

# Estimating the burden of hepatitis B virus infection in Laos between 2020 and 2021: A cross-sectional seroprevalence survey

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## Summary

**Background** Laos is considered highly endemic for persistent infection with hepatitis B virus (HBV). To eliminate this burden, it has gradually implemented universal anti-hepatitis B immunisation of newborns over the past two decades.

**Methods** Using VIKIA<sup>®</sup> HBsAg, a rapid test for the qualitative detection of the HBV surface antigen, we conducted between Sep 1st, 2020 and Aug 31st, 2021 the largest prospective prevalence survey ever in Laos. This survey included blood donors (BD,  $n = 42,277$ ), patients attending care in capital and provincial hospitals ( $n = 37,347$ ) including attending mothers ( $n = 20,548$ ), HIV-infected patients ( $n = 7439$ , recruited from 2009 to 2020), students from the Health Sciences University ( $n = 609$ ), and outpatients ( $n = 350$ ) coming for diagnosis at the Center Infectiology Lao-Christophe Mérieux in Vientiane. In total, 88,022 persons were tested, representing approximately 1.22% of the national population. To reach a reasonable estimate of HBsAg prevalence in Laos, we segmented the population according to three variables, age ( $\leq 20$  years as a cut-off), sex, and geographical origin. BD values were used to estimate HBsAg prevalence in patients aged  $< 20$  while hospital survey prevalence was used to estimate the prevalence in those aged older than 20 years.

**Findings** We observed an HBsAg seroprevalence ranging from 2.6% in blood donors to 8.0% in HIV-infected patients. In BD, men were significantly more at risk to be carriers than women (RR = 1.2,  $P = 0.00063$ ). For BD, attending mothers, or HIV-infected patients, HBsAg was significantly more prevalent in northern Laos (5.1–8.4%) than in central (2.0–8.1%) or southern parts of the country (2.2–6.9%), thereby delineating a North-to-South gradient.

**Interpretation** We considered that HBsAg prevalence probably ranges between 5.0% and 6.0% of the total population. Thus, we consider that Laos may no longer be highly endemic for chronic HBV infection but rather a country with intermediate endemicity.

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**Keywords:** Hepatitis B virus; Southeast Asia; Laos; Endemic country; Universal Immunization

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### Research in context

#### *Evidence before this study*

According to a PubMed search (2007–2021) using “Hepatitis B surface antigen” [MeSH] AND “Laos” [MeSH], Laos is considered as a country highly endemic for persistent infection with hepatitis B virus (HBV) with more than 8% of the national population infected. The country is also frequently mentioned for its high incidence of primary liver cancer that results either from infections with HBV or with liver fluke. To put an end to the endemic circulation of HBV, the Lao government decided two decades ago, in 2001, to implement gradually the universal immunization of newborns against the virus.

#### *Added value of this study*

In the largest serological survey ever conducted in Laos, we screened, in 2020–21, the sera of around 80,000 participants (1.22% of the total population) for the presence of HBV surface antigen (HBsAg). We observed a North-to-South decreasing gradient of chronic infections in the country and a global rate of HBsAg carriage ranging between 5.0% and 6.0%.

#### *Implications of all the available evidence*

According to our survey, it appears that Laos might be no longer a country where HBV is highly endemic but rather a country of intermediate endemicity. This remarkable achievement, obtained within one generation by the poorest country of southeast Asia, represents an example to follow, and a source of hope for many similar countries affected by a heavy burden of persistent HBV infection around the world.

## Introduction

Laos is a tropical landlocked country in Southeast Asia belonging to the lower-middle income countries (LMICs), ranking 99<sup>th</sup> in the Global Health Security Index.<sup>1</sup>

With a little over 7.2 million inhabitants, it is underpopulated (32 inhabitants/km<sup>2</sup>) in relation to its neighbours and life expectancy at birth is one of the lowest in the region (67.9 years).<sup>2</sup> While the population living with HIV (0.3%) or the incidence of tuberculosis (149/100,000 inhabitants) are low by regional standards, infection death rates before five years (488/100,000 inhabitants) is the highest in Southeast Asia.<sup>3</sup>

Historical estimates of hepatitis B in Laos came from infectious disease screening surveys of immigrants to the United States of America around four decades ago. During these campaigns, that frequently mixed participants originating from different countries of the region (Laos, Cambodia, Vietnam), the rate of hepatitis B virus

(HBV) surface antigen (HBsAg) positivity was very high (e.g. 12.6–20.0% in the 1980s).<sup>4</sup> In the following decade, however, it appears that Lao people established in the US were often members of specific ethnic groups (Hmong, Mien) that could be considered as underserved in comparison to the rest of their homeland populations.<sup>5</sup> The worrying situation of hepatitis B in Lao refugees persisted until recently.<sup>6</sup> Taken together, these data indicate that chronic hepatitis B was and still is a serious health problem in many segments of Lao society and perhaps for the entire population.

Laos is, thus, considered to be a country with a high (>8%) prevalence of chronic infection with hepatitis B virus (HBV) in the adult population.<sup>7</sup> As a consequence, severe liver diseases including cirrhosis and cancer are estimated to be common in the country.<sup>3,8</sup> This is especially true for liver cancer, for which the age-standardized incidence rate is one of the highest in the world, both for women and men (14.2 and 35.7 cases for 100,000 respectively in 2021).<sup>9</sup> This major Public Health issue prompted the Lao government to progressively implement a nationwide anti-hepatitis B immunisation campaign of newborns, starting in 2001. Laos is currently applying the Expanded Programme of Immunisation of the WHO. In this frame, newborns should receive a first injection of anti-hepatitis B vaccine at birth. It is followed at weeks 6, 10, and 14 by three additional injections of vaccine.<sup>10</sup> Importantly, there is no difference of procedure for newborns from carriers or non-carriers of HBsAg. Only 34% of newborns were covered by the birth-dose administration by 2011 while it was ranging between 58% and 66% in 2020.<sup>11,12</sup> This same year the coverage of newborns by the three doses of anti-hepatitis vaccine was 83%.<sup>12</sup> There are therefore still substantial efforts to be made to protect Laotian babies from birth. To improve coverage, it was shown that it was possible to store anti-hepatitis B vaccine outside the cold chain to extend its availability.<sup>13</sup> Significant dropouts were also noticed, particularly in provinces with higher rates of poverty.<sup>10</sup> Despite the imperfect immunisation status of children, investigators found they had a much lower HBsAg prevalence than in mothers (1.0% vs 7.0%) and observed within a few years a two-fold reduction of HBsAg carriage in toddlers (0.75% vs 1.82%).<sup>14</sup> The situation for children has improved recently due to the systematic anti-hepatitis B immunisation of newborns that started to be implemented two decades ago.<sup>7,15</sup> In addition, serological evidence of improved protection against hepatitis B is already detectable in older schoolchildren or young students (11–18 years) in both the capital and provinces.<sup>16</sup>

