Results from Nationwide Hepatitis B Serosurvey in Cambodia using Simple and Rapid Laboratory Test: Implications for National Immunization Program

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Abstract. Chronic liver infection and cancer in the western Pacific region is disproportionate to the population globally. This study provides the first nationwide estimates of hepatitis B surface antigen (HBsAg) seroprevalence in Cambodia among children five year of age. Using a simple and rapid test for HBsAg and multi-stage stratified cluster sampling design, we estimated HBsAg seroprevalence to be 3.5% (95% confidence interval = 2.4–4.8%) among five-year old children. Triangulating the results with other studies, we demonstrate the importance of interrupting perinatal transmission and one-time catch-up vaccination of older children born before nationwide introduction of vaccination for effective hepatitis B control in Cambodia and for reaching the disease control goal of less than 2% chronic infection rates among children ≥ 5 years of age. The results demonstrate the feasibility of conducting nationwide serosurveys using simple and rapid tests to evaluate the impact of hepatitis B vaccination programs in lieu of standard enzyme-linked immunosorbent assays.

INTRODUCTION

Worldwide, an estimated 350 million persons have chronic hepatitis B virus (HBV) infection. The Western Pacific region, with only 28% of the global population, accounts for almost half of all chronic HBV infections worldwide. This report uses geographic regions as defined by World Health Organization (WHO). The Western Pacific region of WHO includes 37 countries and areas including China, Japan, South Korea, The Philippines, and Vietnam. With an estimated 160 million persons chronically infected with HBV living in the Western Pacific region, hepatitis B is estimated to cause almost 890 deaths per day, a mortality rate almost comparable to tuberculosis in the region. With few exceptions, most countries in Western Pacific region were estimated to have a chronic HBV infection rate of ≥ 8% before introduction of hepatitis B vaccination. Despite the perceived high disease burden and availability of highly efficacious vaccine since the early 1980s, the hepatitis B vaccine was introduced and expanded in some of the poorest countries in the region (e.g., Cambodia, China, Lao PDR, and Vietnam) only in 2002 with external financial support from the Global Alliance for Vaccines and Immunization.

Countries need to evaluate the impact of vaccination programs on disease burden to fine-tune the immunization strategies to achieve maximum impact and to continue public funding or to advocate for domestic funding once the external support ends. Considering the limited utility of acute hepatitis B disease surveillance for evaluation and monitoring the impact of hepatitis B vaccination, especially in the short-term, alternative modes of surveillance, for example, population-based serosurveys, are needed. The WHO also recommends measuring reductions in the seroprevalence of hepatitis B surface antigen (HBsAg) in representative population-based serosurveys to evaluate hepatitis B vaccination programs. However, conducting representative population-based serosurveys in poor developing countries with limited laboratory infrastructure and financial resources may be demanding. There is little documented literature on conducting serosurveys for hepatitis B in resource-poor settings to evaluate or monitor the vaccination programs.

This report presents the experiences and results from a population-based serosurvey conducted in Cambodia, one of the poorest countries in the Western Pacific region and situated in Mekong sub-region with a gross national income of $540 per capita in 2007. The prevalence of HBV infection is assumed to be high in Cambodia, but not well documented, with few empirical population-based estimates of chronic HBV infection. The country introduced the vaccine for infants in 2002 on the basis of presumed high disease burden as suggested by high rates of chronic HBV infection (7.8–13%) in blood donor surveys (WHO, 2002, unpublished data). A seroprevalence survey among Cambodian immigrants (15–92 years of age) in Australia estimated a seroprevalence of 8%. This rate was consistent with the rate reported among adults (16–60 years of age) in a serosurvey conducted in rural areas of Takeo Province in Cambodia in 1990–1991, but lower than the rate of 14% reported among southeast Asian migrants in United States in 1980s. In 2001, the National Immunization Program and the National Institute of Public Health in Cambodia conducted the first and the only population-based, age-stratified serosurvey by sampling 2,257 people in a small geographic area, Kampong Chhnang Operational District, in collaboration with PATH, an international non-governmental organization (PATH, 2001, unpublished data). The survey results showed an HBsAg prevalence rate of 11.8% among adults 20–35 years of age with seroprevalence increasing from 3.4% among children 4–5 years of age to 9.2% among persons 14–15 years of age.

