Utilising a Cohort Study of Hepatitis B Virus (HBV) Vaccine-Mediated Immunity in South African Children to Model Infection Dynamics: Can We Meet Global Targets for Elimination by 2030?

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RUNNING HEAD: HBV vaccine responses and model of timescale to elimination

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ABBREVIATIONS

- HBcAg – Hepatitis B core antigen
- HBeAg – Hepatitis B envelope antigen
- HBsAg – Hepatitis B surface antigen
- Anti-HBc – antibody to hepatitis B core antigen (antibody mediated by exposure to infection)
- Anti-HBe – antibody to hepatitis B envelope antigen
- Anti-HBs – antibody to hepatitis B surface antigen (vaccine-mediated antibody)
- ART – anti-retroviral therapy
- COSAC – coinfection in South African children
- EPI – Expanded Programme on Immunisation
- HBV – hepatitis B virus
- HIV – human immunodeficiency virus (type 1)
- KReC – Kimberley Respiratory Cohort
- PMTCT – prevention of mother to child transmission
- RTHB – Road to Health Book
- WHO – World Health Organisation

ABSTRACT

Background: Sustainable Development Goals and the World Health Organisation (WHO) Global health sector strategy on viral hepatitis have set a challenge for the elimination of hepatitis B virus (HBV) infection as a public health concern by the year 2030. Based on current estimates of 250 million individuals with chronic infection, an intensive scale-up of preventive interventions will be required to achieve this goal, alongside enhanced diagnosis and treatment. Although a safe, effective HBV vaccine has been part of the Expanded Programme on Immunisation since the mid-1990's, the extent to which enhanced immunization can contribute to these elimination targets is currently uncertain. We set out to characterise the epidemiology of HBV infection and the prevalence of vaccine-mediated protection in a cohort of South African children in order to inform a model of HBV transmission and prevention. This has allowed us to develop robust, evidence-based insights into the extent to which scaling up vaccination and prevention of mother-to-child transmission (PMTCT) might ultimately contribute to HBV elimination, and to assess the extent to which the targets for 2030 are realistic.

Methods and findings: We studied a cohort of 310 children (136 HIV-positive; 174 HIV-negative) aged 6-60 months in Kimberley, South Africa. We screened for HBV infection (HBsAg) and exposure (anti-HBc); these were each present in 3 children (<1% prevalence). A vaccine-mediated antibody (anti-HBs) titre ≥10 mIU/ml was present in 238/310 children (77%). The mean Anti-HBs titre in HIV-negative participants was significantly higher than among HIV-positive children ($p<0.0001$). Comparing the 136 HIV-positive children with an additional group of older children, also with HIV infection (n=92, age >60 months), we demonstrated significantly higher antibody titres in the younger group ($p<0.0001$). We used observations made in this cohort, alongside previous estimates of HBV transmission and epidemiology, and published population statistics for South Africa, to underpin a model for HBV infection dynamics. We used this model to investigate the influence of prevention strategies, focusing on vaccination and PMTCT. Current vaccination efforts can be predicted to reduce population prevalence by ~20% in the first 25 years, but can bring the prevalence of HBV e-antigen (HBeAg)-positive chronic carriers down by ~40% in the same time period. There is additional benefit in providing catch-up vaccination, with higher short-term impact but little long-term difference. Combining neonatal vaccination with robust PMTCT is the most effective population-level strategy to secure short-term impact, but coverage of both interventions needs to be high. Overall, the model demonstrates that with strategies and resources already available, sustained control with significant, positive public health impact is possible, although time to elimination is substantially longer than that proposed by current goals.

Conclusions: At the level of an individual cohort, these data reflect the substantial overall success of HBV immunisation, with <1% of children now becoming infected with HBV in an endemic setting, despite the high population prevalence of HIV infection. These findings endorse the existing efforts of vaccine campaigns in protecting individual children from chronic HBV infection. However, we go on to demonstrate that vaccination alone is not sufficient to eliminate this endemic virus; moreover, to move towards the target of elimination, major improvements in vaccination deployment and coverage are required, and enhanced efforts are required to provide diagnosis and treatment to antenatal women to prevent vertical transmission. Realistic targets, rather than focusing on a complete elimination of the public health threat posed by HBV by the year 2030, may be better aiming for a substantial reduction in prevalence, which will come about through vaccination, PMTCT, and reduction of HBeAg-positive carriage. The magnitude of the elimination challenge, and the long time periods of sustained investment that will be required, underline the crucial importance of parallel investment into diagnostics, advocacy, policy, education and ongoing research into HBV cure strategies.

INTRODUCTION

The World Health Organisation (WHO) has recently published strategies targeting the elimination of viral hepatitis as a major public health threat by 2030 (1,2). The magnitude of this challenge becomes apparent on reviewing the burden of mortality and morbidity attributed to these infections: overall, hepatitis viruses are responsible for an estimated 1.4 million deaths annually. Of these, 47% are directly attributable to hepatitis B virus (HBV) (1), which is currently estimated to be responsible for chronic infection in 240-260 million individuals worldwide (3). The highest prevalence of HBV infection, frequently >8%, is reported in Africa and South East Asia (4), but African populations are under-represented in the current literature.

In South Africa, HBV is endemic: the estimated prevalence among adults is 6-11% (4–6), with a disproportionately higher prevalence occurring in rural regions (7,8). Conventional parenteral transmission routes are well recognised, but a substantial proportion of infections in South Africa have traditionally been attributed to horizontal transmission within households during early childhood (9). In a recent study in Kimberley, we demonstrated cases of HBV infection among children with HIV (10), despite the inclusion of the HBV vaccination in routine childhood immunisation programmes in South Africa since 1995 (9).

This vaccine, a safe and affordable recombinant surface antigen protein, has been available for several decades (11,12), and is highly efficacious, generating immunity to HBV in >90% of immunocompetent individuals after a primary course of three doses (11,13,14). Since the mid-1990s, the WHO has encouraged universal coverage of the HBV vaccine through its Expanded Programme on Immunisation (EPI) (15) and the majority of countries now offer three doses of HBV vaccination in infancy. Antenatal screening for HBV, to reduce the risk of vertical transmission, is also advocated by the WHO (16). The administration of the HBV vaccine and HBV immune globulin immediately after birth for babies born to HBV-positive mothers has been demonstrated to reduce the risk of vertical transmission to ≤3%, with high maternal HBV viral loads and HBV envelope antigen (HBeAg) positivity being risk factors for transmission despite prophylaxis (17).

EPI guidelines recommend universal administration of the HBV vaccine, with advice being to provide the primary dose in the first day of life (3,18). However, there are several challenges to the success of this strategy. In South Africa, the first dose is conventionally delayed until age 6 weeks with subsequent doses at 10 and 14 weeks, leaving a window during which vertical transmission can occur peripartum or in the early weeks of life (19). Coverage of the third vaccine dose is difficult to ascertain with confidence; estimates for coverage in the first

year of life range from 56-97% (9). South Africa's high HIV prevalence (estimated 12.7% (20)) poses a further challenge to the success of national HBV initiatives, as being HIV positive can increase the risk of peripartum transmission of HBV, and the HBV vaccine has been demonstrated to have reduced efficacy in HIV positive individuals (21–24).

Vaccine modelling studies, which include 'number needed to vaccinate' (NNV) approaches, can be used to inform and advise on intervention strategies and to develop cost-benefit analyses for vaccination programmes (25). Such models are based on parameters that include vaccine efficacy, and the incidence, prevalence and transmission rates of a given infection (26). A recent modelling study has considered the contribution of vaccination towards the global elimination of HBV (27), but the broad approach and inclusion of vaccination as one of many potential interventions makes it difficult to single out the role and influence of immunisation. Although HBV is one of the most robust and widely used vaccines, to the best of our knowledge there is no specific HBV vaccine model in the published literature.

In order to investigate HBV vaccine coverage and vaccine-mediated immunity in South Africa, we set out to investigate HBV sero-epidemiology. We then used these data from one specific site to prime a model that illustrates HBV dynamics and transmission, providing us with a tool to investigate the impact of preventive interventions. The model allows us to assess the extent to which current elimination goals are realistic, and to develop an understanding of specific ways in which prevention strategies can be developed and improved, underpinning wise deployment of limited resources, and providing insights that inform our approach to the challenges of measuring progress - with the ultimate goal of elimination.