The elimination of hepatitis B and C has been scheduled for 2030 by the World Health Organization,<sup>17</sup> which necessitates a massive diagnostic effort to identify previously undetected virus carriers and correctly assess the disease burden in the country. Despite many seroprevalence surveys conducted in the last decade, the

situation of chronic infection with HBV is still only partially characterized in Laos, and unexpected results, such as geographical differences, are observed.<sup>18</sup>

To this aim, we conducted a large serological survey using the VIKIA point-of-care rapid test that explored the HBsAg status of 1.22% ( $n = 88,022$ ) of the total Lao population.<sup>19</sup> This survey included five categories of participants: blood donors, hospitalized patients, HIV(+) patients at the Center Infectiology Lao-Christophe Mérieux (CILM), students from the University of Health Sciences in Vientiane, and persons from the general population coming to the CILM for diagnostic purposes.

## Methods

### Study design and participants

This study was conducted between Sep 1<sup>st</sup>, 2020 and Aug 31<sup>st</sup>, 2021 except for HIV(+) cohort. Laboratory staff were trained in the proper use and interpretation of the diagnostic kit and good data collection at each study site. The study included all ages. For minors (below 18), an oral informed consent of the parents was obtained. Healthcare professionals explained the procedure to each participant and allowed them to freely decide if they wished to participate in the study. After obtaining oral informed consent, the doctor gave information about hepatitis B, its prevention, and treatment, and then conducted a face-to-face interview where data such as sex, age, occupation, address, and place of birth were recorded. Blood was then drawn and used to detect HBsAg as well as other parameters requested by the clinician. The HBsAg test result was given to the patient by a doctor at the next visit.

Five distinct cohorts were investigated with VIKIA<sup>®</sup> HBsAg (Biomérieux, Paris, France) (see [Table 1](#)):

- Hospital-based survey (Sep 1<sup>st</sup>, 2020- Aug 31<sup>st</sup>, 2021): Patients ( $n = 37347$ ) primarily coming from
- prenatal care clinics ( $n = 20,548$ ), internal medicine departments, and emergency units, conducted in 8 provincial hospitals (Oudomsay, Sainyaboury, Saisomboun, Vientiane, Khammouane, Savannakhet, Salavan, and Champasak), and five central hospitals (Mahosot, Mittaphap, Mother and Child, Setthathirath, and 103-Hospital). Persons included were those willing to participate. No specific selection of patients with symptoms or with suspected HBV infection was introduced. For persons under 18, the consent of both the parents and the participant himself was sought. Persons whose physical or mental conditions prevented informed consent were excluded.
- Blood donors (Sep 1<sup>st</sup>, 2020- Aug 31<sup>st</sup>, 2021): All 42,277 blood donors who presented themselves at blood banks during the study period were tested for HBsAg. Eighteen blood banks participated in the survey, one in the capital and in each province of Laos. According to the routine procedure implemented in the Blood bank network of Laos, another rapid test, SD Bioline (Standard Diagnostics, Yongin, Korea), was used to confirm positivity of VIKIA<sup>®</sup>. Out of 2161 positive donors, 59 were serodiscordant. A commercial ELISA test (HBsAg ultra sens, Human GmbH, Wiesbaden, Germany) was performed for further validation or invalidation of these 59 samples (see Supplementary material for details about sensitivity and specificity of the tests). As an outcome, 56 samples were invalidated, and three were confirmed. In addition, another series of 1268 randomly-chosen negative samples were tested by ELISA (HBsAg ultra sens), 9 were found positive by ELISA (0.7%). In this Laotian series, the overall sensitivity of VIKIA<sup>®</sup> was 99.6%, while its specificity was 95.7%.
- Students from the University of Health Sciences, Vientiane: Volunteer students ( $n = 609$ ) in the 2<sup>nd</sup>

	Blood donors survey ( $n = 42,277$ )	Hospital survey ( $n = 37,347$ )	HIV survey ( $n = 7439$ )	University of Health sciences survey ( $n = 609$ )	CILM survey ( $n = 350$ )
<b>Sex ratio (M:F)</b>	2.42 (29,929/12,348)	0.28 (10,672/26,675)	1.27 (4173/3266)	0.79 (269/340)	0.69 (136/214)
<b>Age (years)</b>					
mean $\pm$ SD	26.6 $\pm$ 8.9	33.6 $\pm$ 14.1	17.8 $\pm$ 15.9	21.5 $\pm$ 1.3	33.8 $\pm$ 14.2
median (IQR)	24 (19–32)	30 (24–40)	9 (5–31)	22 (20–23)	33 (23–43)
<b>HBsAg(+) (%)</b>	2.6 (1131/45,858)	6.4 (2380/37,347)	8.0 (595/7439)	3.3 (20/609)	10.0 (35/350)
<b>Blood banks (n)</b>	19	na	na	na	na
<b>Hospitals</b>	na	18	na	na	na
<b>Diagnostic centre (CILM)</b>	na	na	1	1	1
<b>Provinces (n)</b>	18	10	8	1	1
<b>North (%)</b>	20.7 (8775)	16.0	10.7	na	na
<b>Center (%)</b>	71.1 (30,023)	64.7	77.8	na	na
<b>South (%)</b>	8.2 (3479)	19.2	11.5	na	na

**Table 1: Demographic, biological, and geographical features of the different series analyzed.**

to 5<sup>th</sup> years of the curriculum were recruited between Jan 1<sup>st</sup>, 2021 and Apr 30<sup>th</sup>, 2021.