Although data from blood bank and other surveys as presented above supported the national strategy to introduce hepatitis B vaccine for infants, the Ministry of Health in Cambodia expressed a need for population-based national estimates to enable them to evaluate the impact of national immunization program post-vaccine introduction. Considering the limited financial, human, and laboratory resources, it was proposed to conduct a nationwide HBsAg serosurvey using alternative simple and rapid tests rather than the traditional enzyme-linked immunosorbent assays (ELISAs). In addition, an attempt was
made to examine the risk factors for HBV infection by collecting self-reported data on established risk factors (e.g., injection safety, blood transfusions, surgery/dental procedures) and other socioeconomic factors that may have implications for public health and immunization policies.

The primary objectives of the study were 1) to assess chronic HBV infection rates as measured by point sero-prevalence of HBsAg among children five years of age born before start of national hepatitis B vaccination program in Cambodia in 2002, and 2) to assess the operational and programmatic feasibility of doing nationally representative serosurvey to estimate HBsAg sero-prevalence using simple and rapid tests in a poor developing country setting with minimal laboratory support.

MATERIALS AND METHODS

The field work for the survey was carried out in early March 2006 and lasted for two weeks. The study used a stratified multi-stage cluster random sampling design. The sample was restricted to children five years of age, which was justified because the risk of acquiring chronic HBV infection is the highest in the first five years of life. In addition, the age of five years provides the earliest opportunity to evaluate impact of infant hepatitis B vaccination program five years after vaccine introduction, especially for countries such as Cambodia that have introduced the hepatitis B vaccine nationwide for infants less than five years ago. Evidence from neighboring hyperendemic countries (e.g., China and Vietnam) in the region suggests that major transmission of hepatitis B infection takes place at birth and in early childhood. Thus, only children five years of age were sampled on grounds of economy with intention of evaluating the hepatitis B vaccination program of Cambodia in the same age group five years later.

The sample size was calculated on the basis of expected HBsAg sero-prevalence of 5% among five-year-old children. The sample size of 1,576 was calculated at 5% level of significance and with precision of ±1.5%. The design effect for cluster sampling was assumed to be 1.75 and loss to follow-up to be 10%.

Sampling. All 24 provinces in Cambodia were stratified into three regions, remote/least developed, medium level of development, and most developed, on the basis of the national classification system, which uses socioeconomic development status and geographic location criteria. In the second stage, all communes in each of the three regions were stratified into urban and rural strata, yielding a total of six strata. A specified number of clusters (communes) are allocated to each of six strata using the probability proportional to size (PPS) based on the proportion of total population of Cambodia in each strata. A total of 60 clusters were sampled at this stage. In the last stage, two villages were sampled from each cluster by PPS methods. In the instance where the two sampled villages in the cluster lacked a sufficient number of children, the next nearest village in the same cluster was selected. For each selected village, researchers obtained a list of children born in 2000, who will be five years of age at the time of survey from the village headman or health worker. From these lists, 26 children were randomly selected from each cluster for the survey.

Questionnaires. A brief face-to-face questionnaire was administered to the parents or caretakers of each sampled child. Information was collected on demographic status of the child (age, sex, place of birth, school enrollment) and on socioeconomic status of the child’s household as measured by mothers’ literacy level and the type of house. In addition, questions were also asked on exposure to potential risk factors for acquiring hepatitis B infection (e.g., injections, blood transfusion, sharing of toothbrush, ear piercing, dental procedures) and history of past hepatitis infection in the child or among the immediate family members.

Testing for HBsAg. Although ELISAs are the most widely used laboratory tests for detection of HBsAg, these are much more expensive and logistically difficult (because of requirements of collection of intravenous blood samples and shipping them in cold chain condition to a centralized laboratory) in field settings during the household surveys. Thus, a simple and rapid (Abbott Determine test strip; Abbott Laboratories, Abbott Park, IL) was selected. The documented sensitivity and specificity of the test ranges from 95% to 100% and from 96% to 100%, respectively, and is quite comparable to standard ELISAs in laboratory and field settings.