MATERIALS AND METHODS

Ethics Approval

Ethics approval for the study was obtained from the Ethics Committee of the Faculty of Health Science, University of the Free State, Bloemfontein, South Africa (HIV Study Ref: ETOVS Nr 08/09 and COSAC Study Ref: ECUFS NR 80/2014). Written consent for enrolment into the study was obtained from the child's parent or guardian.

Study cohort

Children were recruited as part of the Co-infection in South-African Children (COSAC) study, in Kimberley, South Africa, as previously described (10,28). We set the lower age limit of recruitment as 6 months in order to limit the detection of maternal anti-HBs in younger

infants, and the upper limit at 60 months in order to optimise the capture of hand-held vaccine records (Road to Health Book, RTHB) carried by children aged under 5. These children were recruited from two sources:

i. HIV-negative participants (n=174), recruited through the Kimberley Respiratory Cohort (KReC) as previously described (28). These children were admitted to hospital with a clinical diagnosis of respiratory tract infection between July 2014 and August 2016. The majority of KReC children were routinely HIV-tested as a component of their clinical assessment, and were confirmed HIV-negative in 163 cases (93.7%). A further 11 children did not have an HIV test result recorded, but were assumed to be HIV-negative for the analysis based on the clinical data that were available at the time of admission to hospital.

ii. HIV-positive children (n=136) recruited primarily from HIV outpatient clinics, (recruited between September 2009 and July 2016). This includes five children who were recruited into the KReC study but subsequently tested HIV-positive. For HIV-positive children, we recorded date of commencement of ART (anti-retroviral therapy), CD4+ T cell count, CD4+ T cell percentage, and HIV RNA viral load, when these data were available. We recorded these information using the time point closest to the sample that was analysed for HBV serology.

In order to study the influence of age on vaccine-mediated responses, we also collected data from a third group of older HIV-positive children (age >60 months, range 64-193 months, n=92) as previously described (10,28). Where possible, we recorded the number of HBV vaccine doses received based on the RTHB. At the time of undertaking this study, children were immunised with three doses of a monovalent HBV vaccine (Biovac Paed). The characteristics of the cohorts are summarised in table 1 and all metadata can be found in Suppl. data 1.

Table 1: Characteristics of three paediatric study cohorts, comprising 402 children, recruited from Kimberley Hospital, South Africa.

Cohort	HIV negative; KReC (age ≤60 months)	HIV positive (age ≤60 months)	HIV positive (age >60 months)
Number of subjects	174	136	92
Age range in months	8-58	6-60	64-193
Median age in months (IQR)	18 (12-26)	29 (18-40)	137 (122-154)
Sex (% male)	55.4	44.9	45.6

KReC = Kimberley Respiratory Cohort. IQR = interquartile range.

Laboratory assessment of HBV status

Testing for Hepatitis B serum markers and DNA was performed as previously described; for HIV-positive children this is in keeping with recent implementation of HBV screening in Kimberley (10). Briefly, HBsAg testing was carried out in Kimberley Hospital, South Africa using the Magnetic parcel chemiluminometric immunoassay (MPCI; Advia Centaur platform). Confirmatory HBsAg testing was carried out by the UKAS accredited clinical microbiology laboratory at Oxford University Hospitals (OUH) NHS Foundation Trust, Oxford, UK (Architect i2000). For all samples, anti-HBs and anti-HBc testing were carried out by the OUH laboratory (Architect i2000). Limit of detection of the anti-HBs assay was 10 mIU/ml.

Setting a threshold for vaccine mediated immunity

In practice, a threshold for vaccine-mediated immunity is difficult to define, and studies variably quote anti-HBs titres of ≥10 mIU/ml or ≥100 mIU/ml as a correlate of protection. UK recommendations for testing HBV immunity often rely on the more stringent criterion of an anti-HBs titre of ≥100 mIU/ml (29). However, early vaccine studies have highlighted that a titre of ≥10 mIU/ml is likely to be a clinically relevant threshold for protection; a study of children in The Gambia showed that children who attained an anti-HBs titre of ≥10 mIU/ml were most likely to be immune (30), and another study demonstrated increased risk of infection when antibody titres fell <10 mIU/ml (31). Due to the varying use of different thresholds, we have presented our results pertaining to both thresholds of ≥10 mIU/ml and ≥100 mIU/ml.

Statistical analysis

Data from the cohort was analysed using GraphPad Prism v.7.0. We determined significant differences between sub-sets within the cohort using Mann-Whitney U tests for non-parametric data, Fisher's exact test for categorical variables and correlation between data points was assessed using Spearman's correlation coefficient. We calculated multi-variable analysis by logistic regression using the Statistics add-on tool in Google Sheets (https://www.google.co.uk/sheets/about/).

Model of HBV transmission and prevention

A mathematical model of HBV transmission was developed using ordinary differential equations. The model is represented schematically in Fig 1A. We consider the population grouped into categories as follows:

- ***Susceptible*** (S); sub-divided into three age groups, representing infants (S_i, <1 yr of age), children (S_c, age 1-6 yrs) and older children and adults (S_a, >6 years of age);
- ***Carriers*** (C); sub-divided into three groups represented as acute infections (designated I), chronic e-antigen positive (HBeAg+, designated C+) and chronic e-antigen negative (HBeAg-, designated C-);
- ***Recovered*** (R); individuals who have been infected but cleared, rendering them immune.
- ***Vaccinated*** (V); individuals who have received a full vaccine schedule and are assumed to have protective titres of vaccine-mediated anti-HBs.

We used the mid-year population estimates from 2016 published by Statistics South Africa (20) to determine demographic data about life expectancy, fertility rate and infant mortality. Alongside all other parameter descriptions and references, these details can be found in suppl. data 2.

Depending on age at infection, individuals could either sustain an acute infection (I) or become carriers (C), as represented by the age-specific parameters γ, ψ, ε for S_i, S_c and S_a respectively. Chronic carriers were assumed to be initially HBeAg+ (C+), but could convert to HBeAg- (C-) at rate θ, and eventually clear infection at a rate ρ. We assumed that acute (I) and HBeAg+ carriers (C+) had a higher transmission potential than acute and HBeAg- carriers. Depending on the infection status of mothers, individuals could be born susceptible (represented by an input of Z into the S_i class) or be infected at birth (represented by an input of W into the C+ class). Population size is constant with equal births (b) and deaths (μ), with HBeAg+ individuals having a shorter lifespan ($1/\mu'$) than the rest of the population. Acutely infected individuals were assumed to enter a recovered class, R, at a rate σ.

Intervention strategies were considered in the model in the form of reductions in vertical transmission (affecting W and Z), and routine and catch-up vaccination (moving individuals to class V). Routine vaccination could take place at birth (with a proportion Z' entering class V) or effectively within the adult population (as a proportion V_a of those leaving S_c entering class V). Catch-up vaccination was modelled by moving a proportion of susceptible individuals in every age class into the vaccinated class in a single time event (not shown in diagram).

The modelling approach is subdivided into three main steps: (i) fitting to demographic and (ii) transmission backgrounds, followed by the (iii) simulation of single or combined

interventions. In the first two steps we effectively fit unknown model parameters (a, c, β, ρ, θ) to population-based observations using a Bayesian Markov-Chain Monte-Carlo (MCMC) approach. After obtaining posteriors for these parameters, we set them to the obtained medians before numerically simulating interventions. Full model details, fitting output, as well as other results and sensitivity experiments are presented in suppl. data 2.

RESULTS

Serological evidence of exposure to HBV infection

Evidence of current infection with HBV, determined by the detection of HBsAg, was observed in three children (0.8% of the cohort; table 2). None of these three children attended with a written vaccination record (RTHB). Anti-HBc was detected in 0.8% subjects (n=3), one of whom was also HBsAg positive. The other two participants were HBsAg negative, indicating previous exposure to HBV infection and likely viral clearance in these subjects.