- At CILM: Inhabitants of Vientiane ( $n = 350$ ) were referred to our laboratory (Jan 3<sup>rd</sup>, 2021–Jun 21<sup>st</sup>, 2021) by their family practitioners for free first-time HBsAg tests. As for hospital survey, no specific selection of patients with symptoms or with suspected HBV infection was introduced.
- HIV samples: All single patients positive for HIV followed-up in our centre ( $n = 7439$ ) between Jun 1<sup>st</sup>, 2009 and Oct 7<sup>th</sup>, 2020 for HIV load were tested for HBsAg retrospectively. This cohort corresponds to half of the total estimated population of HIV carriers in the country, with patients coming from 8 different provinces. As HIV(+) samples were retrospectively analysed, informed consent was not obtained specifically for the current study. However, patients agreed that their sera could be used later for scientific purposes at the time of sampling.

The study was approved by the Lao National Ethics Committee for Health Research (reference NECHR 364/July 2020). The study adhered to the STROBE reporting guidelines.

### Procedures

VIKIA<sup>®</sup> HBsAg is a rapid immuno-chromatographic (or lateral flow) test for the qualitative detection of Hepatitis B surface Antigen (HBsAg) in human serum, plasma or whole blood. It can be used for prenatal or transfusion screening and during acute infection or chronic carriage of the hepatitis B virus.<sup>19</sup> According to the manufacturers' recommendations, capillary blood by fingerstick was used, dispensing 75  $\mu$ l of whole blood on the device, then one drop of buffer was added. Readings were available within 15 min, though for negative samples, it was recommended to wait up to 30 min to ascertain the result.

### Data analysis and statistical tests

To estimate HBsAg prevalence, we combined three main parameters: age, sex, and geographical origin of participants. Relying on  $\leq 20$  years blood donor data, we estimated HBsAg seroprevalence in the general population  $\leq 20$  by region, and sex. Estimates of HBsAg seroprevalence in older blood donors do not reflect the true prevalence in these age categories as the carriers of HBsAg, once identified by blood banks, are discouraged to do some further donations. Older and, thus, potentially recurrent blood donors do not represent, therefore, an appropriate category to assess HBsAg carriage in the general population. The geographical distribution of sampling in the hospital-based survey was not comprehensive enough (10 regions out of 18) to rely on observed regional differences with sufficient

confidence. To get an estimate of HBsAg carriage in the general population  $> 20$  of Laos, we calculated the mean hospital-based HBsAg prevalence for women (5.6%) and men (9.6%) between 20 and 69 years.

Statistical analyses were performed using the Prism 9.3.1 statistical package (GraphPad, San Diego, CA, USA). Continuous variables are presented by their mean and standard deviation or median and interquartile range depending upon the distribution type. Accordingly, they were compared either by a Student's t-test (Gaussian distribution) or a Mann-Whitney U test (non-Gaussian). Categorical variables were summarized as frequencies and compared either by Fisher's exact test or the Chi-Square test. All the tests were two-sided, and the level of significance was set at  $P < 0.05$ .

### Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. PS, ED, PhPa, and PaPi had access to the dataset and had final responsibility for the decision of publication.

### Results

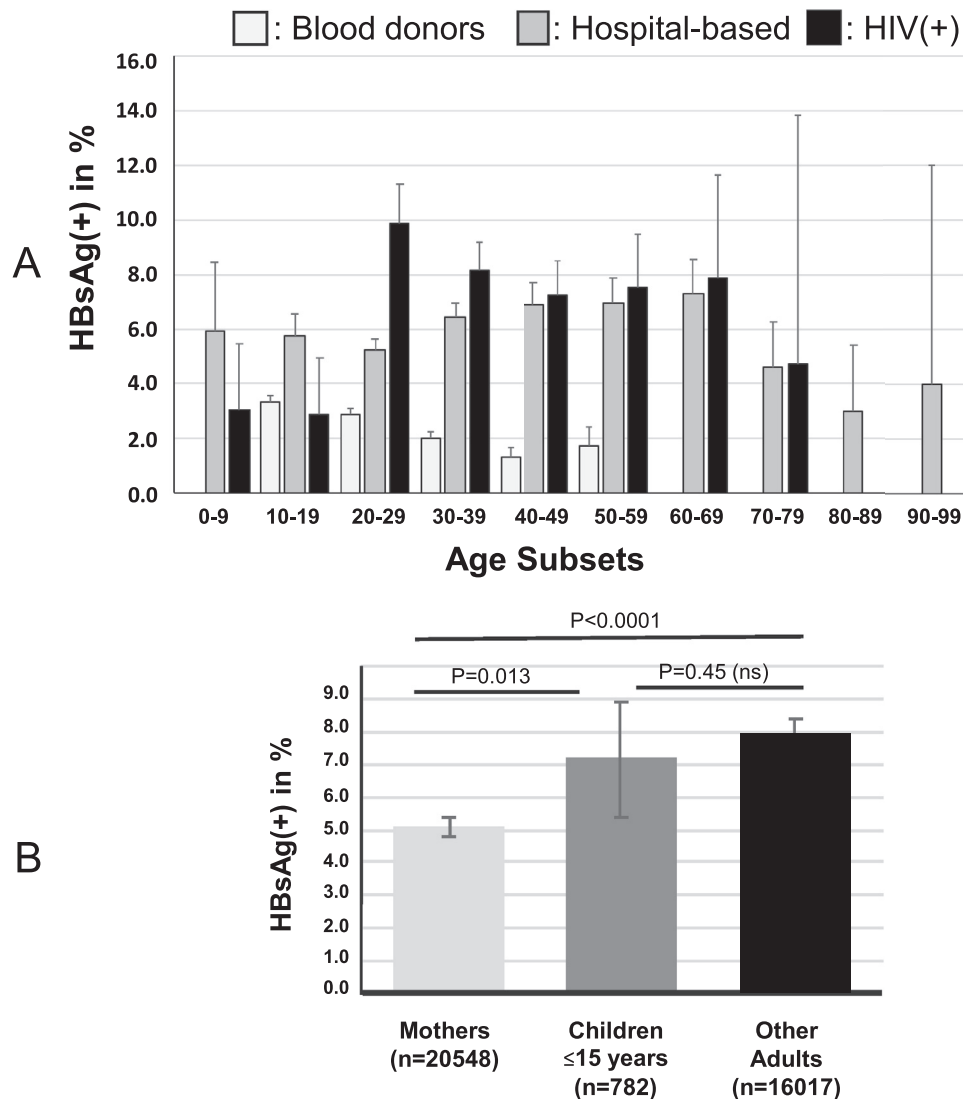
With 80,082 tests, this was the largest seroprevalence survey of the hepatitis B virus surface antigen (HBsAg) ever conducted in Laos. The participants' demographic features and geographic origins are summarized in [Table 1](#).

