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Prevalence of chronic hepatitis B virus infection before and after implementation of a hepatitis B vaccination program among children in Nepal

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Abstract

Background—In Nepal, an estimated 2–4% of the population has chronic hepatitis B virus (HBV) infection. To combat this problem, from 2002–2004, a national three dose hepatitis B vaccination program was implemented to decrease infection rates among children. The program does not currently include a birth dose to prevent perinatal HBV transmission. In 2012, to assess the impact of the program, we conducted a serosurvey among children born before and after vaccine introduction.

Methods—In 2012, a cross-sectional nationally representative stratified cluster survey was conducted to estimate hepatitis B surface antigen (HBsAg) prevalence among children born from 2006 to 2007 (post-vaccine cohort) and among children born from 2000 to 2002 (pre-vaccine cohort). Demographic data, as well as written and oral vaccination history were collected. All children were tested for HBsAg; mothers of HBsAg positive children were also tested. Furthermore, we evaluated the field sensitivity and specificity of the SD Bioline HBsAg rapid diagnostic test by comparing results with an enzyme immunoassay.

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Results—Among 2181 post-vaccination cohort children with vaccination data by either card or recall, 86% (95% Confidence Interval [CI] 77–95%) received ≥3 hepatitis B vaccine doses. Of 1200 children born in the pre-vaccination cohort, 0.28% (95% CI 0.09–0.85%) were positive for HBsAg; of 2187 children born in the post-vaccination cohort, 0.13% (95% CI 0.04–0.39%) were positive for HBsAg (p=0.39). Of the 6 children who tested positive for HBsAg, 2 had mothers who were positive for HBsAg. Finally, we found the SD Bioline HBsAg rapid diagnostic test to have a sensitivity of 100% and a specificity of 100%.

Conclusions—This is the first nationally representative hepatitis B serosurvey conducted in Nepal. Overall, a low burden of chronic HBV infection was found in children born in both the pre and post-vaccination cohorts. Current vaccination strategies should be continued.

Keywords

hepatitis B virus; Nepal; seroprevalence; vaccine

INTRODUCTION

Worldwide, more than 2 billion people have been infected with hepatitis B virus (HBV); approximately 240 million have chronic HBV infection, 20–25% of whom will eventually die from HBV-related liver disease, including cirrhosis and hepatocellular carcinoma [1, 2]. The prevalence of chronic HBV infection, defined by the presence of hepatitis B surface antigen (HBsAg), varies markedly, from <1% in the United States and Australia to >7% in countries like China [3–5]. Regardless of country-specific prevalence, the World Health Organization has recommended at least three doses of hepatitis B vaccine for all infants, including a first dose within 24 hours of birth [6].

Nepal is considered to have low to intermediate endemicity of chronic HBV infection, with an estimated HBsAg population seroprevalence of 2–4% [1]. However, no nationally representative serosurvey has been conducted so the population prevalence is unknown. In blood donors, the prevalence ranged from 0.35%–1.2% [7–10]; small convenience surveys of healthy Nepalese adults males found a seroprevalence of 0.93%–4%, with variations across different regions [11–14].

To protect future generations of Nepalese children, Nepal introduced a single antigen hepatitis B vaccine in a phased manner from 2002 to 2004 for infants. Shortly thereafter, the single antigen vaccine was phased out and replaced with a combination tetravalent vaccine containing diphtheria, tetanus, pertussis, and hepatitis B antigens (DTP-HepB). Beginning in 2009, hepatitis B vaccine has been administered as a pentavalent DTP-HepB-Hib combination vaccine to infants at 6, 10, and 14 weeks of age. Coverage has steadily improved, starting at 27% in 2004 and rising to 82% in 2010 (Figure 1) [15]. Mothers are not routinely screened for HBsAg, and neither hepatitis B immunoglobulin nor a hepatitis B birth dose vaccine (HepB-BD) is routinely administered to newborns to prevent perinatal HBV transmission.

To determine the impact of the vaccination program and the burden of HBV perinatal transmission, Nepal undertook a serosurvey of children born prior to and following vaccine introduction.

METHODS

In April 2012, we conducted a nationally representative cross-sectional three-stage cluster survey among children born from April 2000 to April 2002 (10–12 year olds, pre-vaccine cohort) and children born from April 2006 to April 2007 (5–6 year olds, vaccine era cohort). To assess the contribution of perinatal transmission, mothers of children testing positive for HBsAg were tested for HBsAg.

