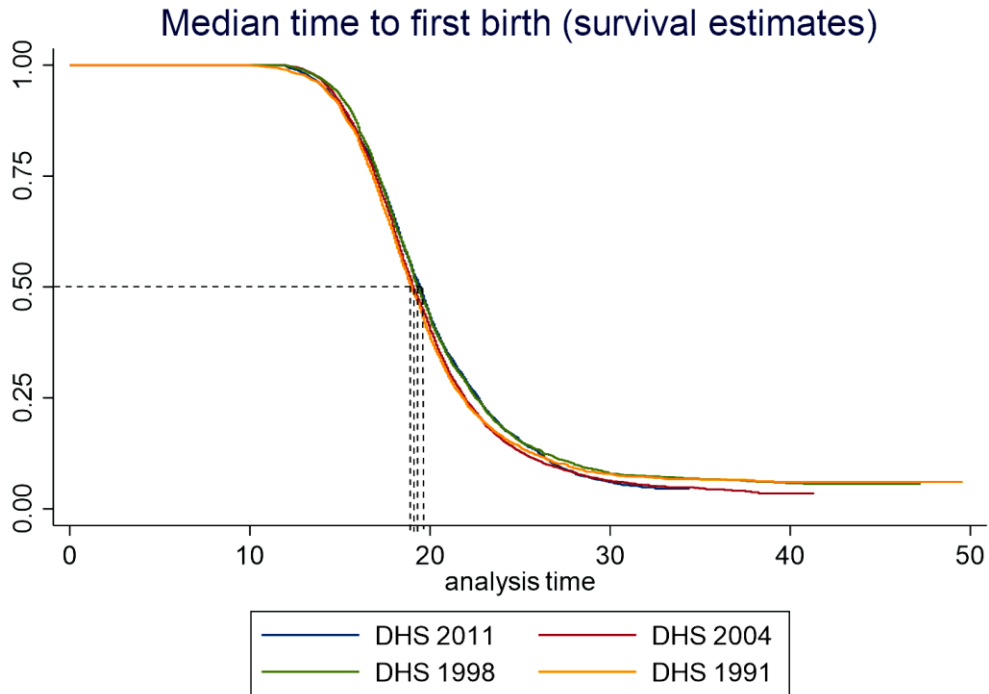


— All index cases
 — Index cases aged 15-29 years
 — Index cases aged 30-59 years

