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Sub-optimal protection against past hepatitis B virus infection where subtype mismatch exists between vaccine and circulating viral genotype in northern Australia

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ABSTRACT

Background: In Australia's Northern Territory, the hepatitis B virus (HBV) subgenotype A2 (subtype *adw2*) vaccine was introduced in 1988 for Indigenous infants. Subsequently, the circulating viral genotype has been identified as subgenotype C4 (subtype *ayw3*). We assessed HBV vaccine effectiveness (VE) in light of this subtype mismatch.

Methods: Participants of the Aboriginal Birth Cohort (ABC) study were recruited at birth (1987–1990), with HBV serology obtained at follow-up waves 3 (2005–2007) and 4 (2013–2015). Participants were immune if HBV surface antibody levels exceeded 10 IU/L. We determined the VE against any HBV infection (anti-HBc$^+$) and against chronic infection (HBsAg$^+$ or HBV DNA$^+$), comparing non-vaccinated participants with those fulfilling United States Centers for Disease Control and Prevention (CDC) criteria for full HBV immunisation.

Results: Of 686 participants in the ABC study, we obtained HBV serology from 388 at wave 4. 181 participants were immune to HBV and 97 had evidence of any infection. Seven participants were chronically infected, of whom five had received three vaccine doses, and anti-HBc seroconversion had occurred subsequent to the three vaccine doses for two of these seven participants. Comparing the 107 participants who had been vaccinated in accordance with CDC recommendations and 127 who had not been vaccinated, VE against any infection was 67% (95%CI, 43–104%). The odds of being anti-HBc$^+$ was 87% lower in participants raised in urban settings compared to those born into families from remote areas (OR, 0.1; 95%CI, 0.03–0.4).

Conclusions: In a setting where there exists a subtype mismatch between vaccine and circulating genotype, the vaccine was largely effective in preventing chronic infection but sub-optimal against any infection. The implications of a high prevalence of anti-HBc seropositivity in this population are unclear and require further study. The fact that anti-HBc seropositivity was strongly associated with remote dwelling suggests ongoing viral exposure in remote settings.

1. Introduction

Hepatitis B virus (HBV) is a global health problem with over two billion people affected and nearly 260 million chronically infected [1,2]. Morbidity and mortality are high, owing to increased lifetime risk of cirrhosis and hepatocellular carcinoma [3,4]. HBV surface antigen (HBsAg) was first isolated from an Indigenous Australian

Abbreviations: ABC, aboriginal birth cohort; Anti-HBc, hepatitis B core antibody; Anti-HBs, hepatitis B surface antibody; CDC, United States center for disease control and prevention; 95%CI, 95% confidence interval; RDH, Royal Darwin Hospital; HBeAg, hepatitis B early antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; IQR, interquartile range; OR, odds ratio; VE, vaccine effectiveness.

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in 1965, and called the 'Australia antigen' [5]. Although Australia as a whole is of low endemicity (1%), the burden of HBV remains significant among Indigenous people, with 2.2–4.0% chronically infected nationally [6,7], and prevalence up to 10% in remote communities [8,9]. Furthermore, Indigenous people from northern Australia are exclusively infected with the C4 genotype (subtype *ayw3*) [10].

The original HBV vaccine became available in the early 1980s and comprised HBsAg extracted from plasma of HBV-infected donors (genotype A2; subtype *adw2*) [11]. Taiwan and Alaska pioneered infant HBV vaccination programmes in 1984, and since then, both have recorded decreased prevalence of chronic infection and incidence of hepatocellular carcinoma [12–14]. Australia's Northern Territory, where approximately 30% of the population is Indigenous, began an HBV vaccination campaign for Indigenous children in 1988, and all children in 1990.

The Aboriginal Birth Cohort (ABC) study was initiated in 1987 to prospectively follow 686 infants born to Indigenous mothers at Royal Darwin Hospital (RDH); from a sample of 1238 babies born between January 1987 and March 1990 [15]. RDH serves a catchment area of approximately 400,000 km^2, encompassing Darwin, and rural and remote communities across the northern parts of the Northern Territory (the 'Top End'). During the recruitment phase, 90% of pregnant Indigenous women from the Top End delivered their babies at RDH [16]. Previous follow-ups of the ABC occurred at mean participant ages of 11 years (1998–2002; wave 2) [17] and 18 years (2005–2007; wave 3) [18]. Here we report the analyses of HBV serology collected between 2013 and 2015 (wave 4). Our aims were to determine vaccine effectiveness (VE) and define predictors of long-term immunity.

