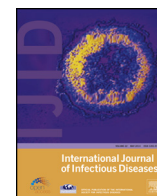


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Seroprevalence of chronic hepatitis B, as determined from dried blood spots, among children and their mothers in central Lao People's Democratic Republic: a multistage, stratified cluster sampling survey



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SUMMARY

Background: There is limited information regarding the prevalence of hepatitis B in Lao PDR, where the hepatitis disease burden is substantial. Thus, reliable seroprevalence data is needed for the disease, based on probability sampling.

Methods: A stratified, multistage, cluster sampling survey of hepatitis B surface antigen (HBsAg) positivity among children aged 5–9 years and their mothers aged 15–45 years was conducted. Participants were selected randomly from the central region of Lao PDR via probability-proportional-to-size sampling. Blood samples were collected onto filter paper and subsequently analyzed using a chemiluminescent microparticle immunoassay.

Results: A total of 911 mother-and-child pairs were collected; the seroprevalence of HBsAg was estimated to be 2.1% (95% confidence interval 0.8–3.4%) among children and 4.1% (95% confidence interval 2.6–5.5%) in their mothers after taking into account the sampling design and the weight of each sample. The children's HBsAg positivity was positively associated with maternal infection and being born in a non-health facility, while the maternal infection status was not associated with any background characteristic.

Conclusions: Lao PDR has a relatively lower HBsAg prevalence in the general population compared to surrounding countries. To ensure comparability to other countries and to future data, rapid field tests are recommended for a nationwide prevalence survey.

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1. Introduction

According to the World Health Organization (WHO) estimate, approximately 360 million people worldwide have a chronic hepatitis B virus (HBV) infection,^{1,2} and almost half of these individuals live in the Western Pacific Region, which comprises only approximately 28% of the global population.³ Every year, an estimated 620 000 people die from HBV-related diseases,¹ and over 90% of them had acquired the virus decades earlier at birth or

during early childhood.^{4,5} In 1992, the WHO recommended that childhood hepatitis B vaccination be included in the immunization programs of all countries.⁶ The Regional Committee for the WHO Western Pacific Region adopted this recommendation in 2005, calling for a reduction in chronic hepatitis B infection to less than 2% among 5-year-old children as an interim milestone towards the final regional goal of less than 1% by 2012.⁷ To achieve this goal, it was recommended that the birth dose coverage be improved to 65% and the third dose of hepatitis B vaccination coverage be improved to 85%.⁸

Hepatitis B surface antigen (HBsAg) is a serological marker of hepatitis B infection and can be detected during the acute and chronic phases of the disease. Therefore, HBsAg seroprevalence

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usually reflects the presence of chronic hepatitis B in a given population, and a serological survey of this marker is recommended to measure the impact of immunization policies.²

Lao People's Democratic Republic (Lao PDR) is a landlocked country located in the centre of the Indochina Peninsula. Since 90% of the land is covered by mountains with deep forest and 80% of the total population lives in rural areas, health services are difficult to provide in Lao PDR. In addition, because the country is bordered by hepatitis B hyperendemic countries such as Cambodia,⁹ China,¹⁰ Myanmar,^{10,11} Thailand,¹² and Vietnam,¹³ Lao PDR has been considered an endemic country.^{14,15} The country initiated its expanded program on immunization (EPI) in selected regions in 1984, and the program became available nationwide in the mid-1990s. According to a report from the Ministry of Health, Lao PDR, immunization coverage for bacille Calmette–Guérin (BCG), the third oral polio vaccine, the third diphtheria, pertussis, tetanus, hepatitis B vaccine, and the first measles vaccine was 60%, 46%, 45%, and 36%, respectively, in 2004. Hepatitis B vaccination was included in the EPI in 2002. Hepatitis B vaccination for newborns was integrated into the EPI starting in 2004; this gradually expanded from central hospitals to rural areas. There have been no previous reports on the infection rates of chronic HBV in the general population. Therefore, population-based seroprevalence surveys for hepatitis B are warranted to better estimate the current prevalence of hepatitis B and to evaluate the impact of immunization policies that focus on preventing mother-to-child transmission.^{2,8}