Table 2: Detailed information and serological profiles of five children from Kimberley, South Africa, with serological evidence of current or previous infection with HBV (based on positive HBsAg (n=3) or anti-HBc (n=2))

Subject	K306	K405	KReC51	KReC151	K093
Cohort	HIV-positive age ≤60 months	HIV positive age ≤60 months	KReC	KReC	HIV positive age >60 months
Sex	F	F	F	M	F
Age (months) at time of sampling	18	37	20	15	118
HIV infection	Positive	Positive	Negative	Negative	Positive
ART[a] (if HIV positive)	Yes	Yes	n/a	n/a	No
Number of doses of HBV vaccine	NK	NK	NK	3	NK
HBsAg result[b]	Detected	Detected	Detected	Not detected	Not detected
Anti-HBc result[c]	Not detected	Not detected	Detected	Detected	Detected
HBeAg result[d]	Not done	Not done	Detected	Not done	Not done
Anti-HBs result[e]	Not detected	Not detected	Not detected	Detected	Not detected
Interpretation	Active	Active	Active	Immunised,	Infected

	infection	infection	infection	infected and cleared	and cleared

[a]ART indicates the participant was receiving anti-retroviral therapy to treat HIV infection; [b]Hepatitis B surface antigen test; [c]Hepatitis B core antibody test; [d]Hepatitis B envelope antigen test; [e]Hepatitis B surface antibody test (vaccine mediated response). KReC = Kimberley Respiratory Cohort. n/a = not applicable.

Documented evidence of vaccination and serological evidence of immunity to HBV in children aged ≤60 months

We were able to collect written documented evidence of immunisations from the RTHB in 90.8% HIV negative (KReC) subjects but only from 6.3% of HIV positive subjects. This means that in the absence of a detectable anti-HBs titre, we cannot reliably distinguish children who are immunised but fail to mount an antibody response from children who are unimmunised. Among children with an RTHB record, 81.3% of HIV-negative and 100% of HIV-positive subjects were recorded as having received three primary doses of the HBV vaccine as stipulated by the EPI.

Across the whole cohort age 6-60 months, 238/310 children (77%) had an anti-HBs titre ≥10 mIU/ml suggesting some degree of vaccine-mediated immunity. The median anti-HBs titre in HIV-negative participants was significantly higher than among HIV-positive children (196.1 mIU/ml, vs. 11.1 mIU/ml, respectively, $p<0.0001$) (Fig 2A). No detectable anti-HBs titre was detected for 3.4% of HIV-negative individuals, vs. 47.8% of HIV-positive subjects ($p<0.0001$). Irrespective of the antibody titre used as a threshold for immunity, anti-HBs was higher in HIV-negative compared to HIV-positive children (Fig 2B, C). We found no significant difference in the anti-HBs titres between male and female participants, either with or without HIV infection (data not shown).

Waning of vaccine response with age

In order to explore the influence of age on titres of vaccine-mediated immunity, we compared the ages of children who achieved anti-HBs ≥100mIU/ml vs those who did not reach this threshold, considering HIV-positive and HIV-negative groups separately. Among HIV-positive children, those with protective antibody titres were significantly younger than those not reaching this threshold (median age 17 months vs. 31 months, $p=0.0008$; Fig 3A). No such difference was observed within the HIV-negative group (Fig 3B). Using a threshold of ≥10mIU/ml, no significant differences were observed in the anti-HBs responses of either the HIV-positive or the HIV-negative groups (data not shown). To expand our view of the HIV-positive group, we also added analysis of an older cohort (92 children aged >60 months),

and demonstrated that anti-HBs titres were significantly lower in this older group (p<0.0001), with only 2/92 subjects (2.2%) achieving a detectable anti-HBs titre of ≥10mIU/ml (Fig 3C).

Anti-HBs titres waned significantly with age up to age 60 months in HIV-positive children (Fig 3D) (p=0.004). No correlation was identified in the HIV-negative cohort (data not shown, p=0.174), but a trend towards a decline was evident over time (Fig 3E).

Stratification of vaccine responses by anti-retroviral therapy (ART) among HIV-positive children

For children aged 6-60 months, we recorded whether or not study participants were treated with ART; this information was available for 79% of subjects. Among these, 71% of HIV-positive study participants were known to be receiving anti-retroviral therapy (ART) at the time of testing for anti-HBs. These children had been treated with ART for varying lengths of time (median 20 months; IQR 6-33 months).

We compared anti-HBs titres of subjects being treated with ART compared to those not currently receiving ART and found no significant difference (p=0.72; 76 children on ART, median anti-HBs 13.3 mIU/ml and 31 children not on ART, median anti-HBs 14.1 mIU/ml). There was also no difference between anti-HBs titres of children on ART for ≤12 months compared with those treated for >12 months (data not shown). We did not examine the effect of ART on anti-HBs titres in children >60 months old due to the low numbers of subjects in this group with a detectable anti-HBs titre (n=2).

Multivariate analysis

In a multivariate analysis, two factors were identified as predictive of a protective anti-HBs titre: age <24 months and HIV-negative status (table 3).

Table 3: Multivariate analysis to identify factors associated with anti-HBs titre ≥100mIU/ml among HIV-positive and HIV-negative children aged 6-60 months from Kimberley, South Africa. Percentages shown represent the proportion of the entire cohort (n=310) who fall into the listed category.

Characteristic	Proportion of group with anti-HBs titre <100 mIU/ml (%)	Proportion of group with anti-HBs titre ≥100 mIU/ml (%)	P-value

Age <24 months	21.2	32.5	0.005
Sex (Male)	27.4	22.9	0.276
HIV (negative)	16.9	39.8	< 0.0001

Among HIV-positive children age 6-60 months, CD4+ T-cell counts and HIV viral load were available for 83% of participants (suppl. data 1). We included age, sex, CD4+ T-cell counts, CD4+ T-cell proportion and HIV viral load in a multivariate analysis for protective anti-HBs responses (table 4). In this model, age <24 months and HIV viral load ≤2.0 $\log_{10}$ were found to be predictors of an anti-HBs titre of ≥100 mIU/ml. Multivariate analysis using the lower anti-HBs titre of ≥10mIU/ml identified only HIV-negative status as a significant association.

Table 4: Multivariate analysis for factors associated with anti-HBs titre ≥100mIU/ml in HIV-positive children age 6-60 months from Kimberley, South Africa. Percentages shown represent the proportion of the entire cohort (n=136) who fall into the listed category.

Characteristic	Proportion of group with anti-HBs titre <100 mIU/ml (%)	Proportion of group with anti-HBs titre ≥100 mIU/ml (%)	P-value
Age (<24 months)	21.1	8.3	0.048
Sex (Male)	40.3	4.7	0.432
Treatment (ART)	54.3	12.9	0.568
CD4+ count (<1000)	33.6	4.7	0.954
CD4+ (<30%)	52.4	5.7	0.462
Viral load (<2.0$\log_{10}$)	18.5	5.6	0.045

Odds of developing an anti-HBs response

An odds ratio (OR) analysis (Figure 4) indicated that being HIV-positive was associated with reduced odds of developing protective anti-HBs titres, based on titres of both ≥10 mIU/ml (OR 26.2, 95% CI 11.2-58.6) and ≥100 mIU/ml (OR 11.6, 95% CI 6.7-20.4). Younger age

(<24 months) increased the odds of having an anti-HBs titre of ≥10 mIU/ml (OR 0.3, 95% CI 0.2-0.5) or ≥100 mIU/ml (OR 0.3, 95% CI 0.2-0.4). Among the HIV-positive subjects only, age <24 months only elevated the odds for developing an anti-HBs response of ≥100 mIU/ml (OR 0.1, 95% CI 0.06-0.4) (Figure 4B). Other characteristics analysed including gender, ART, CD4+ count, CD4+ ratio and HIV viral load were not found to be significantly predictive of anti-HBs titres at either threshold.

Modelling effects of interventions on HBV prevalence

We fitted the transmission model (Figure 1A) to demographic and transmission observations specific to Kimberley, South Africa, Bayesian Markov-chain Monte-Carlo method (see details in Suppl. Data 2). We simulated several scenarios of vaccination and PMTCT interventions deployed first singly (Fig 1 B/C), and then in combination (Fig 5), quantifying 'impact' as reduction in total HBsAg or HBeAg prevalence, and estimating time for prevalence to reach levels for which stochastic extinction would be highly likely.