Years of birth of the index cohort and the maternal cohort by the DHS datasets

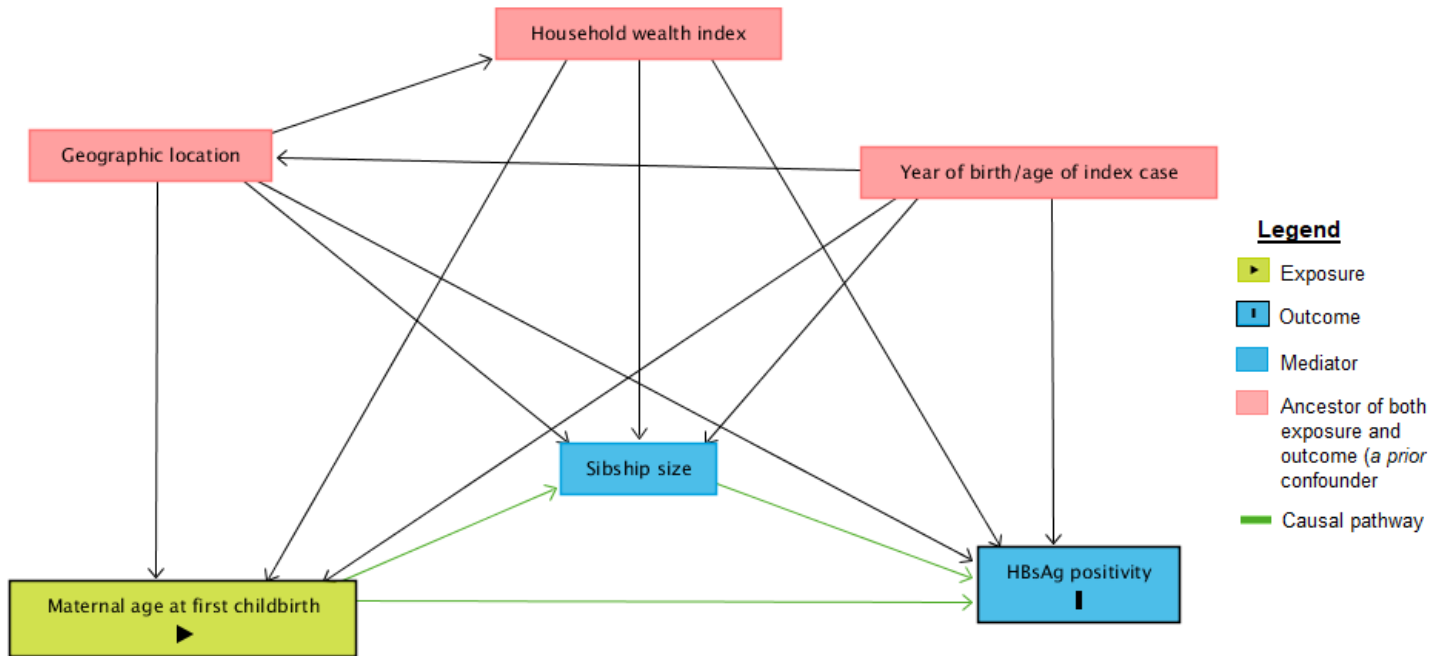
| Type | All index cohort | Old index cohort (blue) | Young index cohort (red) |
|---|------------------|-------------------------|--------------------------|
| Ages of the index cohort in 2011 | 15-59 years | 30-59 years | 15-29 years |
| Years of birth of the index cohort | 1952 - 1996 | 1952 - 1981 | 1982 - 1996 |
| Years of birth of the maternal cohort, assuming the reproductive age of 15-49 years | 1903 - 1981 | 1903 - 1966 | 1933 - 1981 |
| Years of birth of the maternal cohort covered by the DHS 2011 dataset (providing maternal cohort for index cases born between 1977 – 1996) | 1962 - 1981 | 1962 - 1966 | 1962 - 1981 |
| Maternal dates of birth from DHS 2004 (providing maternal cohort for index cases born between 1970 – 1996) | 1955 - 1981 | 1955 - 1966 | 1955 - 1981 |
| Maternal dates of birth from DHS 1998 (providing maternal cohort for index cases born between 1964 – 1996) | 1949 - 1981 | 1949 - 1966 | 1949 - 1981 |
| Maternal dates of birth from DHS 1991 (providing maternal cohort for index cases born between 1957 – 1991) | 1942 - 1976 | 1942 - 1966 | 1942 - 1976 |

Supplementary Figure 3: Computation of median maternal age at first childbirth for Fako division (as an example)



Random-effects ML model for Division of Fako

Supplementary Figure 4: Directed Acyclic Graph



¹ **Minimal set of adjustment for estimating the total effect (MTCT & early childhood transmission due to a large sibship size):** Geographic location, Household wealth index, Year of birth/age of index case