There is limited information regarding the prevalence of hepatitis B in Lao PDR, indicating the need for a population-based household survey to provide a reliable seroprevalence for the disease. However, the sparsely distributed population, less developed infrastructure, and limited human and financial resources in Lao PDR make the conducting of surveys difficult. Filter paper blood collection is simple and inexpensive, and the storage and transport methods are easy. HBsAg testing with dried blood spots (DBS) is suitable for large-scale surveys in resource-limited countries.^{16,17}

The Ministry of Health in Lao PDR plans to conduct a nationwide hepatitis B prevalence survey in the near future. Therefore, it was proposed that a household survey be conducted, as a pilot survey, in the central region of the country, where the infrastructure and transportation are relatively better than in the rest of the country. The primary objectives of this survey were to: (1) assess the operational and programmatic feasibility of a population-based sero-epidemiological survey, and (2) estimate chronic hepatitis B infection rates, as determined from the HBsAg seroprevalence among children and their mothers.

2. Materials and methods

The field work for the survey was carried out at the end of January 2011 and lasted for 10 days. The study used a multistage, stratified, random cluster sampling design. The sample was restricted to children aged 5 to 9 years, and their mothers aged 15 to 45 years on January 1, 2011. The children had to be biologically related to their mothers and not adopted. The rationale for the target age group of children used in this survey was based on the fact that the risk of acquiring chronic HBV infection is highest in the first 5 years of life,^{4,15} as observed in the neighbouring hyperendemic countries.

2.1. Sample size

The sample size was calculated based on the expected HBsAg seroprevalence rate of 15% among children and their mothers. The sample size of 960 was calculated at a 5% level of significance with a precision of $\pm 3.5\%$. The design effect for cluster sampling was assumed to be 2.0 with a response rate of 85%.

2.2. Sampling

Four provinces and the Vientiane Municipality in the central region of Lao PDR, which account for 2.5 million people (approximately 40% of the total population), were selected for the study. The population of Vientiane Municipality is 691 721, of Borikhamxay Province is 225 301, of Khammuane Province is 337 390, of Vientiane Province is 388 895, and of Savannakhet Province is 825 902. There are nine districts in Vientiane Municipality, six in Borikhamxay Province, nine in Khammuane Province, 13 in Vientiane Province, and 15 in Savannakhet Province.

Provinces were considered as strata, yielding a total of five strata. In the first stage of sampling, four districts were selected randomly from each stratum using probability-proportional-to-size (PPS) based on the population data from the 2005 census conducted in Lao PDR. In the second stage, two villages were selected randomly from each selected district by PPS, and a total of 40 villages, as clusters, were selected. Twenty survey teams were formed, and each team comprised a supervisor and two surveyors, nominated from the district health staff. If surveyors could not reach the designated village due to obstacles such as floods or mudslides, the nearest village on the way back to the district centre from the designated village could be selected as an alternative. In each selected village, surveyors developed a list of households that had children aged from 5 to 9 years based on the data from the poverty reduction program, EPI, or other relevant residential lists. From these lists, 24 households were selected randomly via a lottery, and the youngest child within the eligible age range and his/her mother were sampled for the survey. Adopted children were excluded from the lists.

2.3. Questionnaires

A brief face-to-face questionnaire was administered to the sampled mothers. Information was collected on the demographic status and history of the child (i.e., age, sex, place of birth, and immunization history), as well as the socioeconomic status of the child's household, as measured by the maternal educational background and occupation of the household head. Additionally, questions regarding exposure to potential risk factors for acquiring hepatitis B infection (e.g., surgery, blood transfusion, and sharing toothbrushes) and history of past hepatitis infection among other family members were asked.

