PREVALENCE OF HEPATITIS B AND HEPATITIS C VIRUS INFECTIONS IN POTENTIAL BLOOD DONORS IN RURAL CAMBODIA

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Abstract. The aims of the present study were to provide accurate prevalence of acute and occult hepatis B infection and hepatis C infection among potential blood donors in Cambodia and to study the accuracy of ELISA tests used for blood donor screening. A cross-sectional study was performed on samples collected from potential volunteer blood donors (*n* = 1,200) in two districts in rural Cambodia. The samples were tested using the ELISA technique for HBsAg, anti-HBc, and anti-HCV at a local blood bank. To validate the ELISA outcomes, a subset (*n* = 319) was analyzed by Automated Chemiluminescent Microparticle Immunoassay Technique (CMIA) at the University Hospital North Norway. The overall prevalence of the HBsAg positives was 7.7% (95% CI 6.2-9.3); the prevalence of anti-HBc positive samples was 58.6% (95% CI 55.8-61.4), and the prevalence of anti-HCV positive samples was 14.7% (95% CI 12.7-16.7). The prevalence rate of samples being both HBsAg positive and anti-HBc positive was 7.3% (95%CI 5.9 - 9.0), and the prevalence rate of HBsAg negative and anti-HBc positive samples was 51.2% (95%CI 48.4 - 54.1). The overall agreement between the ELISA and the CMIA test results was very high both for HBsAg and anti-HBc (kappa 0.93), and high for anti-HCV measurements (kappa 0.83). However, the false-negative rate for the ELISA anti-HCV test was as high as 15% (95%CI 6 - 30).

INTRODUCTION

Safe blood transfusions is a problem in developing countries where resources are limited and blood-transmitted diseases are endemic. Among transfusion transmitted infections, hepatitis B (HBV) is regarded as the most common, with risk estimates at 1:60,000 in countries where the prevalence is low. In areas where HBV infection is endemic, transmission rates are probably much higher, and infections occur in part due to improper testing (Wang *et al*, 2002; Hollinger, 2008). Blood donor screening for HBV surface antigen (HBs Ag) is carried out in low-income countries. However, HBV transmission may still occur during the ini-

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tial seronegative-window period of an acute infection, and also during late stages where the virus is still present (HBV-DNA positive) even though HBsAg is negative, so-called occult hepatitis B infection (OBI) (Liu *et al*, 2006; Bhattacharya *et al*, 2007). OBI may originate from recovered infections with persistent low-level viral replication, from escape mutants blocking export of antigen, or from reduced HBV replication after co-infection with HCV; HBsAg may or may not be present (Allain, 2004; Niederhauser *et al*, 2008).

The infectivity of OBI is not clear, although several studies report the exclusion of anti-HBc positive donors regardless of an anti-HBs titer probably decreases the rate of HBV transmission by blood transfusion (Hennig *et al*, 2002; Behzad-Behbahani *et al*, 2006). One should take into account many studies of transmission risk may have methodological flaws that make it hard to interpret findings (Hollinger, 2008). Nevertheless, there are clear indications that both the viral load and the immune status of the recipient must be taken into consideration when assuming the risk for transmission of virus is higher in low-income countries, where large populations have deranged immune capacity from chronic malnutrition and endemic diseases. It is therefore urgent we develop scientific estimates of the infectivity of OBI in blood donations (Allain, 2007). In most Western countries the presence of anti-HBc prohibits blood donation and thereby excludes the vast majority of potential OBI cases. However, due to limited resources and the potential exclusion of too many blood donors, this routine is seldom practiced in countries where HBV infections are endemic. Nucleic Acid Amplification (NAT) technology has enhanced accuracy in identification of OBI cases, without excluding blood donors who have previously been HBV infected but who are no longer carrying the virus. However, in low-income countries, especially in rural blood banks, NAT testing may not be financially or logistically feasible (Lieu *et al*, 2006).

Studies from 15 years ago reported prevalence rates of HBV infection in Cambodia of 8% and HCV of 6.5% (Thüring *et al*, 1993). There are reasons to believe that access to modern medicine and vaccines have altered prevalences (Vong *et al*, 2005). However, to the best of our knowledge, no epidemiological studies of hepatitis B and C virus infections have been performed in Cambodia during the last decade. There seems to be large local variations in prevalence rates in Southeast Asia, with Vietnam alone reporting variations of HBV prevalence from 8% to 25%. Studies in Thailand have reported large prevalence variations among different populations (Ishida *et al*, 2002; Nguyen *et al*, 2007). Studies of prevalences in Southeast Asia have been done on relatively small study samples; consequently, the prevalence estimates are imprecise.