The structures of each series regarding age and sex were significantly different. Women were under-represented among blood donors while they were over-represented in the hospital-based survey (71.4%) due to our targeting of maternity departments and antenatal clinics.

HBsAg seroprevalence was 2.6% in the whole series of blood donors. We observed that it tends to decrease in blood donors older than 17–20 years of age (where it peaks above 3.0%) ([Figure 1A](#)). We observed overall prevalence of 6.4% and 8.0% in the hospital-based series and the HIV-infected cohort. In contrast with blood donors, we observed an increasing seroprevalence with age that reaches a plateau of around 8.0% in adults above 20 years in these two series.

The hospital-based series was, however, heterogeneous and hid significant differences in HBsAg prevalence. Mothers attending antenatal clinics or obstetrical departments are inherently healthier on average than other patients admitted to the hospital. Accordingly, HBsAg seroprevalence was 5.0% in attending mothers ( $n = 1044/20,548$ ) while it reached 7.1% in children below 15 years ( $n = 56/782$ ) and 8.0% in the remaining adult participants ( $n = 1280/16,017$ ) ([Figure 1B](#)).

Concerning sex differences in prevalence, males were systematically more often infected than females



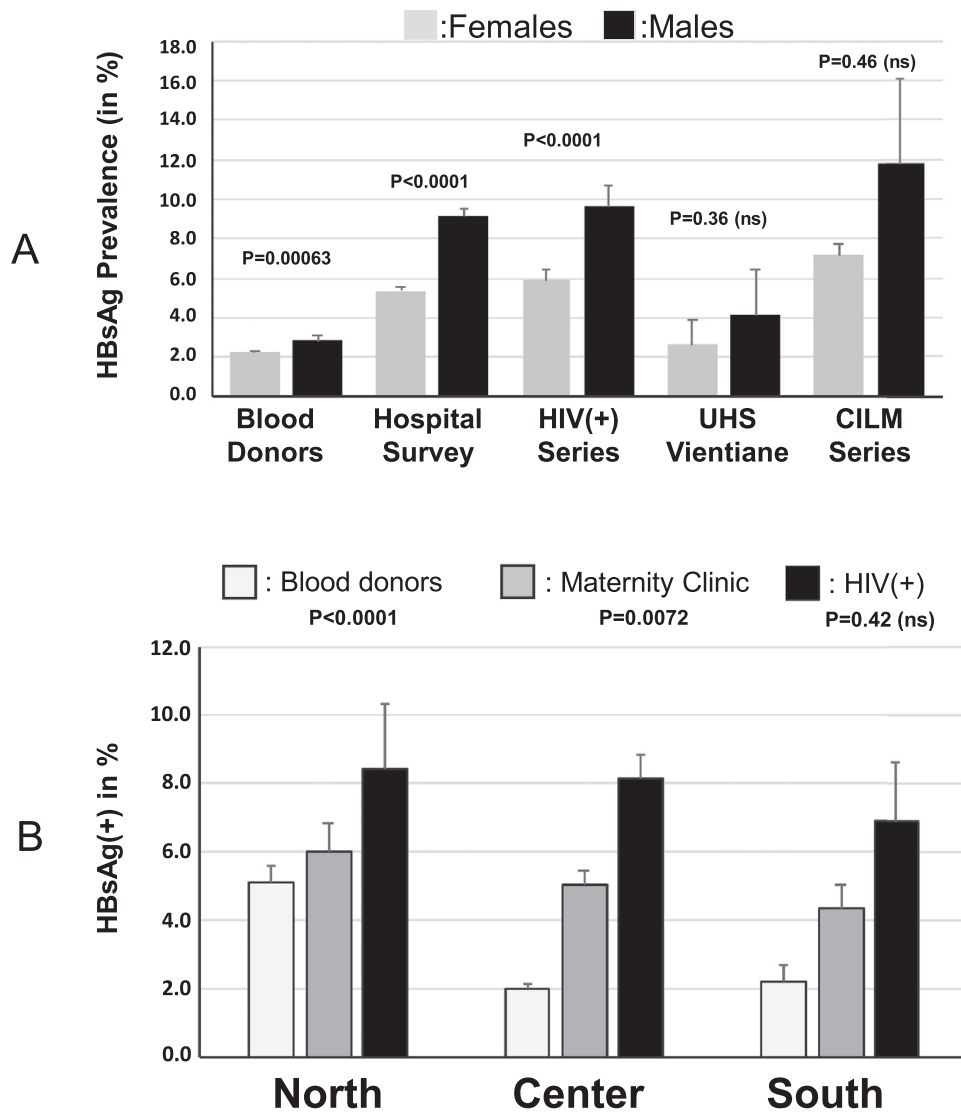
**Figure 1.** Prevalence of Hepatitis B virus surface Antigen (HBsAg) in the different subsets of participants and in the Hospital-based survey. A. Bar chart representing HBsAg prevalence by % in the 42,277 blood donors, the 37,347 patients attending care in the different participating hospitals, and the 7439 HIV-infected patients, according to the different age classes. B. Heterogeneity of HBsAg prevalence in the different components of the hospital-based series. It should be noticed that all patients except those infected with Human Immunodeficiency Virus, HIV(+), were recruited between September 2020 and August 2021. The Human Immunodeficiency virus-positive series [HIV(+)] was enrolled between 2009 and 2020.

(1.2–1.7 fold), and this difference reached the level of significance for blood donors, the hospital-based series, and the HIV(+) cohort (Figure 2A).

Geographic differences in HBsAg prevalence were remarkable. For blood donors and mothers attending maternity departments, HBsAg prevalence was significantly higher in northern than in southern and central regions of the country (Figure 2B and Figure 3A-C).<sup>18</sup> Occupations of the 42,277 blood donors (BD) were known, and when we analysed the prevalence of HBsAg

in the nine major categories, the carriage rate was significantly higher in the North than in the Centre or the South (Supplementary Table 2) in six categories.

Realistic assessment of the current situation for HBsAg prevalence in Laos is crucial to assess any progress in the two decades following the implementation of an anti-hepatitis B immunisation campaign in newborns. An important demographic feature in Laos is the young age of the population, with 42.9% aged 20 or below (median age = 23.0 years).<sup>2</sup> In contrast to



**Figure 2.** Differential Hepatitis B virus surface Antigen (HBsAg) prevalence according to sex or geographic origin. A. HBsAg seroprevalence according to sex in the five population subsets investigated. B. HBsAg seroprevalence according to the place of sampling in blood donors, mothers attending care in maternity departments, and Human Immunodeficiency Virus-infected patients [HIV(+)].