2.4. Testing for HBsAg

Capillary blood was collected onto Whatman 903 filter paper protein saver (Whatman, Maidstone, Kent, UK) via a finger prick and air-dried for at least 60 min; filter papers were kept in sealed plastic bags and transported to Japan. DBSs were stored at ambient temperature in Lao PDR for 7 to 10 days after sample collection, and thereafter at 4 °C for 3 months prior to being tested at the Hepatology Research Centre, National Centre for Global Health and Medicine. Blood samples were extracted from DBSs by punching two bloodstained circles 3 mm in diameter and eluting overnight in 500 μ l of phosphate-buffered saline (pH 7.2). Eluates were tested for HBsAg using a chemiluminescent microparticle immunoassay (Architect i2000SR; Abbott Diagnostics, IL, USA). The relative light unit (RLU) value of each sample was detected with an automated system. The sample was considered to be positive for HBsAg based on comparisons to the RLU value of a calibration sample.

2.5. Consent, confidentiality, ethical considerations, and safety issues

The surveyors explained the detailed survey objectives and procedures to the local authorities and selected mothers and

children verbally and in writing. Written informed consent was obtained from the parents or legal representatives if mothers were illiterate. Informed consent, questionnaires, and the blood collection process were supervised by provincial- and national-level supervisors. The survey proposal was reviewed and approved by the ethics committees of the Ministry of Health, Lao PDR, and the National Centre for Global Health and Medicine, Japan (NCGM-950).

2.6. Data entry and analysis

All of the completed questionnaires were taken to a centralized location, and to reduce any data entry-related errors, the double data entry method was performed in a Microsoft Excel worksheet (Microsoft Office 2007) along with validation and correction. STATA 12 (Stata Corp., College Station, TX, USA) was used for the data analysis. Calculations of the overall prevalence among children and mothers took into account the individual weight of each sample. The Poisson exact method for rare events was used to calculate the 95% confidence interval (CI) of HBsAg prevalence for each region. Chi-square and Fisher's exact tests were used to examine the relationships between the independent variables and HBsAg positivity in the bivariate analysis. Multiple logistic regression models were used to investigate the independent predictors of different households and individual characteristics

for HBsAg positivity. All estimates and standard errors were adjusted for the multistage clustered sampling design to ensure representative and unbiased results. A *p*-value of <0.05 was considered statistically significant.

3. Results

Forty villages from 20 districts in central Lao PDR were selected for the study. The survey teams successfully visited all of the 40 selected villages and conducted the questionnaire and serosurvey. Of the 960 selected mother-and-child pairs, 49 were not eligible for analysis and were excluded from the study (35 pairs did not meet the study age requirement for the child (e.g., 3–4 years old or unknown), 13 pairs did not meet the study age requirement for the mother (e.g., >45 years old or unknown), and one pair did not have a blood sample from the child (reason unknown)). Thus, a total of 911 mother-and-child pairs were included in this analysis.

The baseline characteristics of the selected mothers and their children are summarized in Table 1. The mean age of the mothers was 32.4 years (95% CI 32.0–32.8 years), and the mean age of the children was 6.7 years (95% CI 6.7–6.8 years). Of the children sampled, 453 (49.7%) were male and 458 (50.3%) were female. One hundred and seventy-one children out of 911 had written immunization records at the survey. Among these, immunization

Table 1
Prevalence of HBsAg among children and mothers in the central region of Lao PDR by selected background characteristics