2. Methods

2.1. Data collection

Participants were contacted and provided blood for HBV serology. HBV vaccination histories were obtained from the Centre for Disease Control Immunisation Register, RDH and remote health clinics. Current United States Centers for Disease Control and Prevention (CDC) HBV immunisation guidelines recommend a birth dose of the vaccine. However, the ABC study began during an era when it was acceptable for this initial dose to be given up until 7 days of life [19,20]. Therefore we considered participants adherent to the CDC immunisation schedule if they received: the first dose within seven days of birth; the second dose before 3 months of age, at least 4 weeks after the initial dose; and third dose between 24 weeks and 18 months of age, at least 16 weeks after the initial dose and at least 8 weeks after the second dose [21]. We recorded whether participants had lived in urban or remote settings, and the communities their families were from. Urban locations included Darwin and townships up to 100 km away, as well as Katherine (300 km away) and Kununurra (800 km away). All other locations were considered remote.

2.2. Laboratory methods for serology

Wave 3 serology was performed at the Institute for Clinical Pathology and Microbiological Research at Westmead Hospital, Sydney, Australia and analysed on the Abbott Architect Automated Analyser i2000SR (Abbott Diagnostics, North Ryde, Australia). Wave 4 serology and viral loads were analysed at the Victorian Infectious Diseases Reference Laboratory at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia. The qualitative anti-HBV core antibody (anti-HBc) assay was a two-step competitive chemiluminescence immunoassay (Roche Diagnostics, Melbourne, Australia) where an "index value" <0.9 is considered positive and an index value >1 negative. Quantitative anti-HBV surface antibody (anti-HBs) testing was done using Elecsys Immunoassays (limits of detection, 3.5–1000 IU/L; Roche Diagnostics, Melbourne, Australia). We tested for HBV DNA viral load using the COBAS Ampliprep/Taqman Assay (lower limit of detection, 20 IU/mL; Roche Diagnostics, Melbourne, Australia) on pooled samples, with four samples per pool. Therefore, the detection limit for each sample in this pooled assay was 80 IU/mL, as each sample is in effect diluted 1:3. Individual samples from positive pools were then re-tested using an in-house assay [22] with confirmatory testing on the Realtime M2000 assay (Abbott Diagnostics, North Ryde, Australia).

Participants were classified as: (1) non-immune and non-infected (anti-HBs^-, $HBsAg^-$ and anti-HBc^-); (2) immune by vaccination ($HBsAg^-$, anti-HBs^+ $\geq$10 IU/L and anti-HBc^-); (3) current or past infected (anti-HBc^+); and (4) chronically infected ($HBsAg^+$ or HBV DNA^+). The usual definition of chronic infection requires detection of HBsAg on two occasions at least six months apart. Where possible we reviewed the medical records of all those who were either $HBsAg^+$ or HBV DNA^+ at Wave 4 to determine if they satisfied the stricter definition of chronic infection. However, given the study design we were not able to ascertain the serial results for some participants and elected to define chronic infection based on a single result. An additional subgroup of participants were considered to have been exposed and transiently infected ('natural boosting') as indicated by an increase in anti-HBs level between wave 3 and wave 4 if: $\geq$4$\times$ increased anti-HBs at wave 4 if <100 IU/L at wave 3, or $\geq$2$\times$ increased anti-HBs at wave 4 if $\geq$100 IU/L at wave 3, and all other serological markers negative at wave 4 [23].

2.3. Ethics

Written informed consent was provided by participants and written support provided from each community's local governing bodies. Each wave of follow-up was approved by the Human Research Ethics Committee of Northern Territory Department of Health and Menzies School of Health Research, including the Aboriginal ethics sub-committee (wave 3 reference number 05/26 and wave 4 reference number 2013–2022).

2.4. Statistical analysis

We calculated VE with respect to protection against any (anti-HBc^+) and chronic ($HBsAg^+$ or HBV DNA^+) infection using the risk ratio with chi-square or Fisher's exact test for differences. Logistic regression was used to identify predictors of anti-HBc status. We used linear regression to identify associations with anti-HBs levels. We applied the natural logarithmic transformation on anti-HBs levels when this was the response variable [24]. By contrast, we used base two logarithmic transformations on anti-HBs levels when this was an independent variable to facilitate interpretation of regression models (it is simpler to consider doubling of an antibody level than unit increases, given the left-skewed distribution of the data). Statistical analyses were performed in R (version 3.3.2, the R Foundation for Statistical Computing). Results are presented as geometric means (with accompanying inter-quartile range; IQR) and odds ratios (OR), with accompanying 95% confidence intervals (95%CI).