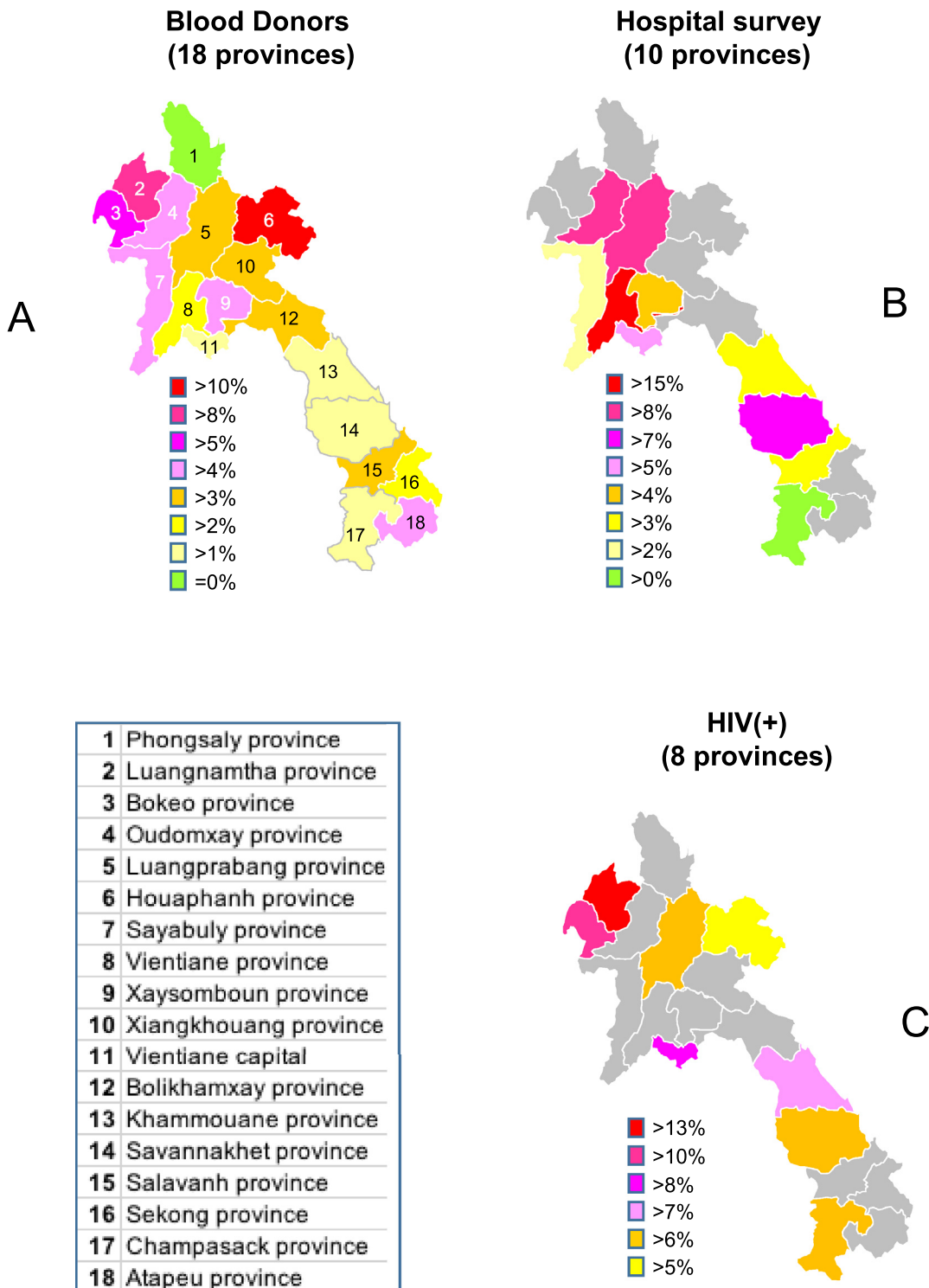
previous generations, blood donors between 17 and 20 would have benefited from the anti-hepatitis B immunisation program, which began in 2001. In addition, they could, for obvious reasons, be considered as first-time donors and thus, HBsAg seroprevalence in this age group should be relatively less biased than in older segments of the population. We observed that men from the northern region of Laos were particularly affected by HBV infection (1.8–2.1 fold the prevalence for women) (see Figure 4A). Sex differences were less pronounced in the Centre (1.0–1.2 fold) and the South (1.2–1.6 fold).

For the population between 20 and 69 years we decided to rely of the hospital survey. We consider that