Figure 1B illustrates how increasing the proportion of infants given birth vaccination has a progressive impact on reducing the population prevalence of HBV infection. Importantly, even with complete coverage of neonates, prevalence is shown to approach zero only after 400 years. These long time-scales are in keeping with an intervention that does not tackle the large established reservoir of chronic infection and targets only a small proportion of the population such that vaccine-induced herd-immunity accumulates slowly over several decades (Figure S6). For similar reasons, implementing robust PMTCT (Fig 1C) would also slowly reduce HBV prevalence, but since it does not prevent infection later in life, PMTCT on its own would have a smaller overall impact than neonatal vaccination.

Having illustrated the impact of these single interventions at a population level, we next used our model to explore the impact of combined interventions, considering vaccination of neonates together with older ages (Fig 5 panels ABCD-1), neonatal vaccination together with PMTCT (Fig 5 panels ABCD-2), and neonatal vaccination together with PMTCT plus 100% catch-up vaccination of all ages (Fig 5 panels ABCD-3).

We first addressed how different combinations of interventions would impact HBV epidemiology in terms of the time required to reduce total prevalence to 0.1% (Fig 5 panels A1-3). Combining immunization of neonates with older ages (>6 years) required the longest time (Fig 5 A1), with improvements seen when combining neonatal vaccination with PMTCT (Fig 5 A2), and shortest periods observed when combining the latter with a one off 100% catch-up of susceptible individuals (Fig 5 A3). In each of these scenarios, reaching 0.1%

prevalence took >200 years, even under full coverage. However, total prevalence may be reduced to 1% (~8 times lower than the level of endemicity before interventions), on much shorter timescales (50 yrs) when neonatal vaccination and PMTCT are combined (Fig S10).

After 25 years, based on a current rate of ~75% vaccination coverage in neonates only (mean of estimated 56-97%), the model predicts a total reduction in prevalence of ~19% relative to the pre-vaccination era (Fig 5 B1-2). If coverage were to be maintained at a stable 95%, impact would be slightly higher with a reduction of 23% (Fig 5 B1-2). The addition of vaccination in older ages (>6 years old) was seen not to add much to this figure (Fig 5 B1). However, PMTCT was seen as highly beneficial, with a combination of 75% reduction in vertical transmission and current neonate coverage achieving 30% reduction in total prevalence (Fig 5 B2). With both interventions being effective at 95%, prevalence could have been reduced by 35%. If vaccination of neonates and PMTCT at 95% had been coupled with an extra catch-up campaign to immunise 100% of the susceptible population, prevalence would be reduced by 43% (Fig 5 B3). The impact of catch-up vaccination was highest in the short and mid-term, but slowed down thereafter.

In considering the overall impact of any intervention, HBeAg+ individuals are the group with the highest public health importance as these present the highest risk of chronic liver disease, including cancer, and high risk of both horizontal and vertical transmission. We found that under current vaccination coverage of 75% of neonates, HBeAg+ carriers could be reduced by 41% in 25 years (Fig 5 D1-2); and, had coverage been 95%, a reduction up to 49% would have been possible. As observed for total prevalence, combining vaccination of neonates with older ages created minimal additional impact (Fig 5 D1). PMTCT, on the other hand, increased the impact of neonatal vaccination alone, with a reduction of 49% predicted for a combination of current 75% vaccination efforts and 50% reduction in vertical transmission. If both interventions had been maintained at 95%, the HBeAg+ carriers could have been reduced by a substantial 75% in only 25 years. As seen before for total prevalence, combining vaccination of neonates with PMCTC and a catchup of 100% would have generally increased impact (Fig 5 D3).

In conclusion, the model demonstrates long time-scales (decades or centuries) to bring about changes in population prevalence of HBV infection based on vaccination of infants, while catch-up vaccination of older age groups does not have a substantial impact. However, combining interventions can have additive effects, and the enhanced influence of key interventions on the high risk HBeAg+ population suggests more cause for optimism.

DISCUSSION

The work presented here is an important foundation for informing strategies that will move us towards the HBV targets enshrined within Sustainable Development Goals and Global Hepatitis Health Sector Strategy (2). With ambitious aims set out to be achieved by 2030, it is crucial that existing resources are deployed in the best possible way, based on a clear understanding of the likely scale and timeline for an impact to be evident at population level. While being optimistic about the improvements we can make in reducing population prevalence of HBV infection, a clear assessment of the challenge ahead is important advocacy for the development of new approaches, with the ultimate long-term aim still being HBV cure.

Studying a cohort of South African children demonstrates the potent effect of vaccination within individual communities: the observation that current and past HBV infections are infrequent in these children (both <1%) is in keeping with other studies that report a substantial decline in prevalence since universal infant HBV vaccination was introduced (22,32). A substantial proportion of HBV transmission in Africa is thought to arise before the age of 5 years (33) and these results are therefore encouraging in demonstrating that vaccination – when deployed correctly - is indeed reducing HBV incidence in early childhood, when the risk of developing chronic infection is at its highest. Although previous studies in the region have indicated that HBV infection is not significantly associated with HIV status (19,21,34), our data do highlight a likely additional vulnerability of HIV-infected children based on lower antibody titres, and a rapid wane to below the threshold of anti-HBs <10 mIU/mL that is associated with protection.

HBV vaccination is given in South Africa at 6, 10, and 14 weeks; this provides a window of opportunity for perinatal HBV transmission (10,19). This is illustrated particularly by one subject within our cohort (KREC-151), an HIV-negative child with serologic evidence of past infection with HBV, despite documentation of the three-dose HBV vaccination schedule and anti-HBs titre of 201.8 mIU/ml. Perinatal transmission can be significantly reduced by the implementation of antenatal screening for HBV and the administration of a birth dose vaccine (35) supplemented with anti-HBV immunoglobulin, although this intervention may not be affordable in resource-limited settings (36). In the case of KREC-151, it seems most likely that vaccination occurred after infection with HBV had already been established. Systematic efforts to deliver the first vaccine dose soon after birth (in keeping with EPI recommendations) could reduce such transmission events, even in settings where HBV immune globin is not routinely available.

Vaccine-mediated immunity to HBV

Anti-HBs seroprevalence was significantly lower in HIV-positive participants when compared to HIV-negative subjects, irrespective of the threshold set for a protective titre. There are two possible explanations for this observation. First, impaired vaccine responses have previously been observed in HIV-positive individuals (22,37–40). However, given the lack of RTHB data for the HIV-positive study participants, we cannot exclude the second possibility, that coverage with the primary HBV vaccine course was lower among these children. This would be in keeping with a previous report that children born to HIV positive parents in South Africa are less likely to complete childhood vaccination programs (41).

Protective anti-HBs titres in HIV-positive subjects were significantly more frequent in subjects <24 months of age than in older subjects. This difference was not identified in the HIV-negative cohort and suggests that the vaccine responses in HIV-positive subjects wane rapidly after immunisation. This waning has been previously reported in both HIV-positive and HIV-negative subjects (32,37). However, loss of the anti-HBs response does not necessarily correlate with loss of clinical protection and anamnestic responses, where anti-HBs titre is very low or absent but immunological memory remains, are thought to occur in a proportion of those vaccinated (42), although this memory may be attenuated in the context of HIV (43,44).

We found no difference in anti-HBs titres between participants on ART and those not on ART, although there are previous reports correlating ART with improved HBV vaccine responses (24,45). A previous study of Kimberley children demonstrated that recovery of CD4+ T cell percentage in HIV-positive children takes a median of five years after initiation of treatment (46); our current study is therefore likely to be underpowered to detect any true effect, given both the relatively short durations of ART treatment, and the small number of children not receiving ART. Interestingly, despite the lack of correlation between anti-HBs responses and treatment with ART, we did find that children with lower HIV viral loads had significantly higher anti-HBs titres, in keeping with previous studies (45,47). Based on current treatment guidelines, all HIV-infected children are now started on ART (48) and the immune reconstitution of this population over time should be anticipated to reduce the differences in vaccine responses between HIV-positive and HIV-negative groups.