The primary aim of this study was to provide accurate estimates of prevalence rates of acute and occult HBV and HCV infections among potential blood donors in rural Cambodia. The secondary aim was to study the accuracy of ELISA tests used for blood donor screening in Cambodian blood banks.

MATERIALS AND METHODS

This was a cross-sectional, epidemiological study with the reference population being potential blood donors in rural areas of Cambodia. The study was carried out in May and June, 2007 in the provinces of Battambang and Pailin, in the Kingdom of Cambodia.

In order to detect differences in prevalences between the two communities

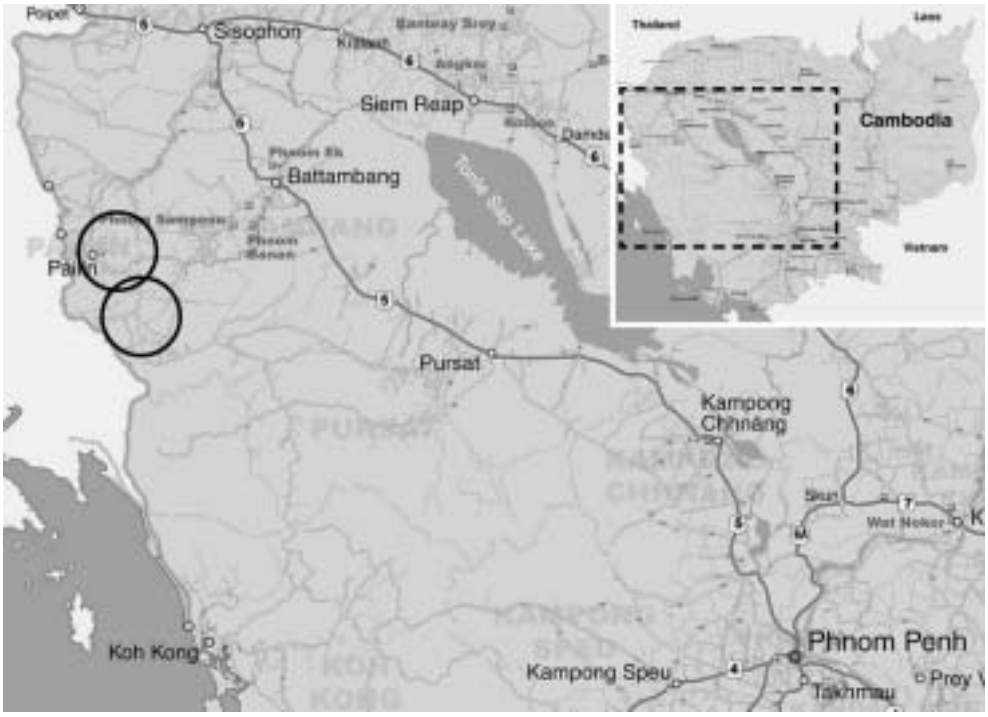

Fig 1–The two study areas in northwestern Cambodia.

of at least 10% with a significance level of 95% and a test power of 90% given a baseline prevalence of HBV of 12%, a total of 1,200 samples were required for the study. The study unit was blood samples collected from 1,200 voluntary participants. Stratified sampling was applied to assess local prevalence variations; 600 samples were collected randomly in one remote area with a less developed infrastructure (Samlot), and 600 samples from one district which had a more developed infrastructure (Pailin, Fig 1). Prior to sampling, the potential participants and health care workers were informed that the study was related to setting up a safe blood transfusion service for the local population. They were also informed that the participation was voluntary and free of charge. The test results for each participant, with medical advice and individual counselling, were given back to them after testing at the Blood Transfusion Center in Battambang. Of the study participants, 677 were females and 523 males, the mean age was 32.8 years, with a range of 18 to 52 years. All participants were living permanently in the study areas and were not previously vaccinated against HBV. For validation of the local ELISA test, a subsample of 319 units was selected from the main sample and blindly re-analysed at the Department of Microbiology, University Hospital of North Norway. The subsample, 120 units for each test, was selected in order to detect test indicator differences of more than 5% with 95% confidence, assuming 2/3 being test-positive units and 1/3 being test-negative (Fig 2). There were no significant differences in the distribution of gender and age among the three subsets (Table 1).

