HUMAN VACCINES & IMMUNOTHERAPEUTICS
2018, VOL. 14, NO. 10, 2491–2496
https://doi.org/10.1080/21645515.2018.1480278

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RESEARCH PAPER

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Hepatitis B seroprevalence among 5 to 6 years old children in the Philippines born prior to routine hepatitis B vaccination at birth

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ABSTRACT

To assess the prevalence of hepatitis B in the Philippines, we conducted a cross-sectional study among 5 to 6 year old children born in 2007–2008, when the birth dose started to be implemented in the country. The study was conducted from 25 July to 22 October 2013 in 24 provinces and used a 3-stage cluster design and probability-proportional to size sampling. Blood was obtained and sera were tested for hepatitis B surface antigen (HBsAg). The survey included 2,769 children, of whom 26% received a timely birth dose (within 24 hours of birth) and 89% received 3 doses of the hepatitis B vaccine. Due to problems in the initial testing algorithm, only 2,407 sera were available for HBsAg testing, 20 (weighted%, 0.86%) were HBsAg positive. By immunization card and recall, among HBsAg positive children, 2 (weighted%, 20%) received a timely birth dose while 17 (weighted%, 85%) received 3 doses of the hepatitis B vaccine. The seroprevalence of HBsAg that we detected was lower than expected. However, there were several limitations in the field and in the laboratory that may have affected the representativeness of the results. Follow up studies need to be conducted to validate these results.

ARTICLE HISTORY
Received 26 September 2016
Revised 6 May 2018
Accepted 20 May 2018

KEYWORDS
epidemiology; hepatitis B; liver disease; Philippines; viral hepatitis

Introduction

Viral hepatitis is a major cause of mortality, responsible for ~1.45 million deaths globally in 2013; 96% of these deaths were due to hepatitis B and C viruses.[1] In May 2016, the 69th World Health Assembly adopted the first global strategy to address viral hepatitis with the goal of reducing new cases of hepatitis B and C by 30% and achieving a 10% reduction in hepatitis-related mortality by 2020.[2] The country members of the Western Pacific Region of the World Health Organization (WHO) have the highest number of hepatitis deaths. Starting in 2003, the region has targeted hepatitis B virus (HBV) for elimination.[3] In 2013, the WHO Regional Committee aimed to reduce chronic hepatitis B (HB) infection to less than 1% among children aged 5 years and older by 2017.[4] To achieve this goal, a high coverage of timely birth dose (given within 24 hours of birth) and ≥3 doses of hepatitis B (HB3) vaccine among infants are recommended.[5]

The Philippines is endemic for hepatitis B, with a prevalence of HBsAg among adults of 16.7% in 2003.[6] HB vaccination of infants was incorporated in the Philippines' Expanded Programme on Immunization (EPI) since 1992 but the program has been unable to cover 100% of the population. In 2007, routine HB vaccination at birth was introduced, resulting in a change in the immunization schedule, with the first dose of HB vaccine given at birth, the second dose at 6 weeks and the third dose at 14 weeks of age. With the introduction of the *Haemophilus influenza*e B (Hib) vaccines in 2012, the country switched to pentavalent vaccines (containing diphtheria, pertussis, tetanus, Hib and HB antigens), The current HB vaccination schedule comprises a first monovalent dose at birth and subsequent doses combined with the other antigens at 6, 10 and 14 weeks of age.[7] Fig. 1 shows the HB vaccine coverage from 2007–2016.

The Regional plan of action for HB control include the conduct of representative seroprevalence surveys to measure the impact of the program and assess the achievement of the targets, which will be supplemented with acute disease surveillance and mortality data.[5] A verification process developed by the WHO requires that seroprevalence surveys be conducted among children 5 years or older born after the start of nationwide infant HB vaccination to assess the seroprevalence rates of different HBV infection markers, especially for HBsAg.[8,9] Studies suggest that 75% of chronic HBV infections are acquired by 5 years of age, either from perinatal or horizontal (child-to-child or from other household members) transmission.[10]