The Abbott Determine™ simple and rapid test is a qualitative, visually read, immunochromatographic test in which HBsAg in a whole blood sample binds to an antibody-selenium colloid that is captured by an immobilized antigen, forming a red line on a nitrocellulose strip. Approximately 50 μL of blood is needed for each test. The test requires an anticoagulant buffer (when using whole blood) and capillary tube, but otherwise no special laboratory equipment is required. The kit is simple and easy to use by front-line health care workers with minimal training. Test results can be visually interpreted after 15 minutes (up to 24 hours). The test is considered robust for use in field conditions in tropical countries lacking refrigeration.

The HBsAg testing was performed according to manufacturer’s instructions. A 1.5 day training session was organized on use of the simple and rapid test and for completion of the questionnaire for surveyors and supervisors. A hands-on practice session was organized for surveyors on the simple and rapid test use at Phnom Penh Pediatric Hospital. The required blood sample was withdrawn from a fingertip using a new, disposable lancet for each child. To ensure the safety of the blood collection procedure, surveyors used a new pair of latex gloves. Immediately after use, all capillary tubes and lancets were placed in safety boxes and disposed safely in incinerators as per the standard guidelines in Cambodia.

The test result is interpreted as positive if a red bar is observed in the patient and the control windows and interpreted as negative if a red bar is observed only in the control window (Figure 1). No red bar in either window (or a red bar in only the patient window) indicated an invalid test result.

Consent, confidentiality, ethical considerations, and safety issues. Local authorities and the parents or guardians of sampled children received oral and written information about the study and were given the option of not participating. Instructions and information emphasized that participants would only be tested for HBsAg. A written consent was obtained from the parents/caregiver of each participating child. The survey was reviewed and approved by the Cambodian Ethical Standards Committee of Ministry of Health and by the Cambodian Ministry of Health.

Data entry and analysis. All the filled questionnaires were brought to a centralized location and the data were entered using Epi Info™ software (Centers for Disease Control and Prevention, Atlanta, GA). Double data entry along with
validation and correction were performed to reduce any data-entry related errors. STATA software (Stata Corp., College Station, TX) was used for data analysis. The Poisson exact method for rare events was used to calculate 95% confidence intervals (CIs) of HBsAg infection rates for each region caused by stratified sampling design. Chi-square tests were used to examine the relationship between independent variables and the outcomes of interest in the bivariate analysis. Multivariate logistic regression was used to investigate the independent association of different household and individual characteristics with the odds of being HBsAg positive. All estimates and standard errors were adjusted for the multistage clustered sampling design to give representative, unbiased results. We considered results statistically significant when P < 0.05.

RESULTS

Background characteristics of the sampled children. Against a calculated sample size of 1,576, only 1,561 children could be sampled from the selected villages, and data were excluded from three children because of inconsistent data either on age or the laboratory test. The results from remaining 1,558 children are presented below. Among the sampled children, 51.6% were female, 8.3% came from the least developed region, 53.3% came from moderately developed region, and 38.3% came from most developed region. The regional distribution of sampled children reflected the regional distribution of total population.

HBsAg seroprevalence rate and correlates. At the national level, 3.5% (95% CI = 2.4–4.8%) of all the sampled children were positive for HBsAg. Table 1 shows the HBsAg seroprevalence by background and other characteristics of the sampled children. Statistically significant differences in HBsAg seroprevalence were observed by sex (2.2% in females versus 4.8% in males), region of residence (8.6% in the least developed region versus 3.2% in the most developed region), history of yellowing of eyes/skin/colored urine in the child (8.3% versus 3.1%) and history of liver disease in mother or in any maternal relative (9.6% versus 3.1%).

Higher HBsAg seroprevalence observed by history of blood transfusion, history of having had at least one surgical procedure, and being taken to a dentist was not statistically significant. However, no clear differences were observed by mother’s educational status, number of siblings (an indicator of overcrowding), history of having at least one injection, history of liver disease on the paternal side, body(ear) piercing, or with sharing of toothbrushes with other family members.