Blood collection and analysis

The collection of samples took place at

Table 1
Distribution of demographical variables in the total sample and three subsets for agreement analysis

	Total sample *n*=1,200	Subsets		
		HBsAg *n*=120	anti-HBc *n*=120	anti-HCV *n*=120
Gender ratio F/M	677/523	58/67	68/52	67/53
Mean age (SD)	32.8 (10.2)	32.7 (10.4)	32.6 (10.4)	39.1 (8.9)
Range of age	(18 - 52)	(18 - 52)	(18 - 52)	(18 - 52)

health centers and villages in the study areas. From each participant one blood sample of five ml was drawn into a sterile vacuum tube by a trained laboratory technician. The serum samples were set aside for spontaneous coagulation for 30 minutes before centrifugation. After centrifugation, serum was pipetted into two new tubes for further analysis. The serum samples were kept in a portable cooling box at 4ºC and then taken to Battambang Blood Transfusion Center for analyses within three days. The main sample was analyzed using the ELISA technique (Monolisa® BioRad). The actual ELISA test has a claimed sensitivity and specificity for HBsAg of 100% and 99.94%; for anti-HBc of 99.53% and 99.5%; and for anti-HCV of 100% and 99.8%, respectively (Biswas *et al*, 2003). The subsample of 319 units was re-analyzed using an Automated Chemiluminescent Microparticle Immunoassay Technique (CMIA, Abbott, Wiesbaden, Germany). On CMIA testing of HBsAg, specimens with concentration values less than 0.05 IU/ml were considered negative and those that had values higher or equal to 0.05 IU/ml were considered positive. The CMIA analysis of anti-HBc and anti-HCV is based on the ratio of signal to cut-off value (S/CO). An S/CO value less than 1.00 is classified as negative, and a value higher than 1.00 is classified as positive. Units with ratios in the range of 0.90-1.00 are classified as "equivocal" and were re-analysed twice (Murray *et al*, 2007; www.abbottdiagnostics).

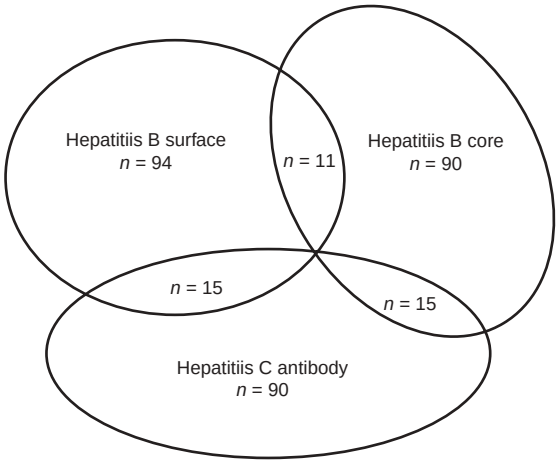

Fig 2–Venn diagram describing the composition and over-lap of subsets for CMIA analysis. The numbers within each area indicate the number of study units.

Statistical model

Continuously distributed variables are expressed as mean values with 95% confidence intervals constructed by the Student procedure. Categorical variables are presented in contingency tables with 95% confidence intervals for two-tailed comparison

Table 2
Comparison of prevalence rates between subpopulations, differences given by 95% confidence intervals.

ELISA	Samlot (*n* = 600)	Pailin (*n* = 600)	95% CI difference
HBsAg	6.5 %	8.8 %	-0.7 - 5.3
Anti-HBc	58.0 %	59.2 %	-6.7 - 4.4
Anti-HCV	17.0 %	12.3 %	0.7 - 8.7

between groups (Agresti, 2002). Kappa (κ) analysis is used to express agreement between test methods; κ-values in the interval of 0.4-0.6 are classified as having acceptable agreement, values of 0.6-0.8 as having high agreement, and values in the range of 0.8-1 are classified as having very high agreement (Altman, 1999). The data was stored in an Excel database and analyzed with JMP and Confidence Interval Analysis software packets (JMP 6.0.2. SAS Institute; CIA version 1.2).

Ethical considerations

The participants consent was given both orally and in writing. Free medical counselling based on test outcomes was given to all study participants. The informed consent forms were distributed by local health workers to potential participants, so the participants could have a full understanding of the study aims and study process. The consent form was signed on-site when the blood samples were drawn. The consent forms and written files of demographical and laboratory data were stored in locked steel shelves at the Trauma Care Foundation head office in Battambang, Cambodia. Access to non-anonymous data was restricted to members of the research team. The data was stored and processed according to approved guidelines from the Norwegian Social Science Data Service (ref. no. 13702) and the National Ethics Committee for Health Research of the Ministry of Health, Cambodia (ref. 023 N.E.C.H.R., 2/4/2007).