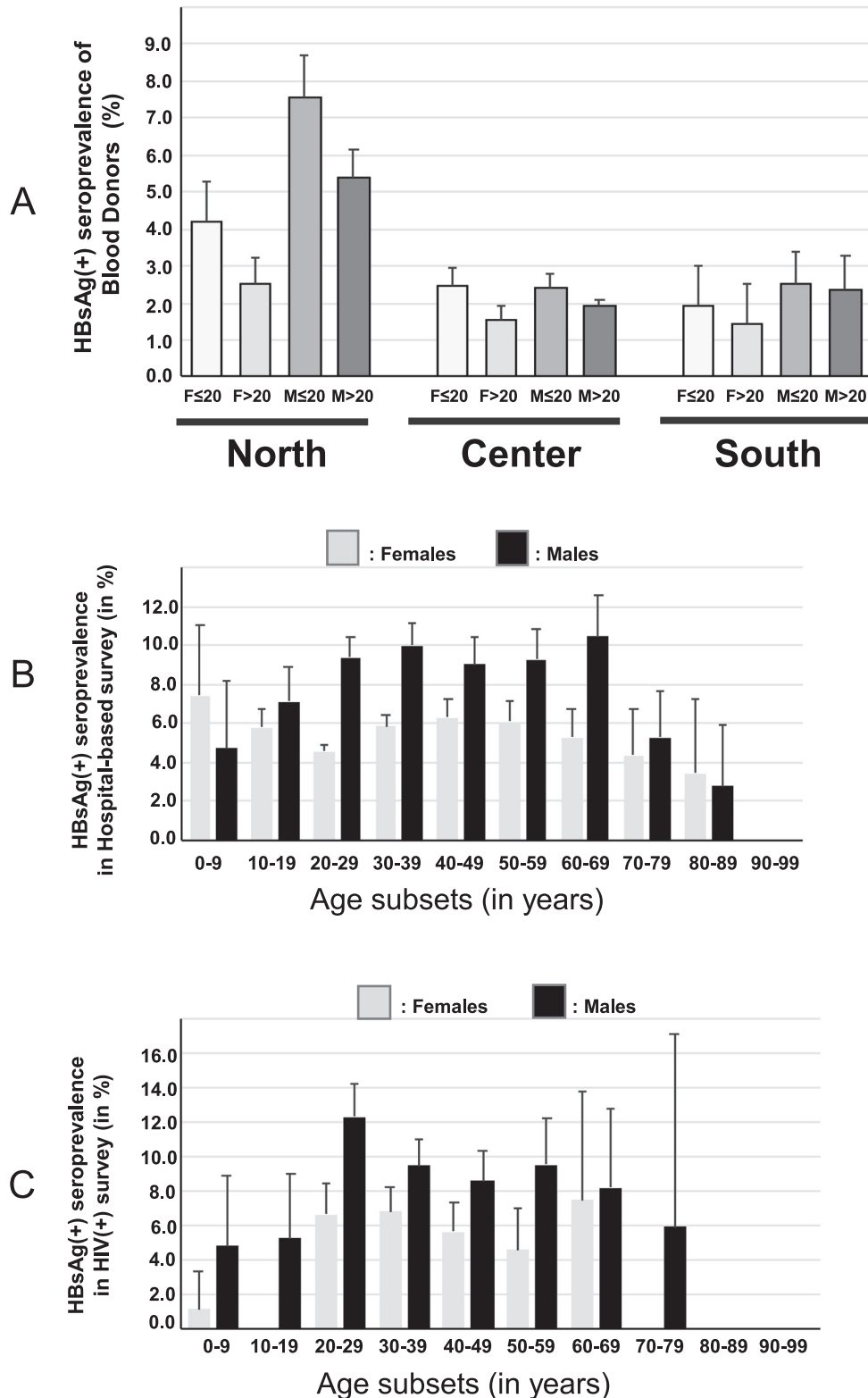
the 21–69 years subset of patients attending care at the hospital represents approximately a random sample of the corresponding general population that did not benefit from the systematic anti-hepatitis B immunization campaign implemented in the country. The mean HBsAg prevalence for women was 5.6% and while it was 9.6% in men (see Figure 4B). Seroprevalence in the hospital series was not so different from that observed in the HIV-infected cohort (6.2% and 9.6%), which represents a population of patients often considered to be in the upper range of HBsAg prevalence in Southeast Asia (Figure 4C).<sup>20</sup>

Overall, once normalized for the population structure and size in each region, according to our estimates,



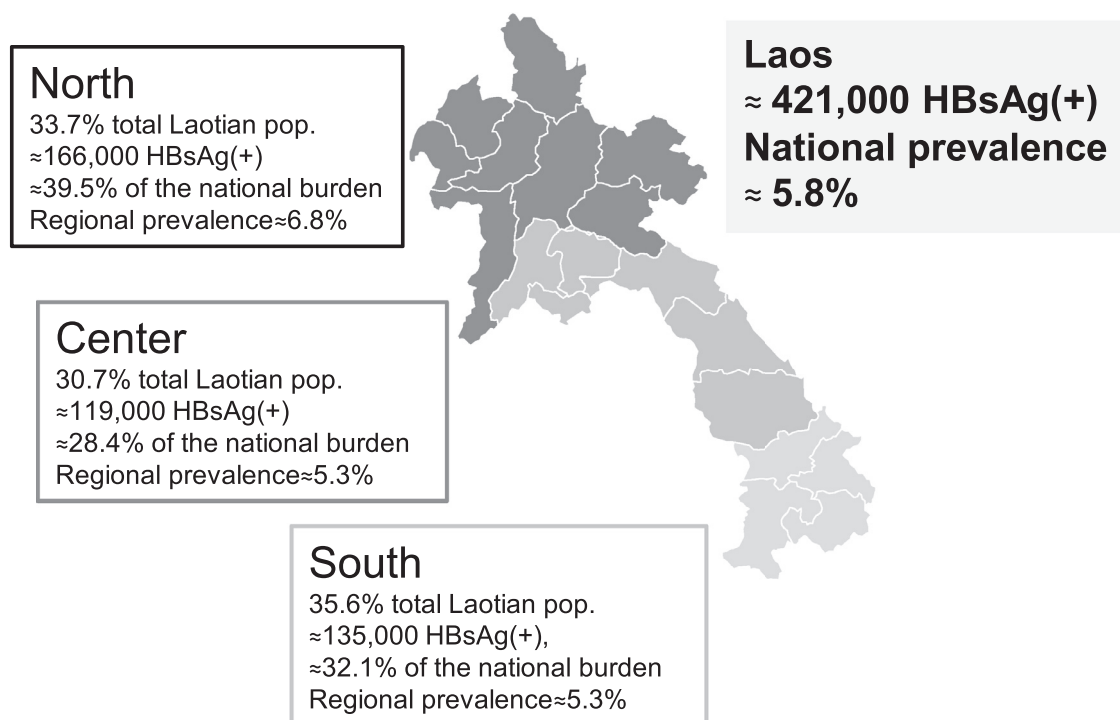


**Figure 3.** Map of Laos representing the prevalence of Hepatitis B virus surface Antigen (HBsAg) in the different provinces A. In blood donors, B. In patients attending care in hospitals. C. In Human Immunodeficiency Virus-infected patients [HIV(+)].



**Figure 4.** Bar-Charts differentiating Hepatitis B virus surface Antigen (HBsAg) prevalence according to age and sex. A. Bar chart representing HBsAg prevalence in blood donors according to sex, age, and region of origin. B. Bar chart representing HBsAg prevalence according to sex and age in the hospital-based cohort. C. Bar chart representing HBsAg prevalence according to sex and age in Human Immunodeficiency Virus-infected patients [HIV(+)].





**Figure 5.** Estimated distribution and seroprevalence of Hepatitis B virus surface Antigen (HBsAg) in the three large regions of Laos.