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3. Results

3.1. Study participants

Among the 686 ABC participants, 459 (217 males; 25.9 ± 1.1 y ears of age) were followed-up in wave 4 between September 2013 and June 2015 (Fig. 1). 29 participants had died before wave 3 and a further eight had died between waves 3 and 4. No deaths were attributed to HBV infection. HBV serology was obtained from 388 participants at wave 4; 52 participants were born before the vaccination programme was implemented in May 1987, and the remainder were born afterwards, with the last vaccine dose administered in January 2000. 271 wave 4 participants had received at least a single dose of the vaccine before wave 4; of whom 158 had received the first dose within the first week of life and 107 had fulfilled CDC criteria for HBV vaccination (29 of these participants had received a birth dose). Maternal HBsAg status was available for 19 participants who followed up at wave 4, among whom, six of the mothers were $HBsAg^+$. Five infants born to these women received anti-HBs immunoglobulin. HBV serology was obtained from five of these participants at wave 4, four of whom received anti-HBs immunoglobulin (i.e. one participant had received anti-HBs immunoglobulin at birth, but did not consent to HBV serology at wave 4). Finally, there were 70 participants in wave 3 who received a booster vaccination, of whom 57 were followed up at wave 4. A more detailed analysis of this subset of participants will be presented in a subsequent report.

3.2. Hepatitis B virus chronic ($HBsAg^+$ or $HBV\ DNA^+$) and any ($anti\text{-}HBc^+$) infection

3.2.1. Chronic infection

Seven participants had either detectable HBsAg and/or HBV DNA at wave 4, and five of these participants (participants #1, 2, 4, 6 and 7) had definitive evidence of chronic infection (Table 1). Participant #1 may have been infected perinatally or during childhood, as she had evidence of HBV infection on two occasions, and had not been immunised in infancy. Participant #2 was $HBsAg^+$ at both waves 3 and 4, but interestingly $anti\text{-}HBc^+$ at wave 3 and $anti\text{-}HBc^-$ at wave 4. Other serological records obtained from participant #2 showed he had been $anti\text{-}HBc^+$ five months before his wave 4 visit. He was infected with subgenotype C4 with no vaccine escape mutations identified. Participant #3, who had been vaccinated according to CDC criteria together with a 4th dose of vaccine at 5 years of age, was $HBV\ DNA^+$ with an equivocal HBsAg at wave 4 and seroconverted from $anti\text{-}HBc^-$ to $anti\text{-}HBc^+$ between waves 3 and 4. Participant #7 had been born to a $HBsAg^+$ mother and had received anti-HBs immunoglobulin at birth. He subsequently received three doses of vaccine during childhood and seroconverted from $anti\text{-}HBc^-$ to $anti\text{-}HBc^+$, and from $HBsAg^-$ to $HBsAg^+$ between waves 3 and 4, with detectable HBV DNA at wave 4. Participants #3 and #7 represent episodes of breakthrough infection later in life. Three participants (#2, #4 and #5) had already been $HBsAg^+$ at wave 3. Of the remaining $HBsAg^+$ participants at wave 4, HBsAg status was missing at wave 3 for participants #1 and #6. Of the five children born to $HBsAg^+$ mothers and with wave 4 serology, only one was $HBsAg^+$ at wave 4, and this participant only seroconverted between wave 3 and wave 4 (participant #7).

3.2.2. Any infection

There were 97/388 participants (25.0%; 52 males and 45 females) with evidence of current or past HBV infection at wave 4, increasing from a proportion of 22.5% (93/414) at wave 3. Among these wave 4 participants, 24/97 completed the vaccination schedule as per CDC criteria, and 46 in total received three or four doses of the vaccine before wave 3. Six participants received a single dose of the vaccine and four received two doses of the vaccine. 41 $anti\text{-}HBc^+$ participants remained unvaccinated at wave 4.

Among the 304 participants with serology at waves 3 and 4, 16 additional participants became $anti\text{-}HBc^+$ at wave 4. Of these 16, seven were immunised in accordance with CDC criteria, 15 resided remotely, two had detectable HBV DNA (125 and 84 IU/L, corresponding to participants #3 and #7 in Table 1) of whom one was $HBsAg^+$. There were 16 participants who were $anti\text{-}HBc^+$ at wave 3 but $anti\text{-}HBc^-$ at wave 4.