RESULTS

The prevalence of HBsAg in the study population (*n* = 1,200) was 7.7% (95%CI 6.2 - 9.3), the prevalence of anti-HBc 58.6% (95%CI 55.8 - 61.4), and the prevalence of anti-HCV 14.7% (95%CI 12.7-16.7). The prevalence of anti-HCV indicates past or present HCV infection. The prevalence rate of samples being both HBsAg positive and anti-HBc positive was 7.3% (95%CI 5.9 - 9.0); the prevalence rate of samples being HBsAg positive but anti-HBc negative was 0.3% (95%CI 0.1-0.8), and the prevalence of HBsAg negative and anti-HBc positive samples was 51.2% (95%CI 48.4 - 54.1). In regard to regional differences, the prevalences of HBsAg and anti-HBc between the two study districts were not significantly different. However, the prevalence of anti-HCV was significantly higher in Samlot District than in Pailin (Table 2, Fig 3).

The agreement between the ELISA and the CMIA test results was very high, with kappa values higher than 0.8 for all three tests (Table 3). The rate of false negative ELISA test outcomes was zero for HBsAg (95%CI 0 - 9), 5% for anti-HBc (95%CI 1 - 17), and 15% for anti-HCV (95%CI 6 - 30).

DISCUSSION

We report for the first time a major cross-sectional study of the endemicity of HBV and the prevalence of past and present HCV infections in rural Cambodia. The

Table 3
Agreement analysis of ELISA and CMIA test outcomes. The results are expressed in observed numbers and kappa-values with 95% confidence intervals.

	CMIA					
	HBsAg		anti-HBc		anti-HCV	
ELISA	Negative	Positive	Negative	Positive	Negative	Positive
Negative	41	0	38	2	34	6
Positive	4	75	2	78	3	77
Kappa (95%CI)	0.93 (0.86-1.00)		0.93 (0.85 - 1.00)		0.83 (0.72 - 0.94)	

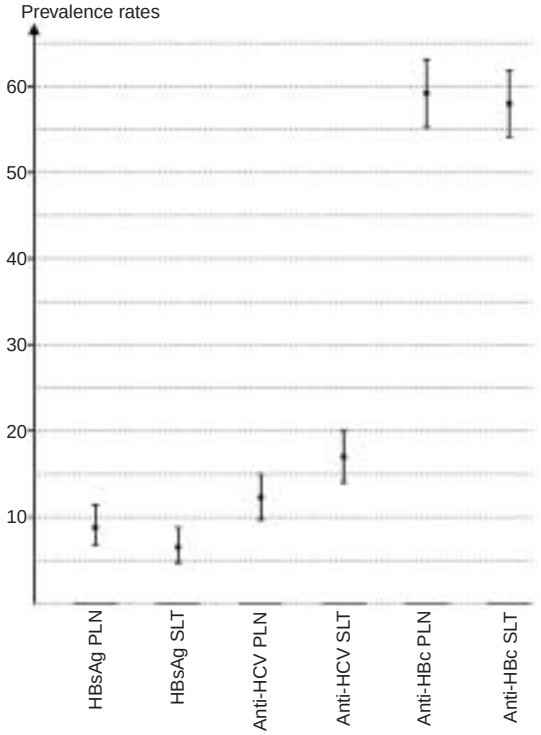

Fig 3–The prevalence of HBsAg, anti-HCV and anti-HBc in the two subpopulations, Pailin (PLN) and Samlot (SLT). The results are expressed with vertical 95% confidence interval bars.

study documents high prevalence rates of actual infections in potential blood donors in the study area. Not surprisingly, the actual study also documents a very high prevalence rate of anti-HBc positive results indicating that HBV infections have been endemic in the study area for decades. In a recent study from rural Vietnam, Viet *et al* (personal communication) reported prevalence rates for HBsAg of 11.4% and for anti-HBc of 51.7%. Compared to our study the HbsAg rate in Vietnam was significantly higher and the anti-HBc positive rate significantly lower; however, the differences are of minor medical importance.