HBsAg seroprevalence decreases along a north to south gradient for all ages, ranging from 6.8% in the North to 5.3% in Central and Southern Laos. The overall HBsAg prevalence in Laos is estimated to be 5.8% (see [Figure 5](#)).

## Discussion

Our study conducted between 2020 and 2021 confirmed the existence of a decreasing North-South gradient of HBs antigen prevalence in Laos.<sup>18</sup> Moreover, the participation of more than 42,000 blood donors allowed us to highlight a reduction in the prevalence of HBsAg in donors aged 20 and under. This last observation suggests that the immunisation policy for newborns implemented from 2001 has undoubtedly already borne fruit and reduced the burden of hepatitis B in the youngest segments of the population.

The different series investigated were characterized by substantial heterogeneities in sex-ratio. The observed under-representation of women among blood donors was expected as the high prevalence of anaemia affecting young women<sup>21</sup> makes them less likely to donate blood. The earliest survey of HBsAg in blood donors in Laos was conducted 17 years ago on 14,000 first-time donors in Vientiane capital and Bolikhamsay province (Central region).<sup>22</sup> The overall prevalence in these donors from the pre-immunisation era was 8.7%, much higher than observed in our series (2.8%). This

difference is probably due to recurrent blood donors in the current survey. It is known that blood banks discourage people already known to be infected with HBV to donate their blood resulting in a lower proportion of infected persons in recurrent donors.<sup>18</sup> However, if we focus on first-time donors aged 17–20, we observe a 2.4-fold decrease from 8.5% to 3.3% ( $P < 0.0001$ , OR = 2.9, 95%CI = 2.5–3.3) in HBsAg prevalence between 2003–2005 and 2021. The sex difference observed at that time (6.2% HBsAg in females and 9.7% in males, 1.56-fold) is similar to what we observed (2.7% vs 3.6%, 1.33-fold). The difference of seroprevalence between males and females is a hallmark of chronic infection with HBV. It was described early on.<sup>23,24</sup> The patho-physiological bases of this difference are not clearly known. Regarding viral factors, a role of the androgen-responsive elements (ARE) present on HBV genome and capable to stimulate HBV replication is sometime mentioned.<sup>25</sup> But, overall, it is considered that the higher susceptibility of males to infection with HBV is due to the lesser immune competence that characterize males in human species.<sup>26</sup>

Additionally, HBsAg(+) donors tend to be older in the current study (24.6±7.7 years) than previously (22.3±6.6 years), suggesting the progressive ageing of HBsAg carriers. The large difference between first-time and other donors was already observed in 2006 when 17–20 years old donors in Vientiane and Bolikhamsay ( $n = 342$ ) born before the immunisation era had an

HBsAg prevalence of 9.3%.<sup>27</sup> A recent first-time donor survey conducted between 2013 and 2015 observed, as in the current study, that HBsAg carriage was much higher in the North (13.1%) than in the rest of Laos (6.6% in the Centre, and 5.6% in the South).<sup>18</sup> These rates were, however, much higher than those observed in first-time donors (17–20 years) in the present study (6.2%, 2.4%, and 2.3%, respectively). We assume that the discordance between that study and ours is because Nouanthong *et al.* includes individuals born in the pre-immunisation era.

In the past decade, a survey of mothers ( $n = 965$ ) recruited throughout the country yielded an estimated HBsAg prevalence of 2.9% ( $P = 0.00027$ ,  $OR = 0.5$ ,  $95\%CI = 0.3-0.7$ ),<sup>28</sup> much lower than the overall rate observed in our work (4.9%,  $n = 20,156$ ). The reason for this difference may possibly be that participants in the present study were in hospital maternity departments, an at-risk medical context, while the previous ones were in villages in a potentially healthier context. Our results also differed from those of other studies conducted in antenatal departments in different provinces, such as a study in Vientiane, which measured HBsAg prevalence at 8.0–8.2% ( $n = 388$ ,  $P = 0.0067$ ,  $OR = 1.7$ ,  $95\%CI = 1.1-2.5$ ).<sup>15</sup> We are, however, in closer agreement with the results of a serological survey of three maternity departments in central provinces and Vientiane Capital. In that study, HBsAg prevalence estimates were 4.1% ( $n = 911$ ), 5.9% ( $n = 3000$ ), and 5.4% ( $n = 13,238$ ), none of which are significantly different than the 5.1% in our work ( $P = 0.16$ ,  $P = 0.22$ , and  $P = 0.30$  respectively, ns).<sup>29</sup> Overall, it seems that there could be considerable variation in HBsAg prevalence in Lao child-bearing women. These differences presumably depend upon the geographical locations of the recruitment centre as we and other have shown that there is a decreasing North to South gradient of HBsAg prevalence.<sup>18</sup> The structures of recruitment might represent another modulator as attending mothers at risk of complications are primarily found in regional hospitals rather than in village health centres.

Few hospital surveys have been conducted in Laos. Recent work in the southern province of Saravan involving 2463 patients observed an overall HBsAg prevalence of 3.8%, characterized by a significant difference by sex (2.4% in females, 5.7% in males). The prevalence of HBsAg was low (2.8%) in the 11–20 years age group ( $n = 643$ ) compared to the 4.8% observed for those aged above 21 years ( $n = 1562$ ).<sup>30</sup> Our survey conducted in Saravan and Champasack ( $n = 3657$ ) yielded higher values with overall HBsAg carriage at 6.5% (5.0% for females and 6.8% for males). As in the study of Xaydalousouk and co-workers, HBsAg prevalence was much lower in patients aged 20 and lower (3.3%) than in older ones (7.1%). These results clearly indicate that the immunisation program first implemented 20 years ago has already benefited populations seeking care at the

hospital. We observed that children attending care in hospitals were significantly more infected than young mothers (Figure 1B). This situation is most probably due to the fact that diseased children in Laos are logically more at risk to be HBV-infected than the healthy segments of the population to whom belong the young mothers.

To the best of our knowledge, there has been no survey of HIV-HBV co-infections in Laos. However, the prevalence of HBsAg in the current retrospective Lao cohort (8.0%) was lower than those observed in studies in Cambodia (10.7%), Vietnam (11.9%), Thailand (11.9%), and more similar to observations from Myanmar (7.6–9.0%).<sup>20,31-34</sup> The higher representation of men in participants with HIV infection plausibly reflects the fact that men who have sex with men are more infected with HIV than other groups in Laos.<sup>35</sup> The low rate of hepatitis B in HIV-infected patients is most probably due to the young age of the series (mean = 17.8±15.9 years), implying that most were born during the immunisation era. It represents further evidence of the efficacy of the immunisation policy conducted in Laos.