Using multivariate logistic regression, higher wave 3 anti-HBs levels, fewer vaccine doses received and being born into a family community from a remote setting predicted anti-HBc seropositivity at wave 4. For every doubling of the anti-HBs level at wave 3,

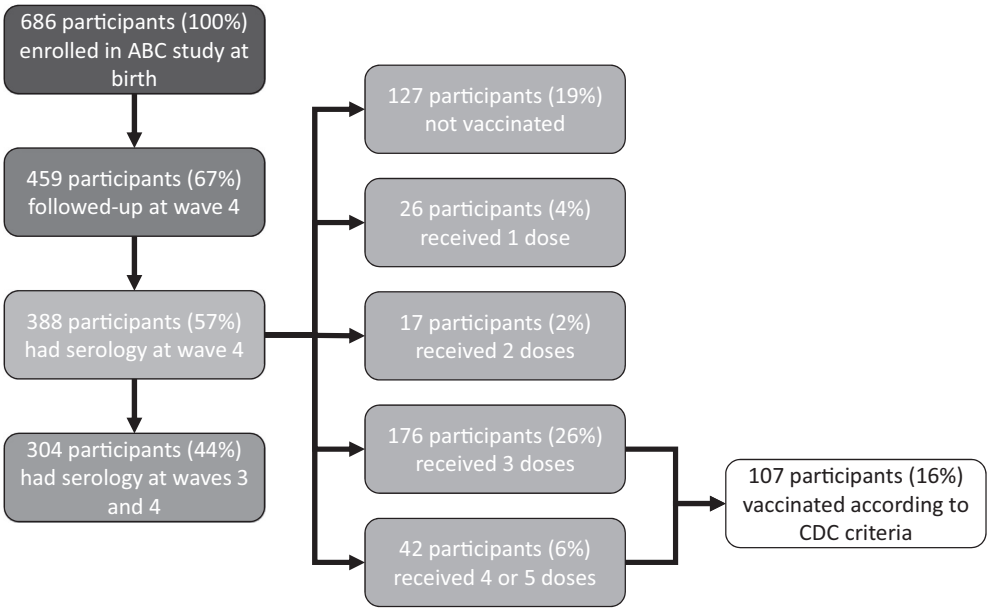

Fig. 1. Outline of participant flow through the ABC study.

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Table 1
Characteristics of the seven participants with detectable HBsAg or HBV DNA at wave 4. Participant #1 was HBsAg$^+$, anti-HBc$^+$ and had undetectable anti-HBs nearly 1.5 years before wave 4. Participant #2 was anti-HBc$^+$ five months before wave 4. Participant #3 had insufficient sample to confirm HBsAg status at wave 4. Participant #6 was HBsAg$^+$ 2 years before wave 4 and had an HBV DNA viral load of 140 IU/L 9 months before wave 4. Participant #7 was HBsAg$^+$ and had an HBV DNA viral load of 33 IU/mL 1.5 years before wave 4. Abbreviations: =, equivocal; y, years; m, months; and d, days.

Participant	#1	#2	#3	#4	#5	#6	#7
Sex	Female	Male	Female	Male	Male	Male	Male
Family community location	Remote	Remote	Remote	Remote	Remote	Remote	Remote
Vaccine 1 age	5.2 y	10.1 y	6 d	11.3 y	–	–	5 d
Vaccine 2 age	5.3 y	10.3 y	43 d	11.5 y	–	–	1 m
Vaccine 3 age	5.7 y	10.7 y	9 m	19.1 y	–	–	11.6 y
Vaccine 4 age	–	–	5.3 y	–	–	–	–
CDC criteria	No	No	Yes	No	No	No	No
Location	Remote	Remote	Remote	Remote	Remote	Remote	Remote
Anti-HBs (IU/L)	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5	<3.5
Anti-HBc	+ve	−ve	+ve	+ve	+ve	+ve	+ve
HBsAg	+ve	+ve	=	+ve	+ve	+ve	+ve
HBV DNA (IU/mL)	102×10^6	2265	125	910	<20	<20	84
HBeAg	+ve	−ve	−ve	+ve	−ve	+ve	+ve
Location	Urban	Remote	Remote	Remote	Remote	Remote	Remote
Anti-HBs (IU/L)	<3.5	<3.5	<3.5	Missing	<3.5	<3.5	7.7
Anti-HBc	+ve	+ve	−ve	+ve	+ve	+ve	−ve
HBsAg	Missing	+ve	−ve	+ve	+ve	Missing	−ve