For hepatitis B infections the results corresponded well with surveys in Cambodia from the 1990s (Thüring *et al*, 1993) and also with recent survey data from a local blood bank claiming a HBsAg-positive rate among donors of 9.2% (95%CI 8.1 - 10.4) (Battambang Transfusion Center, 2008). For HCV infections, however, there is a clear discrepancy. Battambang Blood Transfusion Center reported a prevalence rate of anti-HCV of 2.7% (95%CI 2.1 - 3.4) among healthy blood donor volunteers, which is significantly lower than the estimates of the actual study (95%CI 9.9 - 14.1). The difference between the two prevalence estimates is large and should be of interest to public health providers. The discrepancy may reflect real local variations in HCV prevalence; in our study we found significant differences in the prevalences of hepatitis C virus markers between the two

study areas. Wide prevalence ranges reported may also be due to methodological flaws affecting crude outcomes as well as the reliability of the findings.

In a major cross-sectional study there may be errors related to the sampling of study participants. We cannot rule out that some categories of the population in the study area, drug addicts or other groups carrying social stigma, may have been under- or over-represented in the study sample. However, since participation in the study gave health benefits free of charge for the study participants, we hold that any such bias should be moderate and without systematic or significant effect on the main outcome variables.

Failures in technical sampling and processing in-field may have occured. All procedures were performed according to protocol, without any accidental events being reported, therefore we believe there is minimal impact due to technical errors.

One should scrutinize the accuracy of laboratory analysis. During ELISA analysis, all study units with test outcomes close to the ELISA cut-off level were re-analyzed before the test results were registered in the database. The ELISA technique used for analysis was validated by blinded CMIA analysis at a high-tech medical laboratory, the comparison yielding high agreement indices. However, the kappa-analysis gives only an over-all estimate of test performance; in donor screening the false-negative rate is of particular interest. False-negative rates higher than 5% as reported for anti-HBc and anti-HCV are below standard and mandate exploration of laboratory routines.

There is reason to believe the prevalence estimates reported in the actual study are representative for the reference population of potential blood donors in rural Cambodia. The accuracy of the estimates should, however, be interpreted with care due to considerable local variations in prevalence rates. Further cross-sectional studies in other parts of the country, including an urban population, are mandatory to attain accurate prevalence estimates.

There are implications to the findings in regard to future blood service in rural Cambodia. In the actual study population the rate of surface-antigen- negative-core-antibody-positive participants was high (51%), which raises the question: how many of these are also OBI cases? Accurate estimates cannot be given because the actual study participants were not tested for HBV-DNA. The rate of HBV-DNA detected in HBsAg-negative samples varies with the prevalence of HBV infection. Less than 5% of surface-positive-core-negative blood donor samples had detectable HBV-DNA in developed countries with low HBV infection, whereas the corresponding rate was as high as 24% in high HBV-prevalence areas (Garcia-Montalvo *et al*, 2005; Dhawan *et al*, 2008; Hollinger, 2008). Thus, a conservative estimate indicates that 10% or more of potential blood donors in rural Cambodia may be carriers of OBI. In Southeast Asia, including Cambodia, routine screening of donors for HIV, syphilis, HBV, HCV, and malaria is routinely carried out, but anti-HBc antibodies are not screened for. Screening for anti-HBc and exclusion of test-positive donors may reduce the risk for HBV transmission but half the donor population would then be excluded from donation, which has obvious consequences for blood product availability in the country.

Based on current knowledge it is impossible to estimate the risk for HBV transmission by surface-negative-core-positive blood products in areas of high HBV endemicity. Further studies and better understanding of the effects of HBV-DNA are needed before evidence-based guidelines can be made. One

option for the blood banks of Cambodia would be to keep anti-HBc-positive blood products in stock ear-marked for patients with life-threatening haemorrhage in anti-HBc-positive or HBV vaccinated recipients.

In summary, hepatitis B and C virus infections constitute a major burden in the rural population of Cambodia. The rate of HBsAg-negative-anti-HBc-positive cases is as high as 51%, which may indicate that at least 10% of potential blood donors in the study area may be carriers of occult hepatitis B infection and thus potential transmitters of HBV infection. Precise risk estimates cannot be given, but our findings indicate that precautions should be taken in Cambodian blood banks for anti-HBc-positive donors, by using anti-HBc-positive transfusions only for life threatening emergencies in already infected or vaccinated patients. High false-negative ELISA test rates for anti-HBc and anti-HCV indicate the laboratory test quality in Cambodian blood banks should be explored.

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