Sample size and sampling

The sample size of 2144 for the post-vaccine era children was calculated based on an expected HBsAg seroprevalence of 0.5%, a one-sided precision of +0.5% (Wilson-Score method), a 95% probability of achieving that precision, and a design effect of 2. The sample size for the pre-vaccine cohort was based on the objective of comparing seroprevalence in the 2 groups. A sample size of 1186 was calculated for the pre-vaccine cohort based on a Fisher's exact test assuming pre-vaccination seroprevalence of 2% and post-vaccination seroprevalence of 0.5%, α=0.05, and 80% power.

To ensure adequate representation of Nepal's diverse geographic, economic, and social characteristics, the country was stratified into distinct strata that factored in the population density (urban/rural), the three ecological zones that divide the country north to south (mountains, hills, and terai) and the Kathmandu metropolitan area. The mountain zone consists of rural areas only, and Kathmandu is completely urban. Thus, a total of six strata were created: Kathmandu metropolitan, mountain rural, hill urban, hill rural, terai urban, and terai rural (see supplemental map).

The primary sampling units (PSUs) for the first stage were village development committees (VDCs) in the rural areas and municipalities in the urban/metropolitan areas. Fifty (50) PSUs were proportionally allocated based on the estimated proportion of the total population residing in each stratum. PSUs were selected based on probability proportion to estimated size (PPES) within each stratum: 3 in Terai-urban, 21 in Terai-rural, 2 in Hill-urban, 18 in Hill-rural, 4 in Mountain-rural, and 2 in Kathmandu (see supplemental map). Within each selected PSU, four wards were randomly selected using PPES. Within each selected ward, eleven 5–6 year olds and six 10–12 year olds were chosen by randomly visiting households until the desired number were enrolled. Only one child was selected in each household in each age cohort. In the few instances where a selected ward did not have enough children to meet the required sample size, the remainder were selected from a neighboring ward.

Eligibility

Children were eligible for participation if they were born in the aforementioned time frames. Children were excluded if they were unable to give blood because of severe illness or hemophilia. For each eligible child, informed consent was obtained from a parent and assent

was obtained from the child. If consent or assent was not provided, the child was not enrolled in the survey.

Data Collection

If consent was obtained, a brief questionnaire was administered to the caregiver. The questionnaire collected demographic data, potential risk factors for infection, and vaccination history. If written vaccination history was not available, vaccination history based on caregiver recall was obtained.

Specimen Collection and Laboratory Testing

Approximately 5 mL of blood was collected by venipuncture. In the field, the sample was centrifuged, and the serum was tested using the SD BioLine HBsAg rapid test (Standard Diagnostics, Inc., Korea, sensitivity 100% (95% CI 96.3–100%), reported specificity 100% (95% CI 97.9–100%)) [16]. Mothers of children with positive field-based tests, and who provided consent, had a venipuncture sample of blood taken, and were also tested using the rapid test. Samples from all children who tested positive, a random selection of 10% of those who tested negative, and all samples from mothers of all positive children were tested by enzyme-linked immunosorbent assay (ELISA) for HBsAg (Anti-Surase B96 (TMB), General Biological Corp., Taiwan, 100% sensitivity, 99.6% specific [17]) and total antibody to hepatitis B core antigen (anti-HBc) (Anti-Corase B96 (TMB), General Biological Corp., Taiwan, 100% sensitivity, 99.6% specificity [18]) at the Nepal National Public Health Laboratory following the standard kit protocols.