Multivariate analysis. Table 2 shows the adjusted odds ratio for being HBsAg positive by selected background characteristics as calculated by fitting a multivariate logistic regression model. A total of 1,542 children were included in the multivariate analysis because of missing values on one or more independent variables for 16 children, all of whom were negative for HBsAg. The characteristics of the excluded children were not significantly different from the children included in the sample and are not likely to bias the results. The differences seen in bivariate analysis by sex, region

![Figure 1. Determine™ HBsAg test (Abbott Laboratories, Abbott Park, IL) for detection for detection of hepatitis B surface antigen.](image-url)


of residence, history of yellowing of eyes/skin/urine in the child, and by history of liver disease in the mother remained significant even in the multivariate analysis.

DISCUSSION

The survey estimated aggregate HBsAg seroprevalence rate of 3.5% (95% CI = 2.4–4.8%) among children five years of age with significantly higher seroprevalence among boys.

The results of the survey are comparable to the rate observed in 4–5-year-old children (3.4%) in the seroprevalence survey conducted in Kampong Chhnang operational district, a moderately developed geographic area, in Cambodia in 2001 using a standard ELISA (PATH, 2001, unpublished data), but much lower than the rate observed in a Takeo Province serosurvey (14%) in 1991. The same 2001 survey estimated an HBsAg rate of 2.7% among 9–17-month-old children, which is comparable to rate of 2% observed among children six months to two years of age in an earlier convenience survey performed in rural areas of Takeo province. Assuming that most infections among children 9–17 months of age are acquired through perinatal transmission at the time of birth, the results point to the importance of interrupting mother-to-child transmission by providing a timely birth dose within 24 hours. A rate of 2.7% among 9–17-month-old children implies that almost 79.4% (2.7/3.4) of chronic HBV infections measured by point prevalence of HBsAg result from mother-to-child transmission, although some of these cases may be acute cases and may not lead to chronic infection among children less than five years of age. This finding in turn implies that administering a hepatitis B vaccine birth dose within 24 hours of birth to prevent mother-to-child transmission will be indispensable in reducing the chronic infection rates to less than 2% by 2012, which is the Western Pacific regional goal of hepatitis B control endorsed by all countries in 2005. In addition, high coverage has to be maintained with three doses of hepatitis B vaccine during infancy when the risk of developing the chronic infection is the highest if exposed to infection.

Previous blood donor surveys, surveys among adult Cambodian immigrants in Australia and the United States, a survey in Takeo Province, and the Kampong Chhnang Operational District serosurvey mentioned in the introduction point to high HBsAg seroprevalence rates, ranging from 7.8% to 14%, among the adolescent and adult populations. The observed increase in HBsAg seroprevalence from children five years of age to the adolescent/adult populations is contrary to the established transmission patterns in other countries in the region with similar seroprevalence rates in adult population. This finding implies that more than 40–50% of chronic HBV infections in hyper-endemic countries are acquired during infancy or young childhood when the chance of becoming a chronic carrier is 90% and 30%, respectively. Therefore, in these hyper-endemic settings, one might expect HBsAg rates in five-year-old children, teenagers, and adults to be similar to each other. For example, a serosurvey conducted in China in 1992 estimated the chronic infection rate in 5–10-year-old children and adults greater than 30 years of age to be 10% and 10.5%, respectively. Similar results were obtained from other hyper-endemic countries, such as Vietnam.

The finding of significant increase in HBsAg seroprevalence from 4–5-year-old children to adolescents/adults in cross-sectional surveys in Cambodia may be caused by a cohort effect or an age effect. Cohort effect implies that the current cohorts of adult and adolescent populations were subjected to much higher transmission of HBV infection when they were less than five years of age 15–30 years ago than the current cohorts of children less than five years of age. Before and during the 1990s, private practitioners practiced unsafe injections and sometimes purchased reusable syringes in the Expanded Program on Immunization. In the late 1990s, comprehensive health education programs aimed at preventing infections with human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome were introduced and health professionals and the general public became highly aware that unsafe injections might transmit HIV. Injections administered during the late 1990s and afterwards were getting safer. A rapid assessment of injection practices in Cambodia in 2002 in Takeo and Phnom Penh found little evidence of reuse of needles and syringes. The Expanded Program on Immunization has used only auto-disable syringes since 2002, and private practitioners now use only disposable syringes. This improvement in injection safety may also explain the reduction in seroprevalence from 14% among 3–5-year-old children in 1991 in the Takeo Province survey to 3.4% in a survey in Kampong Chhnang District in 2001 and the current survey.
However, if findings in Takeo Province are not representative, and the true seroprevalence among 4–5-year-old children is less than 5% even in 1991, then an age effect is most likely to explain the high increase in seroprevalence among adolescents and adults, with risk of exposure to infection increasing after the age of five years. In this event, the policy implications will be different. Cambodia introduced hepatitis B vaccination in 2002 in a phased manner and provided vaccination nationwide in 2005. As a result, all the children 5–10 years of age in 2005 and a large proportion of children born between 2002 and 2004 never received vaccination (because of phased introduction) and are still at high risk of being infected with hepatitis B.