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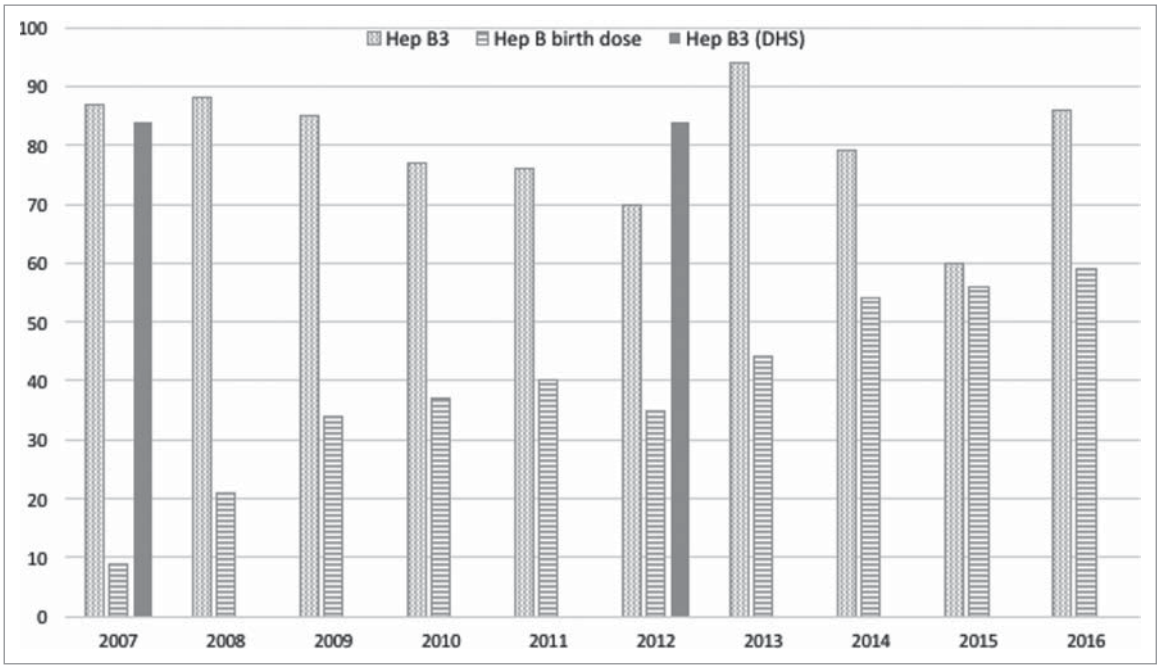

Figure 1. Vaccine coverage of three doses of hepatitis B (HB3) and hepatitis B birth dose (HB_BD), 2007–2016[7] DHS, data based on Demographic Health Survey.

No representative baseline data on disease burden from the period prior to the introduction of the vaccine programme is available from the Philippines and no seroprevalence survey among children aged 5 years has been conducted in the Philippines. To assess the burden of chronic HB infection and the impact of the HB immunization programme in the country, a nationwide survey among children aged 5–6 years was conducted.

Results

From 25 July to 22 October 2013, the survey was conducted in 24 provinces of the Philippines. Five *barangays* (villages) were not included: two gated communities refused to participate, one had insurgency problems and could not be visited, one was a penal colony, hence there were no eligible residents, and one was a commercial district without any residents. These barangays were not replaced. Out of 2,769 children who participated, 32 were ineligible due to age, 2,032 (weighted%, 74%) had an immunization card available for review. Table 1 shows the characteristics of study participants. The majority of the participants were males, Catholics, attended schools and members of the national health insurance program, PhilHealth. 47% of the participants were recipients of the government's conditional cash transfer program, *Pantawid Pamilyang Pilipino Program* or 4Ps. Almost half were delivered in hospitals, while 26% were delivered at home with a skilled birth attendant (SBA).

Hepatitis B vaccination

Among 2,720 participants with information obtained by review of immunization cards or by recall, 567 (weighted%, 21%) received a timely birth dose, i.e. within 24 hours of birth (Table 2). 958 (weighted%, 35%) received the first dose of the HB vaccine more than 30 days after delivery. 2389 (weighted%, 89%) of participants received at least 3 doses of HB vaccine.

Birth in 2008 compared with 2007, maternal education and birthing location were significantly associated with receipt of a timely birth dose of HB vaccine (Table 3). Conversely, sex,

Table 1. Characteristics of study participants.