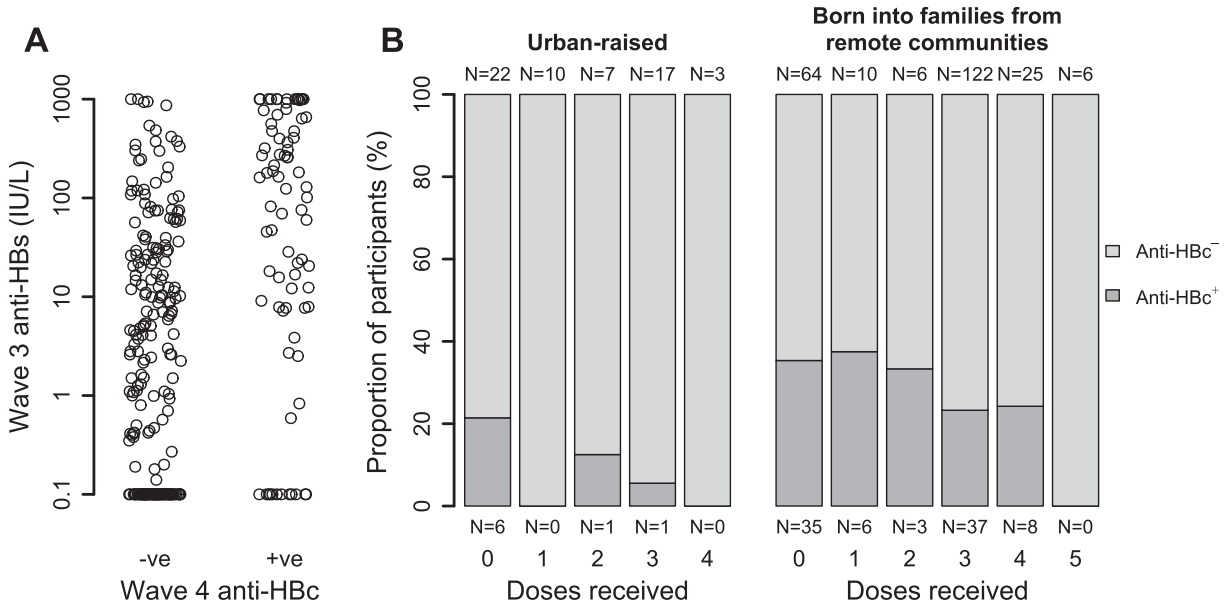

Fig. 2. (A) Participants with higher anti-HBs levels at wave 3 were more likely to be anti-HBc$^+$ at wave 4. (B) Bar graphs illustrating that participants born into families from remote communities were more likely to have been infected with HBV than those raised in urban environments, as well as a dose-dependent protective effect of the vaccine.

Table 2
Characteristics of participants who had been vaccinated according to CDC criteria compared to those who had not received any vaccination at wave 4.

	CDC-vaccinated (N = 107)	Not vaccinated (N = 127)	P-value
Male	42 (39%)	73 (57%)	0.008
Born into families from urban communities	6 (6%)	28 (22%)	0.001
Urban dweller at wave 4	13 (12%)	32 (25%)	0.01
Born before vaccination programme	0 (0%)	28 (22%)	<0.001
Anti-HBs (≥10 IU/L)	49 (46%)	55 (43%)	0.8
Anti-HBc$^+$	24 (22%)	41 (32%)	0.13
HBsAg$^+$ or HBV DNA$^+$	1 (1%)	2 (2%)	1.00

the odds of being anti-HBc$^+$ at wave 4 increased 24.4% (OR, 1.2; 95% CI, 1.2–1.3; $P < .001$; Fig. 2A). By contrast, for every dose of vaccine received, the odds of being anti-HBc$^+$ at wave 4 decreased by 41.0% (OR, 0.6; 95%CI, 0.5–0.7; $P < .001$; Fig. 2B). Finally, the odds of being anti-HBc$^+$ at wave 4 was 87.3% lower in participants raised in urban environments (OR, 0.1; 95%CI, 0.03–0.4; $P = .002$; Fig. 2B).

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3.3. Vaccine effectiveness

Among 107 participants who had completed the CDC HBV vaccination schedule, 24 (22.4%) were anti-HBc$^+$ at wave 4 (Table 2). Of the 127 participants who had not been vaccinated, 41 (32.2%) were anti-HBc$^+$ at wave 4. The VE against any infection (anti-HBc$^+$) was 69.5% in the entire cohort (95%CI, 45–107%; $P = .09$). As a larger proportion of those adherent to the CDC immunisation schedule were born into families from remote communities, this may have had a confounding influence on VE estimates. When we restricted the analysis to the subset of participants born into families from remote communities: 24/101 (23.8%) participants adherent to the CDC schedule and 35/99 (35.4%) participants who had not been vaccinated were anti-HBc$^+$, with VE of 67% (95%CI, 43–104%; $P = .07$). Thus the VE in the entire cohort was similar to that for participants born into families from remote communities.