HBV model

The model we have generated appears to perform robustly based on the population parameters we have included for this population, and we believe this is a novel and important tool for adding to our understanding about transmission dynamics and potential

interventions for HBV. The determinants of an equilibrium in any population depend on a number of factors, which are determined by characteristics and behaviours of the host population (49) as well as potentially by the genetics of the virus. However, where the relevant epidemiological parameters have been defined, we believe the model could robustly be applied to other settings to explore the impact of interventions.

Based on the output from the model, we can demonstrate that targets for 'eliminating viral hepatitis as a major public health threat by 2030' are unlikely to be met, unless there is a major scaling up of both vaccination and PMTCT efforts. These two interventions implemented together, with a focus on neonatal vaccination, offer the best chances of making a significant impact on population prevalence. Perhaps more importantly, the predicted impact is greatest among HBeAg+ carriers, who are at an elevated risk of chronic liver disease and hepatocellular carcinoma, as well as being at higher risk of transmission, and therefore constitute the bulk of the public health burden of HBV.

The model illustrates long time-lines, enumerated in centuries rather than decades, for possible elimination. Although our population data demonstrate a substantial reduction in prevalence of HBV infection in children under the age of five years, which is likely to rest almost entirely on the success of the EPI delivery of HBV vaccination, this intervention is tackling only a small fraction of the total population. As HBV is already endemic, is often clinically silent, and may persist in infected carriers for decades (possibly for a life-time), the time-scale for elimination is long. However, the results of our simulations underscore that we can have a major public health impact even without achieving elimination. Careful adjusting of expectations and aims, according to the scale on which particular changes occur, may inform the setting of realistic targets (e.g. reduction in the prevalence of HBeAg+ carriers could be a useful outcome measure when assessing the impact of interventions across a population). The wrong choice of target and timescale could result in unnecessary abandonment of a strategy that could have a major impact in a few decades.

In conclusion, for all simulated combinations of interventions, the model demonstrates that elimination can only occur on very long time-scales. In the context of single interventions, vaccination in neonates was confirmed as the intervention resulting in highest impact, followed by PMCTC and vaccination in older ages (>6 years). When interventions were combined, the best approach was PMCTC with vaccination of neonates. A catchup campain was demonstrated to be beneficial, but our simulations suggest that for realistic catchup coverages the added value would be minimal.

Caveats and limitations

There are a number of caveats that should be considered when analysing and interpreting the clinical dataset. Different approaches to recruitment of HIV-positive and HIV-negative children may introduce unintentional bias or confounders. Using respiratory admissions to hospital for the KReC cohort provided us with an important opportunity to identify a sufficient number of children quickly, and to acquire blood samples from children who would already be undergoing venepuncture as part of their routine clinical care. However, this approach to recruitment predominantly selected younger children (on average 9.4 months younger than the HIV-positive cohort). This bias towards younger subjects in the HIV-negative group, gave us less data with which to assess waning of the anti-HBs response among these children with age. The KReC children may also be less healthy than a comparable group of HIV-negative children in the community.

When designing the study, we elected to focus on children aged under age 60 months in order to collect data from the RTHB which is not routinely carried by older children. In practice, however, we did not capture good RTHB data from the HIV-positive group who frequently did not bring this record to their clinic visits. Data collection from the RTHB is itself subject to bias, as families who attend with such records may be those who are most likely to have immunised their children, while those for whom data are missing could represent the families in which children have missed vaccine doses.

Our approach to screening for HBV infection in this population is limited by undertaking an HBsAg assay. A more robust assessment, capturing cases of occult infection, would require use of HBV DNA as a screening tool. In practice, this is too expensive for wide-spread deployment and was not practical for this study on the same grounds. The lack of understanding about the biological correlates of HBV-vaccine mediated immunity is another challenge. On pragmatic grounds, we have presented data for anti-HBs thresholds of both ≥10 mIU/ml and ≥100 mIU/ml. In the long-term, better understanding of the correlates of protective immunity are required to tailor vaccine strategies to individuals or cohorts. Recent data suggest that the site of immunization, diurnal timing of vaccine doses, and time of day when samples are collected may also have significant impact on antibody titres (50), although existing data for HBV vaccine do not support this (51). In this study, we did not set out to capture these data, but they might be pertinent for future studies. We have also not addressed issues such as maintenance of the cold-chain and vaccine storage which can also influence efficacy. Finally, vaccine efficacy may be determined by the vaccine received; the children immunised in this cohort would have received the traditional monovalent vaccine

(Biovac Paed), whereas children now being immunised in South Africa will have hexavalent vaccination (HBV/DTP/IPV/Hib, Hexaxim, Sanofi-Pasteur).

Alternative approaches for HIV-positive subjects, such as supplementing the current schedule with booster vaccinations and increased vaccine doses have been trialled with variable results (47). A promising recent study of HIV-positive children found that repeating the primary course of vaccination subsequent to response to ART generated lasting protective immune responses (38).

We present a parsimonious mathematical framework, not including, for instance, the proportion of the population infected with HIV, which may suffer from increased risk for vertical transmission and reduced vaccine efficacy. However, we argue that the increasing proportion of HIV-positive individuals receiving ART should minimize the impact that an HIV subgroup in the mathematical framework could have on the general dynamics of the model. In the population studied here, ART has only been introduced in children achieving certain immunological criteria (as per old treatment guidelines), while in future, infected children will be started on treatment as soon as diagnosed (potentially at birth), which could be predicted to increase vaccine responses to similar levels as seen in the HIV-negative population; further studies will be required to assess this over time. More importantly, the effects of HIV and ART would be mostly in the absolute values of our projections, but not necessarily on the obtained differences in terms of impact between the simulated interventions. We also note that quantifications of time to elimination are problematic in a deterministic model such as ours, but our primary outcome is not to present a quantitative estimate for time to elimination, but rather to demonstrate that the predicted time scale is outside the proposed goals for 2030.

Conclusions

Our results affirm that the HBV vaccine is successful in reducing the prevalence of HBV in children, with current rates of <1% in the South African setting we have studied. This underlines the importance of ongoing immunisation, which is fundamental in preventing infection in the vulnerable early months of life. However, we also highlight that a small number of cases of HBV transmission continue, despite inclusion of the HBV vaccine in EPI, and that a proportion of children (especially those who are HIV-infected) are potentially at risk of infection as a result of low anti-HBs titres, either as a result of incomplete immunisation, or because of poor antibody titres following vaccination. Sustained efforts to vaccinate and boost these children are essential. However, at a population level, although

641 neonatal immunisation is the best single intervention, our model demonstrates that this alone
642 does not offer a route to elimination. Substantial reduction of population prevalence hinges
643 on a combination of measures; the crucial roles of catch-up vaccination for older children
644 and the need for major efforts in PMTCT are highlighted by our model. A meaningful and
645 sustainable campaign to eliminate this infection also requires concerted efforts and
646 investment in case finding and treatment, education, reduction of stigma, and sexual and
647 reproductive health services. Ultimately, the only route to elimination of HBV may be to
648 develop a cure.
649

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FIGURE LEGENDS

Figure 1. Diagram of HBV transmission model and predicted impact dynamics of single interventions on population prevalence of infection.

A: HBV transmission model, showing population groups who are susceptible (S; divided into infants (i), children (c) and adults (a)), Chronically infected (C; divided into eAg-positive C+ and eAg-negative C-), acutely infected (I), recovered (R) and vaccinated (V), and the transitions between these groups. Further details of the model including all parameters are available in suppl data 2. B: Dynamics of total HBV prevalence based on vaccination of newborns (starting at t=0), with proportion vaccinated designated as δn (where δn=1 is equivalent to an intervention that covers 100% of newborns). C: Dynamics of total HBV prevalence, based on implementing an intervention that prevents mother to child transmission (PMTCT, starting at t=0) by a proportion represented by parameter ζ (ζ=1 is equivalent to averting 100% of possible transmission events). Parameters as in Table S1 of Suppl. data file 2.

Figure 2: Hepatitis B surface antibody (anti-HBs) titres mediated by vaccination in HIV-positive (HIV+) and HIV-negative (HIV-) children aged 6-60 months in Kimberley, South Africa.