In the absence of easy and practical ways to rule out an age effect, it is better to err on the side of providing catch-up vaccination to children, especially those less than 10 years of age, if allowed by available financial and human resources. The catch-up vaccination can be provided through one-time school-based campaigns for grades 1–5 if one considers the high primary school enrollment rates in Cambodia.

In this survey, sampling of broader age groups may have helped to answer some of the questions on trends in HBsAg seroprevalence more definitively and would have better informed the decision of catch-up campaigns for the older children. However, this would have meant an increased cost for the survey and would have required more sophisticated logistic arrangements.

This study noted significantly higher HBsAg seroprevalence among male children. The association remained significant in multivariate analysis after controlling the potential confounders. Although this finding has also noted earlier in other studies in the region, the biological basis for this is not clear. The sex differences in immunization coverage are not significant in Cambodia, and boys are as likely or more likely to be immunized than girls. Thus, besides reaffirming the higher HBsAg seroprevalence among boys, this finding has no other implications for hepatitis B immunization policy in Cambodia.

Many studies from other countries have documented differential risk of infection in different socioeconomic groups or geographic areas within a country. The results from Cambodia suggest a statistically significant association with urban areas and least developed geographic areas, and by maternal history of liver disease. The results from demographic and health survey conducted in 2005 showed significantly lower vaccination coverage in least-developed geographic areas and the urban slums. In this instance, the aggregate national immunization coverage may overestimate the impact of vaccination program on overall chronic HBV infection rate. Thus, special efforts to improve vaccination coverage in least-developed regions and urban areas with disaggregated coverage monitoring will be needed while implementing the policy for universal childhood immunization.

An association with other potential risk factors for HBV infection such as history of injections, surgery/dental procedures, and blood transfusions was also examined. The association of being HBsAg positive is not statistically significant with any of these risk factors. However, this finding may be caused by limited sample size with wide confidence intervals. Further studies with sufficient sample size may be needed to further examine this relationship. Nevertheless, injection safety, blood safety, and adequate infection control measures during surgeries and dental procedures are justified on their own right and should be vigorously pursued for multiple public health benefits these interventions offer.

This survey provides a useful baseline against which to compare future assessments of the impact of the hepatitis B infant immunization program. On the basis of demonstrated hepatitis B vaccine efficacy, the impact of a vaccination program can be mathematically modeled by using vaccine coverage rates. However, several other programmatic factors such incidence of vaccine freezing and the timing of first dose may affect the program performance, as demonstrated by a serosurvey conducted in Mongolia in 2002. If one considers the limited utility of routine clinical disease surveillance in monitoring and evaluating the impact of hepatitis B vaccination program compared with other acute vaccine preventable diseases (e.g., measles, polio, diphtheria), representative periodic HBsAg serosurveys are the only way to regularly evaluate the impact of program. Thus, a similar nationwide serosurvey should be conducted in 2010 to evaluate the impact of nationwide vaccination started in 2005.

The results from Cambodia also suggest that nationwide serosurveys using simple and rapid tests are feasible in a poor developing country setting with minimal laboratory infrastructure and poor financial resources. The Western Pacific region has set a goal of reducing chronic hepatitis B infection rates to less than 2% by 2012, and results from this study will provide important lessons to other countries in the region to evaluate their programs towards achievement of these goals. Although this survey was done as a stand-alone survey, the field experience with the survey suggests that this can be easily combined with other nationwide health household surveys (e.g., demographic and health surveys, multiple indicator cluster survey).