Characteristics	n (%)
Male	1440 (53)[d]
Mean Age	5.82 (4.80–6.80)[e]
Birth Year	
2007	1376 (50)[f]
2008	1361 (50) [f]
Urban	1333 (49) [f]
Religion	
Catholic	2273 (85)[g]
Non Catholic Christian	303 (11) [g]
Muslim	43 (1) [g]
Others	65 (2) [g]
Attends school	2607 (97) [h]
Mother's Education Status	
None	34 (1) [i]
Primary	523 (20)[i]
Secondary	1491 (56) [i]
Post-secondary or higher	629 (23) [i]
Unknown	5 (0)[i]
PhilHealth[a] Membership	2012 (75)[j]
4Ps[bb] participant	1269 (47)[k]
Factors potentially related to vaccination	
Birthing Location and Assistance	
Hospital	1300 (49)[l]
Home with SBA[c]	708 (26) [l]
Home without SBA	664 (25) [l]
Heard about hepatitis B	2449 (91)[h]

[a]PhilHealth, Philippine Health Insurance Corporation, the national insurance system; [b]4Ps, Pantawid Pamilyang Pilipino Program, the Philippine government's conditional cash transfer program; [c]SBA – skilled birth attendant; [d]Out of 2732 with information; [e]Standard deviation; [f]Out of 2737 with information; [g]Out of 2684 with information; [h]Out of 2680 with information; [i]Out of 2683 with information; [j]Out of 2678 with information; [k]Out of 2683 with information; [l]Out of 2674 with information.

Table 2. Birth dose of hepatitis B vaccine received by survey participants.

	n (weighted %)
BD[a]≤24hrs (Timely birth dose)	567 (26)[b]
BD≤7 days	751 (34)[b]
Timing of first dose	
0–1 day of birth	567 (21)[c]
2–7 days of birth	184 (7)[c]
8–30 days of birth	331 (12)[c]
>30 days of birth	958 (35)[c]
Never	170 (6)[c]
Unknown	510 (19) [c]

[a]Birth dose of hepatitis B vaccine; [b]Out of 2210 with information; [c]Out of 2720 with information.

residence, religion, 4Ps participation, PhilHealth membership, school attendance and mother's having heard of HB infection were not associated with the timely birth dose of HB vaccine.

Prevalence of Hepatitis B surface antigen

Due to problems with the initial laboratory tests (see Methods below), only 2,407 sera were available for testing. Twenty (weighted %, 0.86%) were positive for HBsAg. In the quality control (QC) testing, there was very good concordance (kappa = 0.89) among 19 specimens with HBsAg results available from both the Research Institute for Tropical Medicine (RITM) and Victorian Infectious Disease Research Laboratory (VIDRL), where QC was performed.

Fourteen (weighted%, 1.19%) of 1,212 children born in 2007 while 6 (weighted %, 0.52%) of 1,195 born in 2008 were HBsAg positive. The HBsAg positive children were distributed in the three major island groups of the country: 12 (weighted%, 0.93%) of 1,305 children in Luzon, four (weighted%, 0.77%) of 515 in the Visayas and four (weighted%, 0.77%) of 587 in Mindanao. Twelve (weighted%, 0.51%) of all children were born at home. Through document review and recall, among children who were HBsAg positive, 17 (weighted%, 85%) received 3 doses of the HB vaccine, and two (weighted%, 20%) received a timely birth dose (Table 4). No significant differences on receipt of 3 doses and timely birth dose of HB vaccine were seen among children who were HBsAg positive compared to those who were HBsAg negative.

Discussion

This is the first nationwide HB seroprevalence survey among children aged 5–6 years of age that was conducted in the

Table 3. Receipt of hepatitis B birth dose (BD) among participants.

Characteristics	n (%) [95% CI]	p
Sex		0.99
Males who received a BD ≤24 hours	297 (26)[a] [23–28]	
Females who received a BD ≤24 hours	270 (26)[b] [23–28]	
Birth year		0.01
Children born in 2007 who received a BD ≤24hours	259 (23)[c] [21–26]	
Children born in 2008 who received a BD ≤24hours	308 (28)[d] [25–31]	
Urban/rural residence		0.11
Children living in an urban area who received a BD ≤24hours	306 (27)[e] [25–30]	
Children living in a rural area who received a BD ≤24hours	261 (24)[f] [22–27]	
Religion		0.12
Catholics who received a BD ≤24hours	485 (26)[g] [24–28]	
Non-Catholic Christians who received a BD ≤24hours	58 (24)[h] [18–30]	
Muslims who received a BD ≤24hours	4 (12)[i] [3–27]	
Other religion who received a BD ≤24hours	20 (33)[j] [22–47]	
4P Participation		0.1
Children in the 4P program who received a BD ≤24hours	251 (24)[k] [21–26]	
Children NOT in the 4P program that received a BD ≤24hours	315 (28)[l] [25–30]	
PhilHealth[a] membership		0.90
Children with PhilHealth who received a BD ≤24hours	429 (26)[m] [23–28]	
Children without PhilHealth who received a BD ≤24hours	137 (26)[n] [22–30]	
Maternal education		<0.0001
Children whose mothers had no education who received a BD ≤24 hours	6 (24)[o] [9–44)	
Children whose mothers had primary education who received a BD ≤24 hours	76 (18)[p] [14–21]	
Children whose mothers had secondary education that received a BD ≤24 hours	314 (26)[q] [23–28]	
Children whose mothers had university or higher education who received a BD ≤24 hours	168 (32)[r] [28–36]	
Children whose mothers had unknown education who received a BD ≤24 hours	2 (40)[s] [5–85]	
School attendance		0.26
Children in school who received a BD ≤24hours	556 (26)[t] [24–28]	
Children NOT in school who received a BD ≤24hours	10 (18)[u] [9–30]	
Factors potentially related to hepatitis B vaccination		
Birthing location and assistance		<.0001
Hospital births who received a BD ≤24 hours	437 (41)[v] [38–43]	
Home births attended by SBA who received a BD ≤24 hours	93 (15)[w] [13–19]	
Home births NOT attended by SBA who received a BD ≤24 hours	35 (7)[x] [5–9]	
Heard about hepatitis B		0.25
Mother knew about Hepatitis B who received a BD ≤24 hours	529 (26)[y] [24–28]	
Mother DID NOT know about Hepatitis B who received a BD ≤24 hours	37 (22)[z] [16–29]	