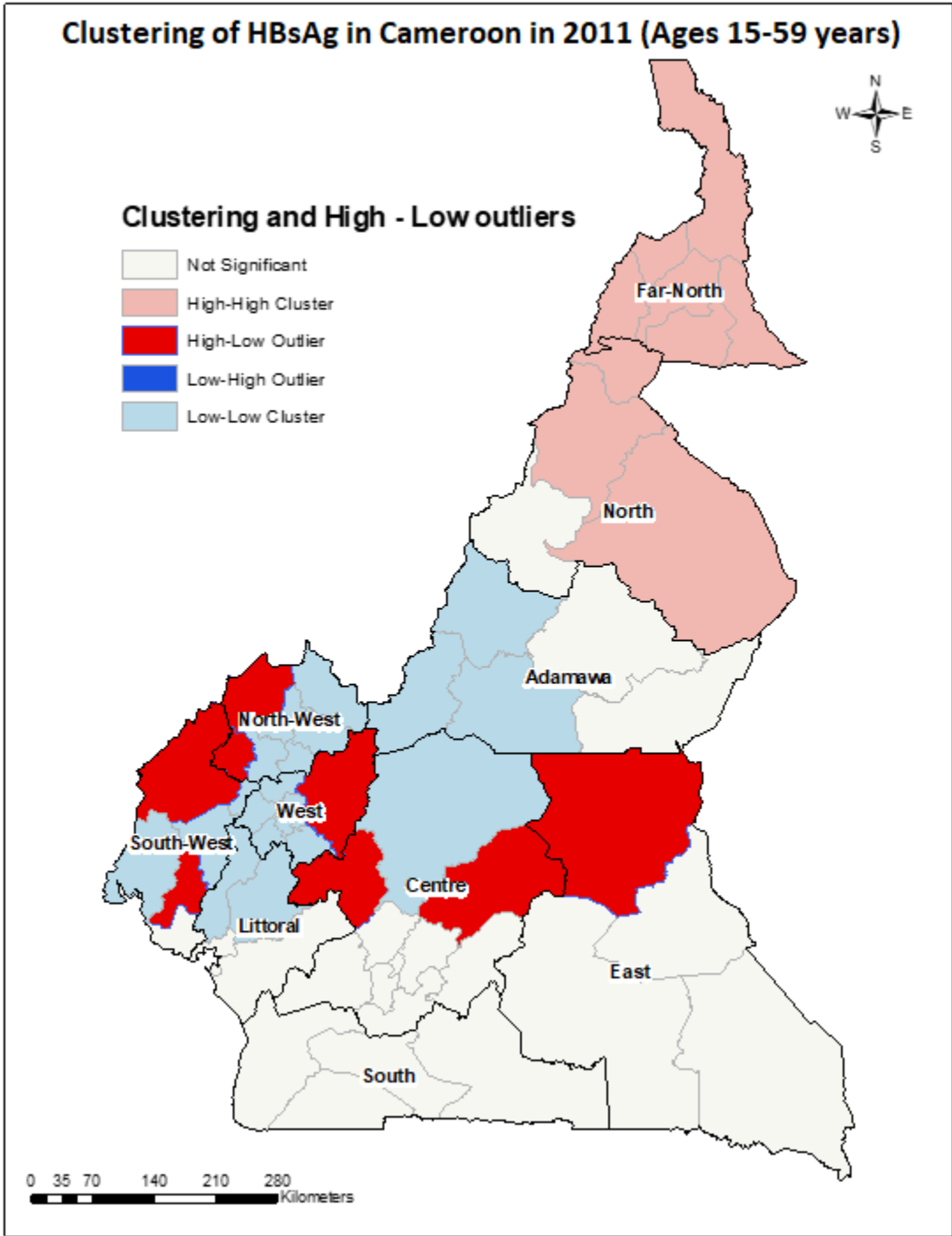
Minimal set of adjustment for estimating the direct effect (MTCT only): Geographic location, Household wealth index, Sibship size, Year of birth/age of index case. This blocks the pathway through sibship size (early childhood transmission due to a large sibship size).

In the analysis (scatter plots), all *a priori* confounders were shown to be associated with both the exposure and the outcome.

Effect modification: Possible effect modification of the association between maternal age at birth of the first child and chronic HBV infection by age/period of birth based on *a priori* knowledge.