A: Scatter plot representing vaccine-mediated antibody titres, indicating median and interquartile ranges, for HIV-positive and HIV-negative children (p-value by Mann Whitney U test). B: Proportion of HIV-positive and HIV-negative children with anti-HBs ≥10 mIU/ml (p-value by Fisher's Exact Test). C: Proportion of HIV-positive and HIV-negative children with anti-HBs ≥100 mIU/ml (p-value by Fisher's Exact Test).

Figure 3: Relationship between age and vaccine-mediated Hepatitis B surface antibody (anti-HBs) titres in HIV-positive and HIV-negative children in Kimberley, South Africa.

A, B: Ages of children attaining anti-HBs titres ≥100 mIU/ml for HIV-positive (panel A) and HIV-negative children (panel B) in cohort age 6-60 months. Median ages, interquartile ranges and p-values by Mann-Whitney U test are indicated. C, D: Relationship between age and vaccine-mediated Ab titre among HIV-positive children including those age 6-60 months and an older cohort age >60 months (range 64-193 months; see table 3). P-value by Mann Whitney U test (panel C) and by Spearman's rank correlation test (panel D). E: Anti-HBs titre for HIV-positive and HIV-negative children according to age. Each point represents the mean

titre for the group of children aged ≤12 months (1 yr), 13-24 months (2 yrs), 25-36 months (3 yrs), 37-48 months (4 yrs), 49-60 months (5 years).

Figure 4. Odds ratios for protective response to HBV vaccination in children age 6-60 months in Kimberley, South Africa.

Odds ratios are shown for Anti-HBs titre <10mIU/ml and <100mIU/ml in the whole cohort (grey) and in HIV-positive children (black). Statistically significant OR are denoted * and significant p-values are indicated in bold.

Figure 5. Impact of combined interventions on HBV prevalence and time to elimination.

A1-3: time to elimination of HBV based on a threshold of 1 carrier per 1000 individuals in the population. B1-3: Reduction of total HBV prevalence for 25 years after the start of interventions. C1-3: Reduction of total HBV prevalence for 50 years after the start of interventions. D1-3: Reduction in HBeAg+ carriers (model class 'C+') for 25 years after the start of interventions. A/B/C/D: In all cases, the x-axis shows the proportion of neonates vaccinated. Top row: neonatal vaccination combined with routine vaccination at older ages (>6 years old, y-axis). Middle row: neonatal vaccination combined with reduction in vertical transmission (PMTCT, y-axis). Bottom row: neonatal vaccination combined with reduction in vertical transmission (PMTCT, y-axis) after a catch-up campaign covering 100% of the susceptible population. Dashed lines are visual references for 70%, 80% and 90% of the respective interventions. Parameters as in Table S1 of supplementary data file 2.

SUPPLEMENTARY DATA LEGENDS

Suppl data 1. Metadata for three paediatric cohorts recruited in Kimberley, South Africa, including longitudinal CD4+ T cell and viral load data for paediatric HIV cohort age ≤60 months in Kimberley, South Africa.

Suppl data 2. Variables used to inform a population-based model of HBV transmission and prevention, with supporting references and a complete methods description for model of HBV transmission and prevention.