[a]Out of 1,158 with information; [b]Out of 1,052 with information; [c]Out of 1,111 with information; [d]Out of 1,099 with information; [e]Out of 1.128 with information; [f]Out of 1,082 with information; [g]Out of 1,871 with information; [h]Out of 245 with information; [i]Out of 34 with information; [j]Out of 60 with information; [k]Out of 1,062 with information; [l]Out of 1,143 with information; [m]Out of 1,684 with information;[n]Out of 523 with information; [o]Out of 26 with information; [p]Out of 433 with information; [q]Out of 1,215 with information; [r]Out of 530 with information; [s]Out of 5 with information; [t]Out of 2,150 with information; [u]Out of 57 with information; [v]Out of 1,079 with information; [w]Out of 592 with information; [x]Out of 533 with information; [y]Out of 2,038 with information; [z]Out of 169 with information.

Table 4. Hepatitis B vaccination status and HBsAg status of children.

	n (weighted %)	p
HBsAg status and birth dose (BD)		0.69
Children who are HBsAg positive who received a BD ≤24 hours	2 (20)[a]	
Children who are HBsAg negative who received a BD ≤24 hours	495 (26)[b]	
HBsAg status and receipt of 3 doses of Hep B vaccine (HB3)		0.65
Children who are HBsAg positive who received HB3	17 (85)[c]	
Children who are HBsAg negative who received HB3	2059 (88)[d]	

[a]Out of 10 with information; [b] Out of 1,935 with information; [c]Out of 20 with information by card or recall, [d] Out of 2,333 with information by card or recall.

Philippines. Our study included children who were born just as the provision for a birth dose was being implemented and therefore provides baseline information prior to widespread birth dose implementation. In addition, these children would have passed through the period of highest risk of chronic infection. The prevalence of HBV infection of 0.86% in this age group falls within the Regional goal of achieving <1% prevalence by 2017. This figure is somewhat lower than expected and has to be interpreted with caution.

There are limitations to our study. First, because of the discrepancies in the anti-HBc serologic results from RITM and the QC results of VIDRL, the designated WHO regional reference laboratory for HB, the planned algorithm was not followed. Because of the discrepant results with different test kits, step by step procedures were reviewed and ELISA equipment were inspected which did not reveal possible sources of error. All available sera were therefore re-tested for HBsAg. Upon re-testing at RITM, there was very good agreement with the VIDRL QC results. The reasons for the discrepant anti-HBc results were not fully understood but the use of different test kits and different procedures in the two laboratories, with different performance may be factors. Another serosurvey is instead planned to be conducted soon.