At wave 4, there were 2/127 (1.6%) non-vaccinated participants with detectable HBsAg, and 1/107 (0.9%) participant who had followed CDC immunisation timelines with an equivocal HBsAg and low-level HBV DNA. VE against chronic infection (HBsAg$^+$ or HBV DNA$^+$) was 50% ($P = 1.00$; Fisher's exact test).

3.4. Immunity

The proportion of participants immune to HBV (i.e. anti-HBs $\geq$ 10 IU/L) was similar at wave 4 (127/388; 46.6%) compared to wave 3 (186/415; 44.8%). Of those who were anti-HBs $\geq$ 10 IU/L, 69/181 participants were also anti-HBc$^+$, 86/181 had been previously vaccinated and were anti-HBc$^-$ (six with a single vaccine dose, four with two doses and 76 with at least three doses), and 26/181 were anti-HBc- but also without a history of vaccination (Table 3). 11/181 participants demonstrated higher levels of anti-HBs at wave 4 than at wave 3, potentially indicating transient HBV infection leading to natural boosting (none of these participants had received a booster dose after wave 3). If we were to consider any detectable anti-HBs level as indicating immuno-reactivity, then 204 participants had anti-HBs levels greater than the lower limit of detection of the assay at wave 4. Among participants who had received at least one dose of the vaccine, the wave 4 geometric mean anti-HBs level was lower at 3.2 IU/L (IQR, 0–54.0 IU/L; Fig. 3A) compared to 4.9 IU/L (IQR, 0–57.7 IU/L; Fig. 3B) at wave 3, suggesting waning of anti-HBs levels between waves 3 and 4. However, the proportion of participants with immunoreactive levels of anti-HBs $\geq$ 10 IU/L increased from wave 3 (150/340; 44.1%) to wave 4 (127/263; 48.3%). These results were largely invariant to the exclusion of participants who received a booster dose, except for the proportion of participants who were immunoreactive to anti-HBs (52.7% at wave 3 and 52.6% at wave 4). The dichotomy illustrated in Fig. 3A and B suggests a subgroup whose immunity waned below the level of detection, and an alternative subgroup whose anti-HBs level increased due to natural boosting following exposure to virus.

Parameters measured at wave 3 correlated with anti-HBs levels at wave 4. Participants raised in urban environments had 65.3% lower anti-HBs levels than those born into families from remote communities (OR, 0.3; 95%CI, 0.1–0.9; $P = .02$). The older participants were, the higher the anti-HBs level (OR, 1.6; 95%CI, 1.1–2.2; $P = .007$). The number of vaccine doses received (OR, 1.2; 95%CI, 0.9–1.5; $P = .20$) and male sex (OR, 1.0; 95%CI, 0.5–1.9; $P = .96$) were not associated with anti-HBs levels at wave 4.

4. Discussion

The ABC study has provided some of the largest, longest, and most detailed follow-up of Indigenous Australians to date, with observation spanning across 27 years in over 400 participants since birth. Nearly 40% of participants received at least a single dose of the HBV vaccine, with 16% fulfilling CDC criteria. Chronic HBV infection was uncommon but for two participants, anti-HBc$^+$ seroconversion with detectable HBV DNA occurred despite prior complete vaccination courses. Additionally, evidence of at least transient breakthrough infection among 22% of those with timely vaccination was unexpectedly high.

4.1. Vaccine effectiveness

Decreased HBV VE in ABC participants was potentially multifactorial. Firstly, there was a mismatch between the endemic virus and that targeted by the vaccine. The ABC participants received a vaccine based on the A2 subgenotype common in Northern Europe and the United States but serodiscordant with the endemic C4 subgenotype [25]. HBV/C4 has a genotype C backbone, with an S gene recombined from genotype J resulting in subtype *ayw3* [10,25]. The significance of subtype mismatch was first proposed by Stramer et al. who detected non-A2 subgenotype HBV infection, despite earlier vaccination, in five blood donors [26]. Similar findings to ours have been noted in the Gambia where the dominant circulating strains (genotype E with *ayw4* and *ayw2* serotypes) and the vaccine (*adw2*) are mismatched, with 10–27% of vaccinated persons found to be anti-HBc$^+$ [27]. Secondly, vaccine escape G145R mutations in the PreS/S region have been identified previously in up to 4% of Indigenous Australians with HBV/C4 [10,25]. There were insufficient viral titres to determine if such mutations were present in ABC samples. Participant #2's viral sequence was determined for clinical purposes, however, a vaccine escape mutant was not observed. Finally, concerns that freezing of the vaccine during transportation resulted in diminished immunogenicity may have been pertinent to ABC participants living in remote areas [28,29].