	n	(%)	Children (5–9 years old)			Mothers (15–45 years old)		
			HBsAg-positive	%	95% CI	HBsAg-positive	%	95% CI
Mother's age, years (n=911)								
15–20	9	(0.99)	0	0.00%		0	0.00%	
21–25	97	(10.65)	1	1.03%	(0.00–3.01)	5	5.15%	(0.68–9.63)
26–30	274	(30.08)	8	2.92%	(0.91–4.93)	13	4.74%	(2.22–7.28)
31–35	266	(29.20)	6	2.26%	(0.46–4.05)	14	5.26%	(2.56–7.96)
36–40	177	(19.43)	4	2.26%	(0.05–4.47)	6	3.39%	(0.70–6.08)
41–45	88	(9.66)	2	2.27%	(0.00–5.45)	2	2.27%	(0.00–5.45)
Mother's ethnicity (n=911)								
Lowland Lao	783	(85.95)	19	2.43%	(1.35–3.51)	36	4.60%	(3.13–6.07)
Highland Lao	83	(9.11)	1	1.20%	(0.00–3.60)	3	3.61%	(0.00–7.71)
Hmong	5	(0.55)	1	20.00%	(0.00–75.53)	0	0.00%	
Unknown	40	(4.39)	0	0.00%		1	2.50%	(0.00–7.56)
Main transportation to the nearest health facility (n=906)								
On foot	78	(8.61)	1	1.28%	(0.00–3.83)	5	6.41%	(0.85–11.97)
Bicycle	23	(2.54)	1	4.35%	(0.00–13.36)	0	0.00%	
Motor bike	554	(61.15)	15	2.71%	(1.35–4.06)	21	3.79%	(2.20–5.39)
Car	120	(13.25)	2	1.67%	(0.00–3.99)	8	6.67%	(2.14–11.19)
Hand tractor	120	(13.25)	1	0.83%	(0.00–2.48)	4	3.33%	(0.08–6.59)
Other	11	(1.21)	1	9.09%	(0.00–29.35)	2	18.18%	(0.00–45.36)
Time to nearest health facility (n=876)								
≤10 min	174	(19.86)	8	4.60%	(1.45–7.74)	10	5.75%	(2.25–9.24)
>10 to ≤30 min	386	(44.06)	4	1.04%	(0.02–2.05)	17	4.40%	(2.35–6.46)
>30 to ≤60 min	162	(18.49)	5	3.09%	(0.39–5.78)	8	4.94%	(1.57–8.31)
>60 min	154	(17.58)	4	2.60%	(0.06–5.14)	5	3.25%	(0.42–6.08)
Mother's education level (n=911)								
No education	178	(19.54)	2	1.12%	(0.00–2.69)	5	2.81%	(0.36–5.26)
Primary school	404	(44.35)	14	3.47%	(1.67–5.26)	18	4.46%	(2.43–6.48)
Junior high school	201	(22.06)	4	1.99%	(0.04–3.94)	10	4.98%	(1.94–8.01)
High school	113	(12.40)	1	0.88%	(0.00–2.64)	6	5.31%	(1.11–9.51)
College	13	(1.43)	0	0.00%		1	7.69%	(0.00–24.45)
Other or unknown	2	(0.22)	0	0.00%		0	0.00%	
Occupation of household head (n=911)								
Farmer	589	(64.65)	13	2.21%	(1.02–3.40)	19	3.23%	(1.79–4.66)
Fisherman	4	(0.44)	0	0.00%		0	0.00%	
Labourer	118	(12.95)	4	3.39%	(0.08–6.70)	6	5.08%	(1.06–9.11)
Public officer	112	(12.29)	1	0.89%	(0.00–2.66)	7	6.25%	(1.70–10.80)
Factory employee	8	(0.88)	0	0.00%		0	0.00%	
General employee	19	(2.09)	0	0.00%		2	10.53%	(0.00–25.72)
Merchant	54	(5.93)	3	5.56%	(0.00–11.87)	6	11.11%	(2.45–19.77)
Other	7	(0.77)	0	0.00%		0	0.00%	
Family history of liver diseases except mother (n=911)								
Yes	95	(10.43)	1	1.05%	(0.00–3.14)	4	4.21%	(0.98–8.32)
No	816	(89.57)	20	2.45%	(1.39–3.51)	36	4.41%	(3.00–5.82)

HBsAg, hepatitis B surface antigen; CI, confidence interval.

Table 2
HBsAg prevalence among children and their mothers by province

	Children (5–9 years old) n = 911		Mothers (15–45 years old) n = 911	
Vientiane Municipality (n = 186)	2.15%	(0.05–4.25)	3.76%	(1.00–6.52)
Vientiane Province (n = 182)	4	(0.35–5.14)	7	(2.95–10.23)
Borikhamxay Province (n = 179)	2.75%	(1.04–6.78)	6.59%	(2.19–8.98)
Khammuane Province (n = 180)	5	(0.00–2.66)	12	(0.35–5.20)
Savannakhet Province (n = 184)	3.91%	(0.00–3.48)	5.59%	(0.67–5.85)
Total (n = 911)	7	(0.79–3.38)	10	(2.58–5.54)
	21		40	

HBsAg, hepatitis B surface antigen.

coverage for BCG was 95%, for the third oral polio vaccine was 88%, for the third diphtheria, pertussis, tetanus, hepatitis B vaccine was 87%, and for the first measles vaccine was 80%.