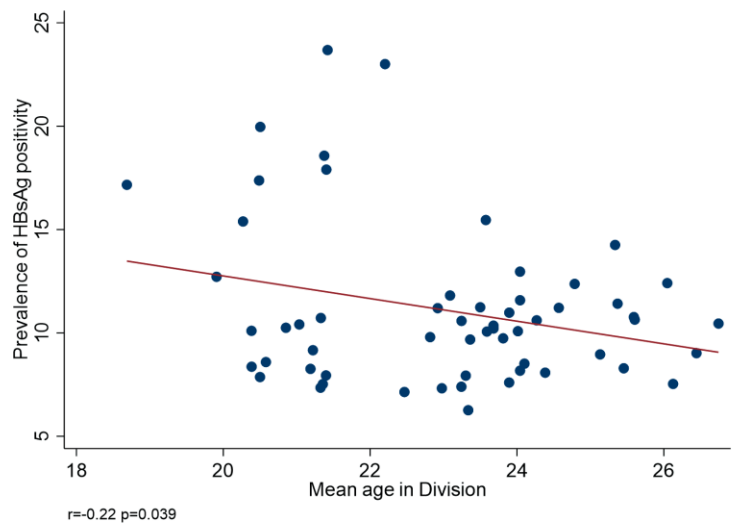
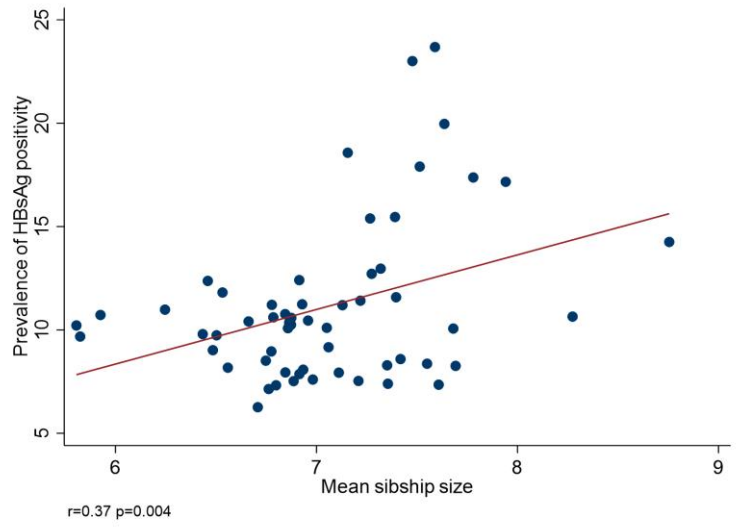
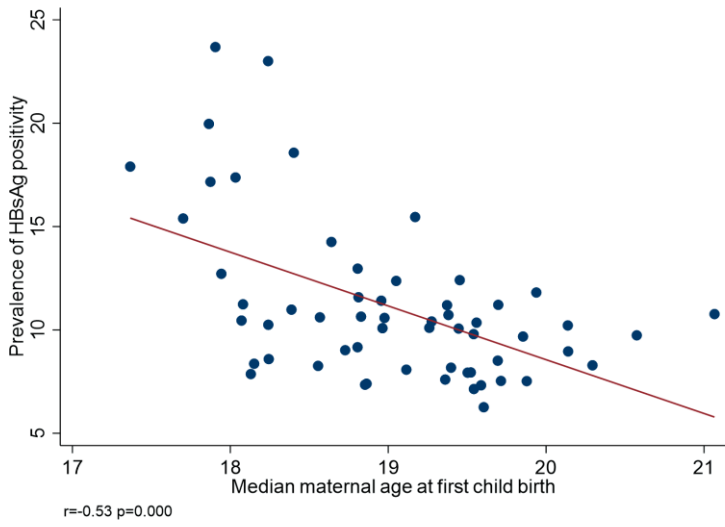
¹ Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology* (Cambridge, Mass) 1999; 10(1): 37-48.

Supplementary Figure 5: Clusters and Spatial Outliers of HBsAg in Cameroon based on Local Moran's I values.