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Nil

CONFLICTS OF INTEREST

None to declare

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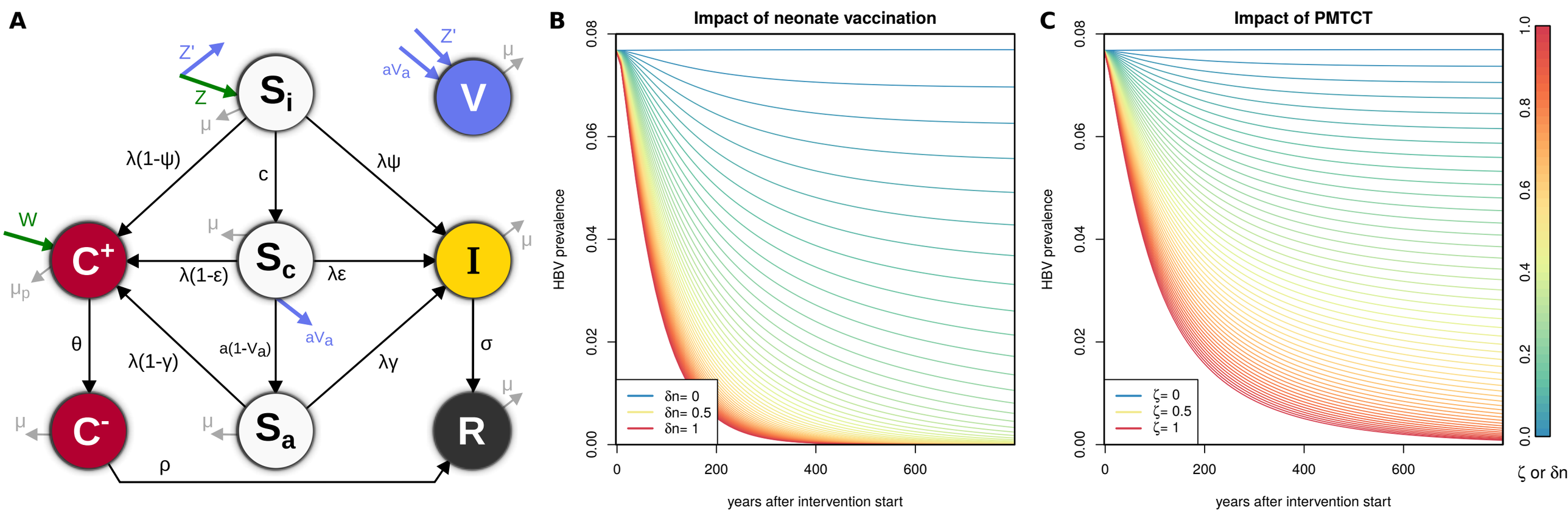
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Impact of neonate vaccination
HBV prevalence
0.00
0.02
0.04
0.06
0.08
0
200
400
600
years after intervention start
δn= 0
δn= 0.5
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Impact of PMTCT
HBV prevalence
0.00
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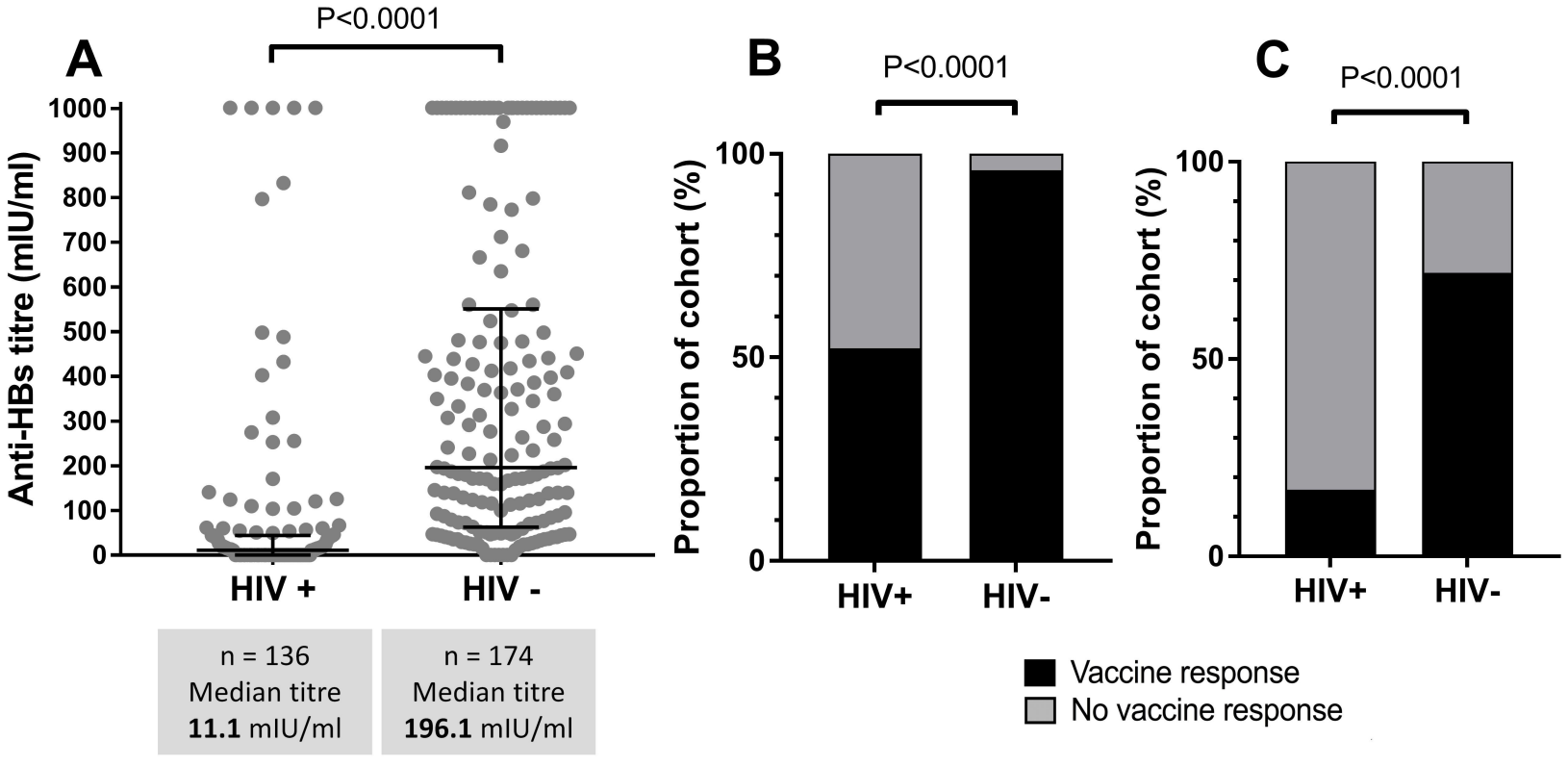
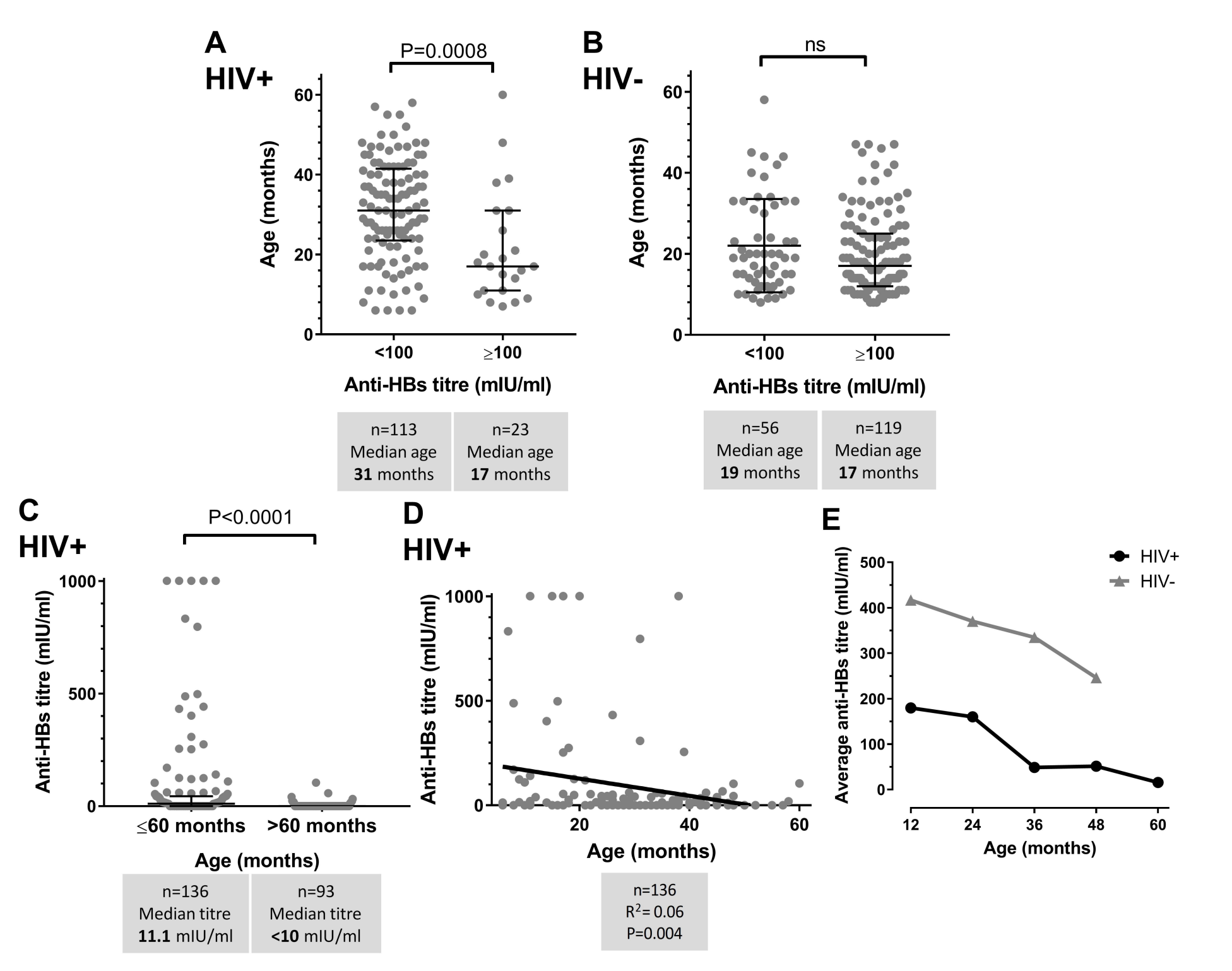
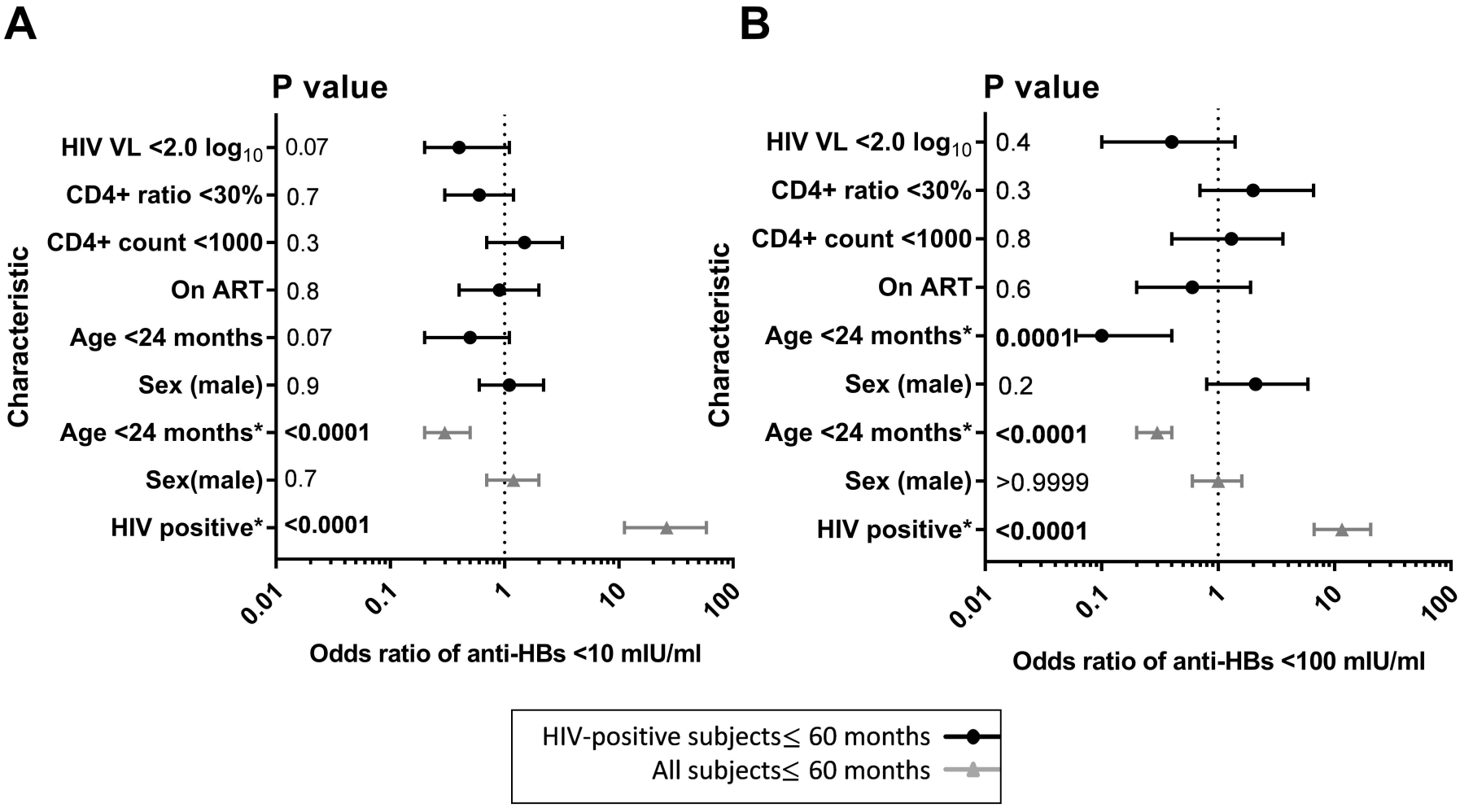
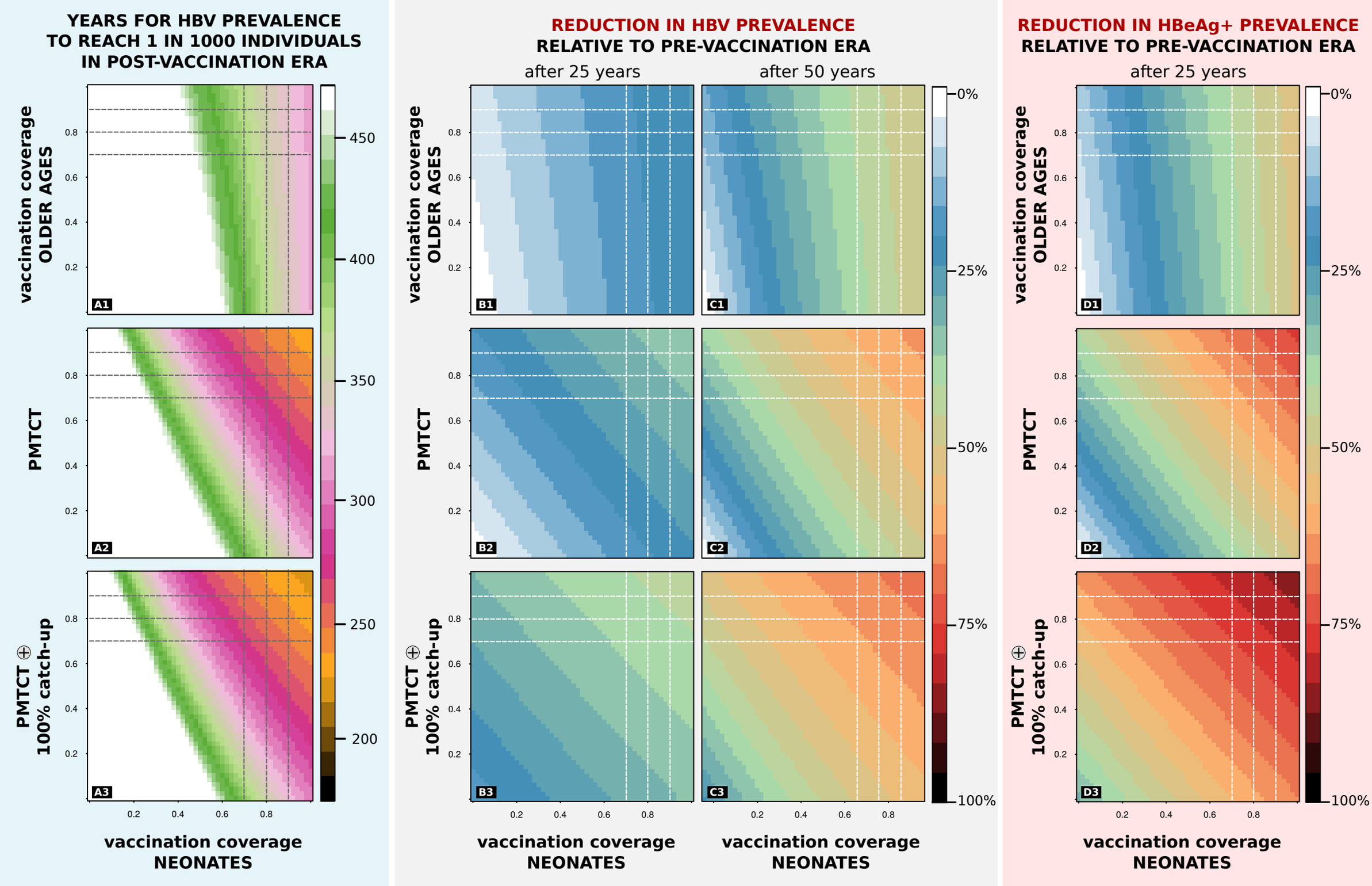