Of the 911 pairs included in the study, 21 children and 40 mothers were positive for HBsAg. Eleven out of 21 HBsAg-positive children (52.3%) had HBsAg-positive mothers, whereas the other 10 were from non-infected mothers. The prevalence was calculated as 2.1% (95% CI 0.8–3.4%) in children and 4.1% (95% CI 2.6–5.5%) in their mothers after taking into account the sampling design and the weight of each sample (Table 2). HBsAg prevalence varied by province, but the differences among provinces were not statistically significant for either children or mothers. In calculating HBsAg prevalence, the design effects were determined to be 1.6 for children and 1.1 for mothers (Table 2).

To determine whether background characteristics affect HBsAg status, a logistic regression analysis was conducted. For the children, maternal HBsAg positivity and being born in a non-health facility were positively associated with hepatitis B infection (Table 3), whereas the other potential risk factors were not. For

Table 3
Adjusted odds ratio for being HBsAg-positive among children aged 5 to 9 years in the central region of Lao PDR by selected background characteristics

Characteristics (Reference group)	Adjusted OR	95% CI	p-Value
Address (Khammuane)			
Vientiane Capital	1.06	(0.23–5.00)	0.934
Vientiane Province	0.94	(0.11–7.88)	0.949
Borikhamxay	1.64	(0.31–8.76)	0.537
Savannakhet	0.88	(0.07–10.63)	0.916
Mother's HBsAg status (Negative)			
Positive	31.31	(12.23–80.16)	0.000 ^a
Mother's age (15–30 years)			
31–45 years	0.87	(0.10–7.50)	0.894
Mother's education level (None)			
Finished primary school or upper	2.83	(0.33–24.49)	0.319
Occupation of household head (Other)			
Labourer	2.14	(0.60–7.65)	0.222
Family history of liver diseases except mother's (None)			
Yes or unknown	0.98	(0.05–18.70)	0.987
Time to nearest health facility (≥10 min)			
<10 min	1.89	(0.70–5.09)	0.193
Sex (Female)			
Male	1.92	(0.67–5.47)	0.207
Birth place (Health facility)			
Non-health facility	3.01	(1.06–8.72)	0.039 ^a
History of HepB vaccination at birth (None or unknown)			
Received	2.58	(0.31–21.71)	0.357
History of HepB vaccination (Never or less than twice)			
Received 3 times	0.48	(0.12–1.92)	0.279
Sharing tooth brush (Never)			
Yes or unknown	1.25	(0.36–4.33)	0.706

HBsAg, hepatitis B surface antigen; OR, odds ratio; CI, confidence interval.

^a p < 0.05.**Table 4**
Adjusted odds ratio for being HBsAg-positive among mothers aged 15 to 45 years in the central region of Lao PDR by selected background characteristics

Characteristics (Reference group)	Adjusted OR	95% CI	p-Value
Address (Khammuane)			
Vientiane Capital	1.03	(0.21–4.91)	0.973
Vientiane Province	2.12	(0.42–10.63)	0.338
Borikhamxay	1.82	(0.38–8.67)	0.426
Savannakhet	1.23	(0.28–5.41)	0.763
Age (15–30 years)			
30–45 years	0.86	(0.45–1.65)	0.633
Mother's education level (None)			
Finished primary school or upper	1.35	(0.25–7.43)	0.709
Occupation of household head (Other)			
Labourer	1.44	(0.41–5.06)	0.538
Family history of liver diseases except mother herself (None)			
Yes or unknown	0.73	(0.14–3.82)	0.689
Time to nearest health facility (≥10 min)			
<10 min	1.35	(0.59–3.05)	0.450
History of surgery (Never)			
Yes or unknown	2.11	(0.83–5.41)	0.111

HBsAg, hepatitis B surface antigen; OR, odds ratio; CI, confidence interval.

the mothers, no background characteristic was associated with HBsAg positivity (Table 4).