4.2. Epidemiology of HBV in the ABC

The HBsAg prevalence at wave 4 of approximately 2% was lower than a recent pooled estimate for Indigenous communities (4.0% HBsAg$^+$) [6], as well as that noted among patients attending urban and regional Indigenous health clinics around Australia (3.9%) [30]. However, both these studies mainly comprised adults born during the pre-vaccination era. The lower prevalence of chronic infection in the ABC participants likely reflects the earlier implementation of infant vaccination in the Northern Territory and thus protection against chronic infection afforded by immunisation.

Table 3
Serological profiles of participants who had received at least one dose of the vaccine compared to those who had never been vaccinated.

	Received $\geq$ 1 vaccine dose		Never vaccinated		
	Anti-HBc$^+$	Anti-HBc$^-$	Anti-HBc$^+$	Anti-HBc$^-$	Total
Anti-HBs$^+$	40	86	29	26	181
Anti-HBs$^-$	15	120	12	60	207
Total	55	206	41	86	388

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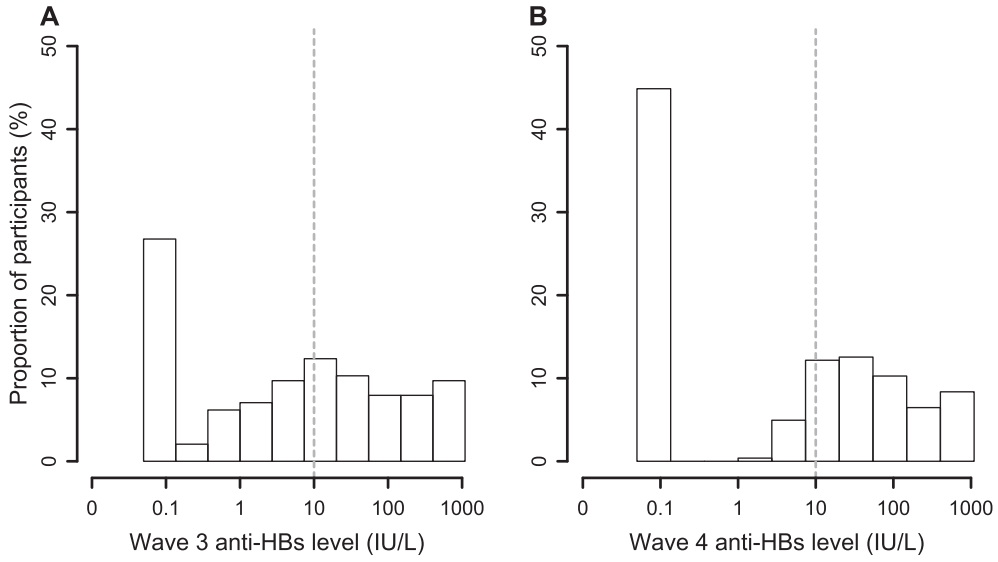

Fig. 3. Histograms depicting the distributions of anti-HBs levels at waves 3 (A) and 4 (B) among participants who received at least one dose of the vaccine throughout life. The vertical dashed line represents the anti-HBs threshold for immunoreactivity (i.e. 10 IU/L). The geometric mean anti-HBs level at wave 3 was 4.9 IU/L, and at wave 4, decreased to 3.2 IU/L. However, the proportion of participants who were immunoreactive for anti-HBs increased from 150/340 (44%) at wave 3 to 127/263 (48%) at wave 4. Compared to wave 3, there were greater proportions of participants who recorded undetectable levels of anti-HBs. Undetectable levels of anti-HBs were assigned values of 0.1 IU/L.

Transient infections were common with 22% of participants anti-HBc$^+$, and 26% either anti-HBc$^+$ or demonstrating an increase in anti-HBs levels between waves 3 and 4. The implications of the high rate of anti-HBc seropositivity remain unclear. Similar rates of transient breakthrough infections in vaccinated persons have been documented in the Gambia (27%) [31], Thailand (up to 27%) [23] and Taiwan [32]. Meta-analyses have determined increased risk for both hepatocellular cancer (OR, 6.1) [33] and cirrhosis (OR, 8.9) [34] in those with occult HBV infection (HBsAg$^-$, anti-HBc$^+$, HBV DNA$^+$) compared to uninfected people. No cases of occult HBV infection were identified among ABC participants, and whether isolated anti-HBc$^+$ predisposes to hepatocellular carcinoma and cirrhosis in Indigenous Australians is unknown. However, HBV reactivation with immunosuppression in isolated anti-HBc$^+$ individuals is well described [35] and may be of importance for the 25% of participants with this serological picture. The possibility of ongoing viral replication and viral release from the liver [36], as well as reactivation, especially with B-cell immunosuppression [35], would suggest that transient breakthrough infections may not infrequently be clinically significant.