A
P<0.0001
Anti-HBs titre (mIU/ml)
1000
900
800
700
600
500
400
300
200
100
0
HIV +
HIV -
n = 136
Median titre
11.1 mIU/ml
n = 174
Median titre
196.1 mIU/ml
B
P<0.0001
Proportion of cohort (%)
100
50
0
HIV+
HIV-
C
P<0.0001
Proportion of cohort (%)
100
50
0
HIV+
HIV-
Vaccine response
No vaccine response


A
HIV+
P=0.0008
Age (months)
0
20
40
60
<100
≥100
Anti-HBs titre (mIU/ml)
n=113
Median age
31 months
n=23
Median age
17 months
B
HIV-
ns
Age (months)
0
20
40
60
<100
≥100
Anti-HBs titre (mIU/ml)
n=56
Median age
19 months
n=119
Median age
17 months
C
HIV+
P<0.0001
Anti-HBs titre (mIU/ml)
0
500
1000
≤60 months
>60 months
Age (months)
n=136
Median titre
11.1 mIU/ml
n=93
Median titre
<10 mIU/ml
D
HIV+
Anti-HBs titre (mIU/ml)
0
500
1000
20
40
60
Age (months)
n=136
R2= 0.06
P=0.004
E
HIV+
HIV-
Average anti-HBs titre (mIU/ml)
0
100
200
300
400
500
12
24
36
48
60
Age (months)


A
P value
HIV VL <2.0 log10
CD4+ ratio <30%
CD4+ count <1000
On ART
Age <24 months
Sex (male)
Age <24 months*
Sex(male)
HIV positive*
0.07
0.7
0.3
0.8
0.07
0.9
<0.0001
0.7
<0.0001
Characteristic
0.01
0.1
1
10
100
Odds ratio of anti-HBs <10 mIU/ml
B
P value
HIV VL <2.0 log10
CD4+ ratio <30%
CD4+ count <1000
On ART
Age <24 months*
Sex (male)
Age <24 months*
Sex (male)
HIV positive*
0.4
0.3
0.8
0.6
0.0001
0.2
<0.0001
>0.9999
<0.0001
Characteristic
0.01
0.1
1
10
100
Odds ratio of anti-HBs <100 mIU/ml
HIV-positive subjects≤ 60 months
All subjects≤ 60 months


YEARS FOR HBV PREVALENCE
TO REACH 1 IN 1000 INDIVIDUALS
IN POST-VACCINATION ERA
REDUCTION IN HBV PREVALENCE
RELATIVE TO PRE-VACCINATION ERA
after 25 years
after 50 years
REDUCTION IN HBeAg+ PREVALENCE
RELATIVE TO PRE-VACCINATION ERA
after 25 years
vaccination coverage
OLDER AGES
PMTCT
PMTCT ⊕
100% catch-up
vaccination coverage
NEONATES
A1
A2
A3
B1
B2
B3
C1
C2
C3
D1
D2
D3
450
400
350
300
250
200
0%
25%
50%
75%
100%
0.2
0.4
0.6
0.8