To evaluate the potential risk factors for children acquiring a chronic hepatitis B infection without vertical transmission, logistic regression analysis was conducted for children born from HBsAg-negative mothers (n = 871). A history of regular hepatitis B vaccination (scheduled at 6, 10, and 14 weeks after birth) was negatively associated with the children's HBsAg status (p = 0.04), while immunization after the delivery (within 24 h) was not. Other risk factors, including birth place, did not show any significant association with HBsAg status among children (Table 5).

4. Discussion

To the best of our knowledge, this is the first population-based, cross-sectional survey to estimate chronic hepatitis B prevalence among the general population in Lao PDR. The major findings were: (1) the questionnaire and serosurvey using multistage,

Table 5
Adjusted odds ratio for being HBsAg-positive among children aged 5 to 9 years born to HBsAg-negative mothers in the central region of Lao PDR by selected background characteristics

Characteristics (reference group)	Adjusted OR	95% CI	p-Value
Mother's age (15–30 years)			
31–45 years	1.36	(0.37–6.05)	0.665
Mother's education level (None)			
Finished primary school or upper	3.93	(0.45–33.11)	0.212
Occupation of household head (Other)			
Farmer	3.96	(0.65–23.01)	0.133
Family history of liver diseases (None)			
Yes or unknown	1.17	(0.11–7.74)	0.887
Time to nearest health facility (≥10 min)			
<10 min	3.00	(0.64–13.78)	0.160
Sex (Female)			
Male	3.97	(0.80–19.12)	0.088
Birth place (Health facility)			
Non-health facility	1.38	(0.29–6.58)	0.683
History of HepB vaccination at birth (Not received or unknown)			
Received	2.89	(0.50–15.72)	0.226
History of regular HepB vaccination (≤2 times or unknown)			
3 times	0.11	(0.13–0.93)	0.042 ^a
Sharing tooth brush (Never)			
Yes or unknown	2.04	(0.37–10.68)	0.404

HBsAg, hepatitis B surface antigen; OR, odds ratio; CI, confidence interval.

^a p < 0.05.

stratified random cluster sampling was carried out successfully in the central region of Lao PDR; (2) the estimated prevalence in the general population was 2.1% in children and 4.1% in women of childbearing age; and (3) HBsAg positivity in mothers was positively associated with HBsAg positivity in their children, whereas no condition was significantly associated with HBsAg positivity in mothers.

4.1. Feasibility of the household survey

Multistage, stratified, random cluster sampling is the most reliable strategy for accurately estimating the prevalence of health status and conditions among the general population within a defined geographical area under constraints of limited time and resources.¹⁸ However, in Lao PDR, this sampling strategy has rarely been applied for these purposes. For example, EPI coverage,¹⁹ measles immunization coverage,²⁰ antenatal care,²¹ energy malnutrition,²² and smoking²³ have been assessed via convenience sampling rather than random sampling methods. The exact reasons for not using a probability sampling methodology are not clear. One exception is the Lao Reproductive Health Survey 2005, which focused on population and development planning.²⁴ The Lao Reproductive Health Survey 2005 used a two-stage, stratified cluster sampling method for the household survey and reported standard errors and design effects for each value measured.

The present survey was conducted successfully in terms of operational and programmatic feasibility, because the data collection was completed within 10 days with response rates of 100%, and the calculated design effects were 1.1 to 1.6 for HBsAg prevalence. This sampling methodology can be applied for other purposes as well.

4.2. HBsAg seroprevalence among children and their mothers

There have been no previous reports on the prevalence of hepatitis B in the general population of Lao PDR. The prevalence of chronic hepatitis B, as determined by HBsAg, has been reported previously in blood donors²⁵ and hospitalized patients.²⁶ However, this selection of individuals may result in biased findings, as they may not be representative of the general population. For example, HBsAg prevalence among blood donors may be higher than that in the general population when donors are paid and lower when they are unpaid.²⁷

The surrounding Southeast Asian countries have previously been considered as highly endemic areas for hepatitis B. For instance, data from Cambodia,⁹ China,¹⁰ Myanmar,^{10,11} Thailand,¹² and Vietnam¹³ suggest a prevalence of 8–10%. The results of the present study revealed a lower prevalence than expected. There are a few reasons to explain this finding. First, the central part of Lao PDR is different from other regions (e.g., better health services and cultural differences). Second, the sensitivity of HBsAg testing on blood collected on Whatman 903 filter paper in the field setting may be low. Lastly, the high temperature and humidity may have damaged the HBsAg protein prior to analysis.