Second, the listing of the children's name in communities may not have been correctly and consistently performed. There was an anecdotal report of some BHWs who obtained the lists of children from the target client list (TCL) of the health center. If this was indeed true, then the list obtained from the TCL would have only included residents who obtained health services in the health center. This may have resulted in bias in the selection of the children, since children who do not seek care would have been excluded in the survey. In our study, 47% of the children belonged to families who were 4Ps recipients, considerably higher than the 19.7% poverty incidence of families[11] however, 75% of our participants were PhilHealth members, which is comparable to the reported 79% PhilHealth membership as of 2013.[12]

Third, during the midwives' visits, they may have chosen the children whose parents were easier to convince to participate. These parents may have more knowledge on health and personal hygiene practices, thus may be at lower risk for HBV infection. This again may have resulted in selection bias. During the training, the importance of randomly selecting children and convincing parents was emphasized but it is possible that this was not followed through. Fourth, we had problems recruiting in some areas of the country. We were unable to recruit in Sulu, an area of the country where home deliveries may be higher than in other areas and majority of the residents are Muslims. Indeed, only 2% of the subjects in our study were Muslims while ~5% of the Philippine population belongs to this religion,[13] suggesting that our sample may not be representative of the whole country. However, HBV infection was identified in children residing in the 3 major island groups. At the same time, we were unable to recruit in some gated communities where the affluent population resides. In the 2013 DHS in the Philippines, >91% of the births among the highest quintile were in a health facility and more likely to have received better health care.[14]

A recent modelling study estimated that the HBsAg prevalence in children <5 years in the Philippines as of 2016 was 2.2%,[15] higher than the seroprevalence detected in this study. In our survey, 88% of the children had HB3, similar to the reported coverage of 87% and 88% in 2007 and 2008 respectively, however, the 26% timely birth dose in the survey is higher than the reported 9% and 21% in 2007 and 2008 birth dose coverage, respectively. Reporting of birth dose is problematic in the Philippines because not all facilities report immunization coverage to the EPI,[16] thus the reported birth dose coverage may be lower than it actually is. Prior to 2007, vaccines were mostly available from the private market, but because of the devolved health care set-up some local government units offered HB vaccines to some children thereby increasing the coverage, but this may not have been reported in the EPI. It is unknown what was the coverage of HB vaccines in the private sector and those provided by the local health units, but it may have some impact in decreasing horizontal transmission as the number of infectious people declined.

Despite significant strides, HB vaccine implementation in the Philippines is not without problems. The coverage of HB3 vaccination gradually increased from 45% in 2002 to 87% in 2007 and 94% in 2013, before declining to 60%[7] in 2015 due to pentavalent vaccine stockouts in 2014 and 2015. Although catch-up doses were provided by the EPI to children who missed out on their scheduled doses, it is unclear how many children did not complete the recommended immunization. Implementation of the birth dose of HB vaccine is affected by the relatively high proportion of children still being delivered outside of a health facility (40%).[17] Furthermore, a study conducted in 2011 reported that 8% of hospitals, the majority of which were private facilities, did not provide the birth dose of HB vaccine, nor did they report vaccine coverage to the EPI.[16] Based on these findings, several steps were taken such as the inclusion of the birth dose of HB vaccine in the PhilHealth Newborn Service Package. A previous modelling study suggested that in a setting with 8% chronic hepatitis B prevalence among pregnant women, at least 85% HB3 vaccine coverage and 65% timely HB vaccine birth dose are necessary to achieve the regional goals.[9] It is suggested that a 90% reduction in new chronic infections and 65% reduction in mortality could be attained with a 90% infant vaccination, 80% birth dose coverage together with peripartum antiretrovirals and population-wide testing and treatment.[18] In our study, 89% of the participants received HB3 and 26% received a

timely birth dose, thus the reasons for the lower than expected HBsAg prevalence among 5–6 years olds may have been affected by limitations in the laboratory and in the field.

Our study has limitations however it is the first large-scale attempt to assess the seroprevalence of HBsAg among children. A follow-up survey is planned in the near future to assess the progress in the country's program implementation. Efforts must be made to increase the coverage and improve recording of the HB vaccine birth dose, ensure high vaccine coverage of HB3 and accurately capture this information to allow the country to attain the regional goal for hepatitis B elimination.

Methods

The Philippines, with a population of 98 million in 2013, is divided into 17 regions covering 80 provinces, 143 cities and 1,491 municipalities that are further subdivided into barangays (villages). We conducted a nationwide cross-sectional cluster survey among children born between January 1, 2007 and December 31, 2008 in the Philippines whose parents provided informed consent for participation in the study. Children who were not able to give blood because of severe illness or haemophilia or whose caretaker did not provide consent were excluded from the study.

Sample size and sampling

To provide an estimate of the national prevalence of HB surface antigen (HBsAg), we assumed a 1% prevalence of HBsAg in this age group, a design effect of 1.5 due to cluster sampling, with 95% confidence level, 0.5% degree of precision, the minimum sample size required was 2,282. After adjusting for drop-outs and non-participation, we planned to enrol 3,000 subjects.