Data management and analysis

The data were double entered and stored in a Microsoft Excel 2010 database (Seattle, WA, USA). Data were analyzed in SAS v9.3 (Cary, NC, USA). Analyses accounted for the stratification, first stage clusters, and stratum-specific weights. Children within each stratum were assumed to have the same probability of selection. For each cohort, unweighted proportions were calculated for population characteristics; weighted proportions were calculated for vaccination status and HBsAg. Wilson 95% confidence intervals (CIs) are given for HBsAg proportions. Rao-Scott chi-square (χ^2) p-values were calculated for comparisons of coverage, reported doses, and seroprevalence between the cohorts. For the subset selected for ELISA testing, Fisher's Exact test was used to compare the proportion who tested positive for Anti-HBc among those who were HBsAg negative in each cohort. Sensitivity, specificity, positive predictive value, and negative predictive value were calculated by comparing the gold standard HBsAg ELISA results to the SD Bioline rapid test results; confidence intervals were calculated based on the Wilson-Score method for a binomial proportion. Samples that had an inadequate volume for follow up testing were excluded. We defined those who were anti-HBc positive and HBsAg negative as having been exposed to HBV. Individuals who were HBsAg positive and anti-HBc status unknown or HBsAg positive and anti-HBc positive were defined as having chronic HBV infection.

Human Subjects Rights and Ethics

The study protocol was approved by the Nepal Health Research Council Institutional Review Board (IRB). CDC determined the activity to be human subject research but CDC involvement did not constitute engagement in human subjects research and did not require CDC IRB review.

Funding

Funding for this survey was provided by the World Health Organization.

RESULTS

Among the pre-vaccination cohort, 1200 eligible children 10–12 years of age were identified, all of whom provided consent to participate. Among the post-vaccination cohort, 2200 eligible children 5–6 years of age were identified, of whom 2187 (99%) participated. All mothers of children who tested HBsAg positive consented to having their blood drawn.

Children in the two cohorts were very similar in many key characteristics (Table 1). The mean age and standard deviation of children was 11±0.7 years in the pre-vaccination cohort and 5±0.5 years in the post-vaccination cohort. Because of the sampling methodology, the underlying geographic and urban/rural distribution mimics that of the population. A majority of children in both cohorts were born at home without the assistance of a skilled birth attendant (SBA).

Vaccination history

Of children in the pre-vaccination cohort, vaccination cards were available for 24 (2%) of 1117 and recall-based information was available for 1092 (99.9%) of 1093 regarding the number of doses of hepatitis B vaccine received; 884 (79%) had zero reported doses, 54 (5%) had 1–2 reported doses, and 178 (16%) had ≥3 reported doses. Of children in the post-vaccination cohort, vaccination cards were available for 71 (3%) and recall-based information was available for 2110 (100%) on the number of doses received was available for 2181 children; 266 (12%) had zero reported doses, 22 (1%) had 1–2 reported doses, and 1893 (87%) had ≥3 reported doses. The proportion of children receiving zero-doses was significantly lower and ≥3 dose vaccination was significantly higher amongst the post-vaccine cohort as compared with the pre-vaccine cohort ($p<0.0001$) (Table 2).

Seroprevalence

HBsAg was detected among 3 of 1200 (0.28%, 95% CI 0.09–0.85%) children in the pre-vaccination cohort and among 3 of 2187 (0.13%, 95% CI 0.04–0.39%) children in the post-vaccination cohort ($p=0.39$) (Table 3). All six children who tested HBsAg positive only had vaccination data provided by recall, and timing of the doses is unknown. Of three HBsAg-positive children in the pre-vaccination cohort, two were reported to have received zero doses of hepatitis B vaccine and one was reported to have received 3 doses; of the post-vaccination cohort, all three HBsAg-positive children were reported to have received 4 doses of hepatitis B vaccine. There was no clustering of HBsAg-positive children in the same district (Table 4). A history of viral hepatitis was reported in siblings of two of the

HBsAg positive children in the older cohort; one of these children also had a father with a reported history of viral hepatitis. Amongst the three HBsAg positive children in the younger cohort, a history of viral hepatitis was reported in one mother (Table 3).

Samples from 343 children were selected for ELISA testing for anti-HBc; 124 in the pre-vaccination cohort and 219 in the post-vaccination cohort. In the pre-vaccination cohort, samples were not of sufficient volume for 21 children (1 HBsAg-positive child, 20 HBsAg-negative children). In the post-vaccination cohort, samples were not of sufficient volume for 41 children (1 HBsAg-positive child, 40 HBsAg-negative children). Of the 4 children testing positive for HBsAg with sufficient serum volume, all 4 (100%) tested positive for total anti-HBc. Of the 101 pre-vaccine children testing negative for HBsAg with sufficient serum volume, 5 (5%) tested positive for total anti-HBc; of the 176 post-vaccine children testing negative for HBsAg with sufficient serum volume, 1 (0.6%) tested positive for total anti-HBc (Fisher's exact p=0.03). (see Supplemental Flow Chart)