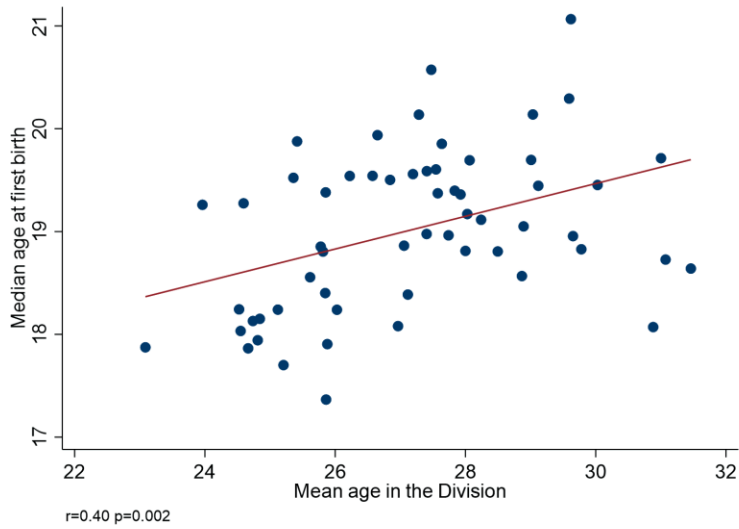
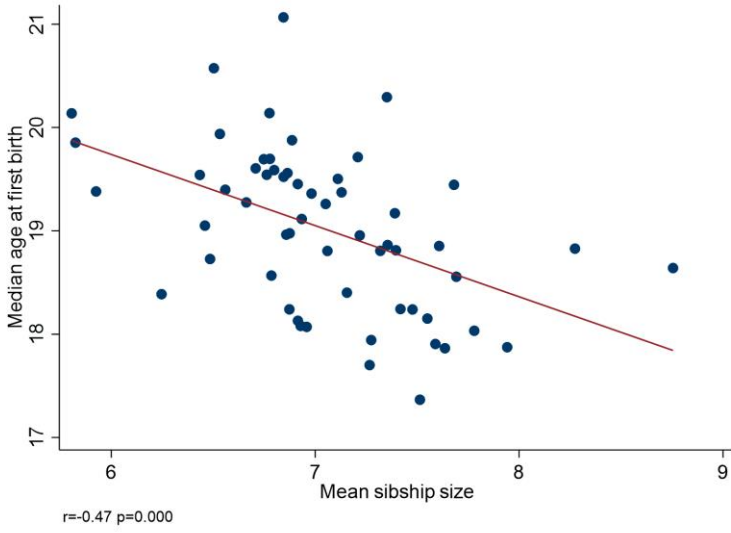


Global Moran's Index: 0.338, p-value: <0.001

Supplementary Figure 6: Ecological correlations between the prevalence of HBsAg and the covariates at division level (N=58 divisions of Cameroon)

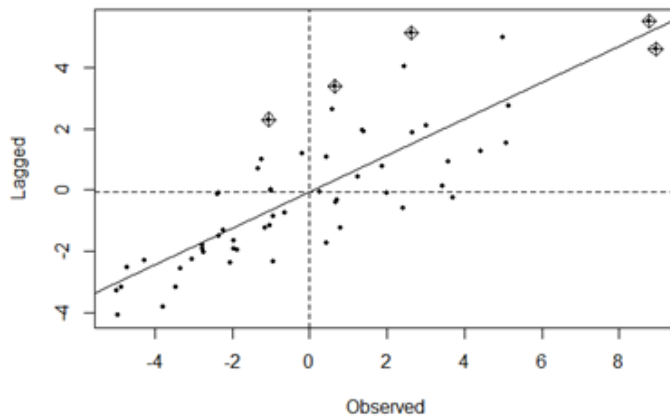


Supplementary Figure 7: Ecological correlations between the median maternal age at first childbirth and the other covariates at division level (N=58 divisions of Cameroon)