A 3-stage cluster design was used for sample selection. Using probability-proportional to size (PPS) sampling method, 25 provinces were selected for the first stage. For the second stage, 12 barangays (villages) per province were selected using PPS. No replacements were allowed. Third, 10 children per barangay were randomly chosen from a list of children aged 5–6 years of age who were residents of the barangay. *Barangay* (village) health workers (BHWs) were requested to prepare this list, by visiting the households in their *barangay* to ensure that all children are included. The midwives were instructed to randomly choose from this list.

Survey

Randomized children were instructed to proceed to the designated local health centers where the medical technologists conducted the survey including the blood draw. In most of the areas, randomized children complied, if there were <10 children, the midwife was instructed to randomly choose another child on the list. After informed consent was obtained from the parent or guardian, information were collected on the following: date of birth, sex, religion, membership in conditional cash transfer program, place of birth (whether health care facility or home), if born at home – presence of skilled birth attendant, mother's level of education, school attendance, receipt of HB vaccine, number of HB vaccine doses, number of days between birth and receipt of first dose of HB vaccine, if less than 3 doses – reason(s) for missed doses. Data were entered in the field with Android smartphones (Sony Xperia E duo) using a tailor made application, developed specifically for the study. Data were sent via SMS to a central server and saved in the phone memory. SMS that failed to transmit centrally were later transferred from the phones to the server at a later date. 5 ml of blood was obtained by venipuncture and sera separated prior to shipment for testing to the RITM.

Laboratory testing

Initial testing followed the algorithm recommended by the WHO[8] which identifies individuals with chronic infection through detection of both anti-HBc and HBsAg.[19] All serum samples were first tested for anti-Hepatitis B core (HBc) antibodies (Monolisa Anti-HBc; Biorad, CA), and samples that tested positive were then tested for HBsAg (Monolisa HBsAg ULTRA, Biorad, CA). 10% of the samples that were negative and all the positive samples tested in RITM were sent to VIDRL for QC. In VIDRL, anti-HBc antibodies were tested using the Murex (Dia Sorin) anti-HBc (total) (96 well format) assay and HBsAg testing was performed using the Murex (Dia Sorin) HBsAg Version 3 (96 well format). Initially, 2,704 specimens were available for testing (33 had no specimens or refused extraction). However, quality control (QC) testing conducted at VIDRL revealed discrepancies with the total anti-HBc results of RITM. Using the VIDRL results as the reference, the RITM anti-HBc results had 74/105 (70.4%) false positives and 12/13 (92.3%) of the equivocal results were false negatives. However, there was very good concordance (kappa = 0.85) between the Monolisa HBsAg results of RITM with the VIDRL Murex HBsAg results. Since the problem appeared to be primarily in the anti-HBc testing, RITM re-tested all available serum specimens (n = 2,407) for HBsAg using Murex (Dia Sorin) HBsAg Version 3 (96 well format), the same test kit used in VIDRL.

Statistical analysis

Data analysis was conducted using SAS v9.3 (Cary, NC, USA) and SUDAAN v10 (Research Triangle Park, NC, USA). Analysis accounted for the three-stage cluster design, applying weights as calculated during the sampling process (the inverse of the probability of selection). This was done with SAS using PROC SURVEYFREQ with a cluster and weight statement. Wilson 95% confidence limits were calculated for proportions that were outside the range of 20%–80%, otherwise Wald 95% confidence limits were calculated (e.g. for seroprevalence). Statistical significance was assessed using t-test for continuous variables and chi-square or Fisher's exact test when data were sparse for categorical variables, with significance set at $p < 0.05$. Re- analysis of additional laboratory data and review of previous statistical results were performed using Stata 13 (College Station, Texas, USA).

Ethics

The study protocol was reviewed and approved by the University of the Philippines Manila-Research Ethics Board and the

Ethics Review Committee of the Western Pacific Regional Office of the World Health Organization (WHO). A list of children who were HBsAg positive was submitted to the DOH. The DOH staff together with staff from the local health centers identified these children. The children and their families were invited to the health centers and were informed of the results, advised and counselled on HB infection.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

We would like to thank Dr. Minal Patel, from the US Centers for Disease Control and Prevention, for her important contributions in protocol development and statistical analysis. Our gratitude extends to all health care personnel from the central and regional offices of the Department of Health and from the different local government units who provided support and/or took part in the implementation of the study; and most of all to all the parents and their children who participated in the survey.

Funding

This study was funded by the World Health Organization.

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