Maternal Infection

Of the three mothers of HBsAg seropositive pre-vaccination cohort children, one mother was found to be seropositive for HBsAg (33%). Of the three mothers of HBsAg seropositive children in the post-vaccination cohort, one mother was HBsAg seropositive (33%). Both HBsAg-positive women by the rapid test were positive for anti-HBc and for HBsAg by ELISA. One HBsAg-positive woman reported a history of viral hepatitis; none reported a history of liver cancer or cirrhosis in themselves or their parents.

Rapid test sensitivity and specificity

Samples from 349 persons (343 children and 6 mothers) were selected for follow-up testing to evaluate the sensitivity/specificity of the SD Bioline HBsAg rapid test. Serum samples for two children who tested negative on the rapid test were of insufficient volume to undergo ELISA testing and were excluded. The sensitivity and specificity of the rapid test was 100% (68%–100%) and 100% (99%–100%) respectively. The positive predictive value and negative predictive value of the rapid test was 100% (68%–100%) and 100% (99%–100%) respectively (Table 5).

DISCUSSION

This nationally representative serosurvey sought to obtain information on the impact of the hepatitis B vaccination program, the burden of perinatal transmission, and the field sensitivity and specificity of a rapid HBsAg test. Tremendous improvement in hepatitis B vaccine coverage has been achieved over the past decade. In this survey, 16% of children born prior to nationwide introduction of hepatitis B vaccination had received ≥3 doses, compared with 86% of children born after introduction. The prevalence of exposure to HBV, defined as the presence of anti-HBc, was 5% in the pre-vaccination cohort and 0.6% in the post-vaccination cohort. The burden of chronic HBV infection was low in both cohorts: 0.28% in the pre-vaccination cohort compared with 0.13% in the post-vaccination cohort.

The prevalence of HBsAg seropositivity in both cohorts was lower than previously reported [7–14]. Reasons for this are unclear, as neighboring countries such as China have a much

higher burden of disease [3]. To detect a difference between a seroprevalence of 0.13% and 0.28%, >12,500 children would need to be enrolled in each group. As a result of the low burden of chronic disease found in the pre-vaccine cohort, we were unable to document the impact of the vaccination program. However, this study did demonstrate a significant decrease in anti-HBc from 5% in the pre-vaccination cohort to 0.6% in the post-vaccination cohort. Despite not demonstrating a statistically significant difference in chronic HBV infection, hepatitis B vaccination should continue to be provided for free to all infants as part of the routine immunization program, as recommended by the WHO Strategic Advisory Group of Experts on Immunization [6]. Furthermore, room for improvement exists within the vaccination program. While 90% of children reported obtaining 3 doses of hepatitis B vaccine in 2012, significant heterogeneity has been seen throughout Nepal, partially due to healthcare access challenges [15, 19]. These disparities must be addressed in order to reach every child in every community with hepatitis B vaccine as well as all other vaccinations.

One of the objectives of this survey was to assess the contribution of perinatal HBV transmission to disease burden. Of the six children who were HBsAg positive, only two had mothers who had chronic HBV infection. We assume these two children acquired the infection perinatally, though we did not undertake confirmatory genetic analysis. HepB-BD has been repeatedly shown to decrease perinatal = transmission, and WHO recommends HepB-BD for every country [6, 20–23]. Though the introduction of a HepB-BD would decrease perinatal transmission, the cost-benefit of introducing a HepB-BD is unclear in a country with a low disease burden and where 64% of newborns are born at home without a skilled birth attendant [19]. Establishing a HepB-BD program to successfully vaccinate these newborns born at home within the recommended 24 hours would require significant financial and human resources. Given competing public health interests and the low burden of disease, a universal HepB-BD program might not be a high priority currently. Targeting a HepB-BD program to high risk populations might be considered; however, this nationally representative serosurvey was unable to reflect the area-specific variations in chronic HBV infection that might exist in the country. For example, given the historically higher burden in China, we suspect the Tibetan population living in Nepal as well as those living near the Chinese border could have a higher burden of chronic HBV infection [3]. Sub-regional or sub-group childhood serosurveys or antenatal screening of pregnant women could help to identify higher risk populations; once identified, HepB-BD could be implemented in select areas or populations to prevent perinatal transmission.