4.3. Risk factors for acquiring hepatitis B

The present study revealed the mother's HBsAg status and child's place of birth to be significantly associated with the child's infection status (Table 3). Other risk factors, such as male child,⁹ ethnicity,¹³ family history of liver diseases,⁹ and the place of residence^{9,13} were found not to be associated. This may be the result of the limited sample size.

The HBsAg positivity results for the children and their mothers indicated that transmission was horizontal in almost half of the

hepatitis-infected children. A potential risk factor for this was the number of routine hepatitis B vaccinations (≤ 2 times; Table 5). This is important, especially in a country such as Lao PDR, where the rate of delivery by a skilled birth attendant is less than 30%. The WHO emphasizes that hepatitis B immunization should be given during the first 24 h after delivery; however, a first vaccination even at >24 h may greatly contribute to the prevention of hepatitis B infection from horizontal sources. Moreover, the importance of regular immunization during infancy cannot be overemphasized.

4.4. Study limitations

There are several limitations that need to be discussed. First, the survey design, which applied PPS, was based on population data from the latest national census in 2005. Since 2005, the population of the country has increased from 6 200 000 to 6 600 000, and the number of districts has increased (separated) from 141 to 143. During the same period, the number of villages has decreased (merged). However, no updated population data have been made available since 2005. Therefore, the present study may have under- or over-estimated the population in selected villages. Moreover, the hepatitis B immunization policy has expanded over the last 10 years, and therefore our sampled children may have, in part, represented the pre-vaccine era.

Another limitation of this study is that the seroprevalence survey was conducted using Whatman 903 filter paper. HBsAg is heat-stable and resistant to drying,^{28,29} and DBSs are convenient and relatively cheap, thus DBSs have been used previously for seroprevalence surveys on hepatitis B.^{17,30,31} However, the sensitivity and specificity of field samples are still not well documented. Therefore, DBS field test results should be compared to other methods like ELISA and rapid tests in the future. Additionally, seroprevalence surveys should be repeated over time, along with hepatitis B control measures, and the methodology should ideally be comparable to that used in neighbouring countries and areas. Given the comparability of the results, the use of a widely available rapid field test, such as the Alere Determine HBsAg test card (Alere Medical Co., Ltd, Chiba, Japan),⁹ is recommended for nationwide surveys. The sensitivity and specificity of the Determine rapid test have been reported as 97–100% and 100% from field studies in Vietnam³² and China,³³ thus the rapid test is recommended by the WHO.³⁴

4.5. Conclusions

In the present study, an HBsAg prevalence survey targeting children and their mothers was conducted successfully in the central part of Lao PDR. The prevalence was estimated to be 2.1% (95% CI 0.8–3.4%) for children aged 5 to 9 years, and 4.1% (95% CI 2.6–5.5%) for mothers aged 15 to 45 years, after taking into account the sampling design and the weight of each sample. The prevalence of hepatitis B in Lao PDR was found to be lower than that in other Southeast Asian countries, as well as the prevalence reported in previous studies conducted in Lao PDR. Given the comparability of the findings, the use of an HBsAg rapid test is strongly recommended for future nationwide surveys.

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Ethical approval: The surveyors explained the detailed survey objectives and procedures to the local authorities and selected mothers and children verbally and in writing. Written informed consent was obtained from the parents or legal representatives if mothers were illiterate. Informed consent, questionnaires, and the blood collection process were supervised by provincial- and national-level supervisors. The survey proposal was reviewed and approved by the ethics committees of the Ministry of Health, Lao PDR, and National Centre for Global Health and Medicine, Japan (NCGM-950).

Conflict of interest: The authors have no conflict of interest regarding this research.

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