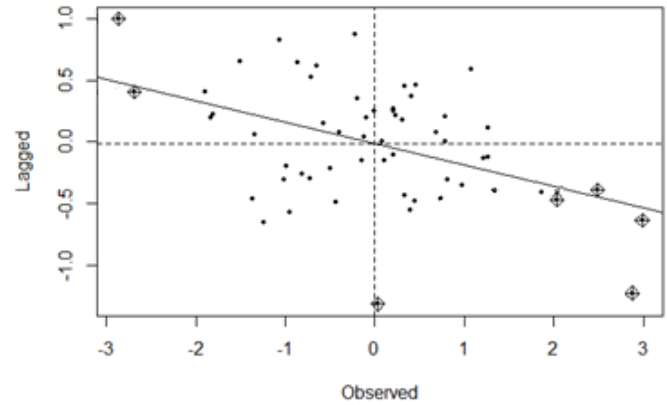


Supplementary Figure 8: Performance of the ESF model in adjusting for spatial autocorrelation

Moran plot of residuals of OLS ($I = 0.60, p < 0.001$)

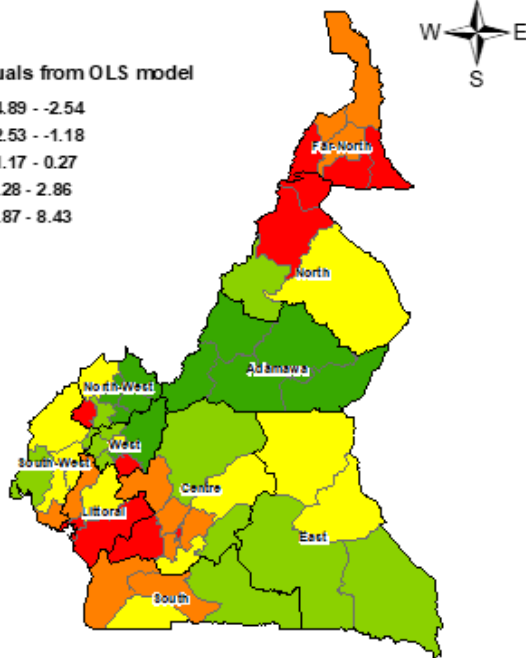
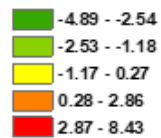


Moran plot of residuals of ESF ($I = -0.17, p = 0.527$)



Plot of residuals from Ordinary Least Squares model

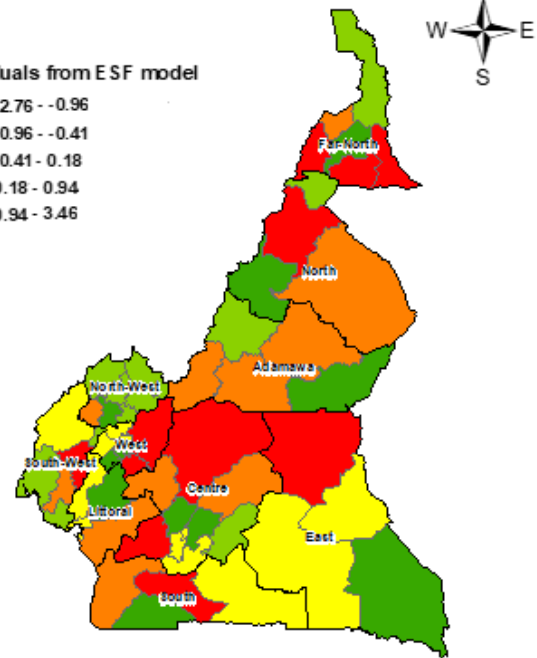
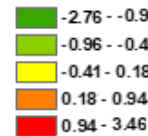
Residuals from OLS model



0 40 80 160 240 320 Kilometers

Plot of residuals from Eigenvector Spatial Filtering model

Residuals from ESF model



0 40 80 160 240 320 Kilometers

* Choropleths of the residuals from the models. There are aggregations of the residuals from the OLS model indicating spatial autocorrelation, while the residuals from the ESF model are randomly distributed implying minimal spatial autocorrelation in the ESF model.

OLS: Ordinary least squares; ESF: Eigenvector spatial filtering

Supplementary Figure 9: Scatter plot with linear model fitted showing interaction between the current age and the association between the division-level median maternal age at first childbirth and the division-level prevalence of HBsAg