Finally, as hepatitis B control goals are considered for various regions, large surveys, usually requiring testing of >3000 children, are required to document the achievement [24]. However, such surveys are expensive as they require the hiring of trained phlebotomists to draw a venipuncture sample, cold chain capacity in the field to store the specimens, the procurement of ELISA kits, and a high quality laboratory to conduct the testing. Rapid testing is a reasonable alternative to ELISA for epidemiologic surveys due to the lower cost of testing and simpler logistics [25–29]. In the first documented field sensitivity and specificity of the SD Bioline HBsAg rapid test kit, we found 100% sensitivity and specificity; this helps to provide further evidence that rapid test-based hepatitis B serosurveys are a reasonable alternative to the ELISA gold-standard. Our findings are consistent with the laboratory sensitivity and specificity seen in other evaluations [30, 31].

This study had several limitations. First, the rapid HBsAg test used in this study is not the gold standard, and therefore the HBsAg prevalence in this study might be lower than the true seroprevalence; however, the ELISA double testing showed the test was 100% sensitive in this study. Vaccination card retention for this survey was extremely low; vaccination data collected by recall are not as reliable as vaccination data abstracted from vaccination cards, especially since we asked the parents about an event that happened more than 5 years ago. Because of this, we did not feel it was sound to further analyze factors associated with under-vaccination. Reasons why card retention were so low in this study population are unknown. As mentioned, we were unable to demonstrate a statistically significant difference between the two cohorts as the study was not powered to detect the small difference between 0.28% and 0.13%. Small numbers of HBsAg positive children limited our ability to evaluate risk factors for seropositivity as originally intentioned; small numbers of seropositive children also limited our ability to estimate rapid test sensitivity with precision. Finally, 18% of sera were not available for ELISA testing. We assume these data are missing at random; however, this missing data could bias our estimates of anti-HBc prevalence and sensitivity of the rapid test.

In conclusion, Nepal's first nationally representative hepatitis B serosurvey has shown a very small burden of chronic HBV infection among post-vaccine cohort children despite the lack of a HepB-BD. In factoring whether to introduce HepB-BD as is recommended by the World Health Organization, Nepal must now evaluate this and other available data to determine the cost-benefit in an environment characterized by limited resources and multiple public health priorities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

Anti-HBc	antibody to hepatitis B core antigen
CI	confidence interval
ELISA	enzyme-linked immunosorbent assay
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HepB3	3-dose hepatitis B vaccination coverage

HepB-BD	hepatitis B birth dose vaccine
SBA	Skilled Birth Attendant
WHO	World Health Organization

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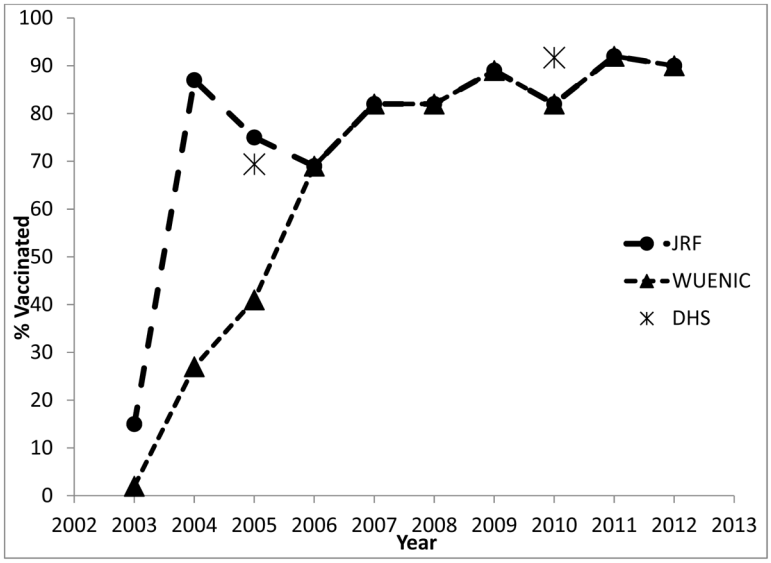

Figure 1.