4.3. Natural immunisation and boosting

Exposure to HBV does not necessarily result in infection of any degree. A small number of unvaccinated ABC participants derived immunity from exposure to the virus, and another minority developed natural boosting. Neither groups became anti-HBc$^+$. If we take an increase in anti-HBs as an indication of transient breakthrough infection in addition to those who were anti-HBc$^+$, then a total of 101 participants experienced transient ('boosting') infections. While the former may have reflected misclassification of vaccination status, immunisation or boosting from exposure to HBV has also been observed in regions of the world that are of intermediate endemicity [23].

4.4. Location matters

Participants whose families were from remote communities had higher levels of anti-HBs and anti-HBc seropositivity, and higher anti-HBs levels at wave 3 were associated with anti-HBc seropositivity at wave 4. This most likely reflects ongoing circulation, natural boosting and horizontal transmission of HBV. Indigenous people living in remote communities experience substantial crowding, which is associated with poorer hygiene and transmission of communicable diseases [37,38]. Liu et al. also found that HBsAg was more frequently identified in Indigenous women living in remote settings than those living in outer regional areas [7]. In areas of higher prevalence, sexual transmission is also more likely and we noted two participants had converted from HBsAg$^-$ to HBsAg$^+$ between waves 3 and 4, a period of age associated with onset of sexual activity. Ongoing exposure to virus is facilitated by having patients with high levels of viraemia. Of patients with chronic HBV/C4 infection, a significant proportion have been demonstrated to be HBeAg$^+$ and with high viral loads (median, 1.14×10^8 IU/ml) [10].

4.5. Study limitations

This is the first large scale longitudinal survey of HBV serology in Indigenous Australians. However, we have captured a narrow cohort born between 1987 and 1990, and findings may not extrapolate to more contemporary birth cohorts.

We encountered multiple challenges in conducting this longitudinal study in a cohort sparsely distributed over a large geographical area. We cannot verify whether participants received the original plasma-derived vaccine or the first-generation recombinant vaccine, as both were available when the ABC study commenced, and we do not have access to the specific records of which vaccine was given. Our definition of chronic HBV infection was based on detection of HBsAg$^+$ or HBV DNA$^+$ on a single occasion and may therefore reflect acute infection rather than chronic infection. However, for most of the participants we were able to find previous results that confirmed chronic infection. Although different laboratories performed the HBV serology at waves 3 and 4, leading to potential inconsistencies in comparison of results, both are accredited to national standards.

We had incomplete records regarding maternal HBsAg and HBeAg status, as well as whether anti-HBs immunoglobulin was given, limiting us from drawing stronger conclusions about the significance of vertical transmission among ABC participants. Nonetheless, perinatal HBV transmission was unlikely given that only 3.5% of women from this region were HBsAg$^+$ in the pre-

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vaccination era [7]. For the five HBsAg$^+$ mothers with available records, anti-HBs immunoglobulin was administered to all infants and two infants received a birth dose of the vaccine. Thus the combination of intermediate HBsAg$^+$ rates and reasonable uptake of immunoglobulin and vaccine should have limited perinatal transmission. Finally, there were 26 participants who were anti-HBs$^+$ but anti-HBc$^-$ in the absence of reported vaccination, an unusual serological profile. As immunisation was not given by study investigators but rather vaccination status was obtained from records, it is possible that the immunisation status of some participants was misclassified.

5. Conclusion

In conclusion, we found sub-optimal effectiveness of the HBV vaccine in Top End Indigenous Australians. The proportion of participants who were vaccinated according to CDC guidelines remained low, and it is almost certain that many anti-HBc$^+$ participants were infected before vaccination. Nonetheless, anti-HBc seroconversion among those who were fully vaccinated remained disproportionately high. There was evidence of at least transient breakthrough infections in 22% of those adherent to the CDC immunisation schedule, and nearly 2% prevalence of chronic infection overall. The long-term implications for individuals who are anti-HBc$^+$ in terms of risk for cirrhosis and hepatocellular carcinoma are unknown. It remains too early to conclude whether a modified vaccine is required or booster doses are needed given the low prevalence of HBsAg$^+$ or HBV DNA$^+$ disease [39,40]. That anti-HBc seropositivity was more strongly associated with remote dwelling rather than prior vaccination or boosting suggests ongoing viral exposure in remote settings.

Conflicts of interest

The authors declare no conflicts of interest in the data collection, analysis and manuscript preparation.

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