Hepatitis B3 vaccination coverage, Nepal, 2003–2012. JRF=Joint Reporting Form [15], WUENIC=WHO/UNICEF estimates for national immunization Coverage [15]; DHS=Demographics and Health Survey [19, 32].

Table 1

Characteristics of enrolled children, Nepal, 2012.

	10–12 year old cohort			5–6 year old cohort		
	Total Respondents	**Number positive**	**%**	**Total Respondents**	**Number positive**	**%**
Male	1200	630	53	2186	1165	53
Religion	1197			2185		
Hindu		985	82		1783	82
Buddhist		125	10		233	11
Muslim		37	3		73	3
Kirant Mundhum		37	3		78	4
Christian		13	1		18	1
Birth Location	1199			2185		
Health Facility		235	20		545	25
Home with SBA[a]		24	2		76	3
Home without SBA[a]		940	78		1564	72
Mother's highest level of education	1197			2177		
None		473	40		800	37
Primary (grades 1–5)		451	38		763	35
Secondary (grades 6–10)		232	19		519	24
Post-secondary		41	3		95	4
CHARACTERISTICS OF RESIDENCE						
Urban (vs. Rural)	1200	168	14	2187	308	14
Geographic Terrain	1200			2187		
Hill		480	40		867	40
Kathmandu		48	4		88	4
Mountain		96	8		176	8
Terai		576	48		1056	48
Region of Nepal	1200			2187		
Far western		120	10		220	10
Midwestern		120	10		220	10

	10–12 year old cohort			5–6 year old cohort		
	Total Respondents	**Number positive**	**%**	**Total Respondents**	**Number positive**	**%**
West		216	18		396	18
Mid		432	36		779	36
East		312	26		572	26

[a]SBA Skilled Birth Attendant

Table 2

Hepatitis B vaccination history[a], Nepal, 2012.

	10–12 year old cohort				5–6 year old cohort			
	Total Respondents	**Number positive**	**Weighted %**	**95% CI**	**Total Respondents**	**Number positive**	**Weighted %**	**95% CI**
Vaccination history by card (vs. recall)	1117	24	2.2	(0.7–3.7)	2181	71	3.2	(0.80–5.6)
Number of hepatitis B doses	1116				2181			
0		884	78.8	(72.7–84.8)		266	12.7	(3.7–21.7)
1–2		54	5.0	(2.4–7.6)		22	1.0	(0.6–1.4)
≥3		178	16.2	(11.0–21.4)		1893	86.3	(77.2–95.4)

[a] Vaccination history was collected from immunizations cards or recall when cards were not available.

[b] CI=Confidence Interval

Table 3

Seroprevalence of hepatitis B surface antigen among 5–6 year olds and 10–12 year olds, Nepal, 2012.

	Total Tested	Number positive for HBsAg	Weighted %	95% CI
10–12 year old cohort (pre-vaccine)	1200	3	0.28	(0.09–0.85)
5–6 year old cohort (post-vaccine)	2187	3	0.13	(0.04–0.39)

Table 4

Characteristics of Children who had hepatitis B surface antigen on the SD Bioline rapid test, Nepal, 2012.

Child	Sex	Age	Mother's rapid test result	Vaccination history	Number of doses of Hepatitis B vaccine	Sibling with reported history of viral hepatitis	Father with reported history of viral hepatitis	Mother with reported history of viral hepatitis
A	Female	11	Positive	Recall	0	+	−	−
B	Male	11	Negative	Recall	0	+	+	−
C	Male	11	Negative	Recall	3	−	−	−
D	Female	6	Positive	Recall	4	−	−	+
E	Male	5	Negative	Recall	4	−	−	−
F	Male	6	Negative	Recall	4	−	−	−

Table 5

Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value of the SD Bioline HBsAg rapid test kit compared with ELISA.

		HBsAg ELISA	
		+	−
HBsAg Rapid Test*	+	8	0
	−	0	339

*2 samples negative by rapid test were not of sufficient volume and were excluded from calculations

Sensitivity: 100% (68%–100%)[a]

Specificity: 100% (99%–100%)[a]

Positive Predictive Value: 100% (68%–100%)[a]

Negative Predictive Value: 100% (99%–100%)[a]

[a]Confidence intervals are based on Wilson-Score method for a binomial proportion.