A remarkable feature of our work was the North-South decreasing gradient of HBsAg prevalence in the country. There are no well-established explanations to this observation recently reported by other investigators.<sup>18</sup> It is noteworthy, however, that the ethnic composition of the northern part of the country differs substantially from that of the center and the south.<sup>36</sup> In the North are established communities speaking Hmong-mien languages described for a long time as affected by higher HBsAg prevalence.<sup>6</sup> Other ethnic communities, speaking Tibeto-Burman languages are also established in the North of the country. Hence, these latter communities are considered by some authors to be the reservoir of the recently described genotype I known to be present in Laos.<sup>37,38</sup>

Our study has some limitations that might have some consequences concerning our estimation of HBsAg prevalence in Laos. The principal problem came from the blood donors that were not identified as first-time or recurrent. It is reasonable to assume, however, that 17–20-year-old are presumably first-time donors, and this study is, therefore, able to provide a fair assessment of HBsAg seroprevalence in populations born after the implementation of universal anti-hepatitis B immunisation. Concerning older segments of the population, our estimation relies on the hospital survey that mechanically tends to overestimate the prevalence of HBsAg as its carriers are more often in position to seek for healthcare than healthy people. In addition, due to a less comprehensive sampling throughout the country, we were not capable to differentiate reliably Northern, Center, and Southern regions to produce estimates.

In conclusion, we observed a significant difference in HBsAg prevalence throughout the country, with

northern provinces being affected by a heavier burden of infection than central or southern regions of the country. New studies are needed both to definitively establish the existence of these differences and to better understand their causes. Given the Lao age pyramid (43% of the population is  $\leq 20$  years) and the implementation of universal anti-hepatitis B immunisation 20 years ago, we consider that Laos is probably no longer highly endemic ( $>8.0\%$ ) for chronic hepatitis B virus infection. From now on, it seems reasonable to consider Laos as a country with high-intermediate endemicity (5.0–7.0%).<sup>7</sup> However, it is fair to keep in mind that our work is only an approximation and that more data is needed to truly demonstrate a significant decrease in the AgHBs prevalence in young people and a modification in the status of Lao from high endemic country to an intermediate one. Finally, our work suggests that a developing country with a predominantly rural population can emerge from high endemicity for chronic hepatitis B virus infection within a single generation. This situation is primarily the result of the demographic dynamism of the populations whose many children are protected from infection by public health measures applied with determination. It is, therefore, a message of hope for many other countries facing similar circumstances.

### Contributors

PhPa and ED designed the project. PS, VL, CS, VS, MM, SP collected the data. PaPi analysed and interpreted the data. PS and PaPi generated figures and tables. PaPi wrote the manuscript. ED, PhPa, AM, and PS edited the manuscript. NS, PV, and SB critically read the manuscript. All authors contributed substantially to the conception, writing and revision of the work and approved the final content of the manuscript.

PS, ED, PhPa, and, PaPi had access and verified the data reported in the manuscript.

### Data sharing statement

These data will be available with publication with investigator support, and after approval of a proposal.

### Declaration of interests

All authors declare no competing interests.

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### Supplementary materials

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### References

- Bell J, Nuzzo J. Global health security index: advancing collective action and accountability amid global crisis, 2021.
- Fund UNP. UNFPA Lao People's Democratic Republic. 2022.
- Organization WH. Global health observatory data repository. 2022.
- Klontz K. A program to provide hepatitis B immunoprophylaxis to infants born to HBsAg-positive Asian and Pacific Island women. *West J Med.* 1987;146:195–199.
- Hurie M, Mast E, Davis J. Horizontal transmission of hepatitis B virus infection to United States-born children of Hmong refugees. *Pediatrics.* 1992;89:269–273.
- Mixson-Hayden T, Lee D, Ganova-Raeva L, et al. Hepatitis B virus and hepatitis C virus infections in United States-bound refugees from Asia and Africa. *Am J Trop Med Hyg.* 2014;90:1014–1020.
- Ott J, Stevens G, Groeger J, Wiersma S. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine.* 2012;30:2212–2219.
- Sitbounlang P, Marchio A, Deharo E, Paboriboune P, Pineau P. The threat of multiple liver carcinogens in the population of Laos: a review. *Liver Int.* 2021;1:49–59.
- Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin.* 2018;68(6):394–424.
- Phoummalaysith B, Yamamoto E, Xeuatvongsa A, et al. Factors associated with routine immunization coverage of children under one year old in Lao People's Democratic Republic. *Vaccine.* 2018;36:2666–2672.
- Xeuatvongsa A, Pathammavong C, Phounphenhack K, et al. Hepatitis B vaccine birthdose practices in a country where hepatitis B is endemic—Laos, December 2011–February 2012. *MMWR.* 2013; 62:587–590.
- WHO Idp. Immunization dashboard. May, 2nd, 2022.
- Kolwaite A, Xeuatvongsa A, Ramirez-Gonzalez A, et al. Hepatitis B vaccine stored outside the cold chain setting: a pilot study in rural Lao PDR. *Vaccine.* 2016;34:3324–3330.
- Hefele L, Syphan S, Xayavong D, et al. Seroprotection at different levels of the healthcare system after routine vaccination with diphtheria-tetanus-pertussis whole cell-hepatitis B–Haemophilus

- influenzae type B in Lao People's Democratic Republic. *Clin Infect Dis*. 2019;69:2136–2144.
- 15 Black A, Nouanthong P, Nanthavong N, et al. Hepatitis B virus in the Lao People's Democratic Republic: a cross sectional serosurvey in different cohorts. *BMC Infect Dis*. 2014;14:457.
  - 16 Hefele L, Vannachone S, Khounvisith V, et al. Lasting benefit of infant hepatitis B vaccination in adolescents in the Lao People's Democratic Republic. *Int J Infect Dis*. 2020;93:217–223.
  - 17 United Nations transforming our world: the 2030 agenda for sustainable development, 2015.
  - 18 Nouanthong P, Hefele L, Keokhamphue J, et al. Analyses of blood donor samples from eight provinces in Lao PDR suggest considerable variation concerning HBV exposure and carriage. *PLoS One*. 2021;16:e0259814.
  - 19 Chevaliez S, Roudot-Thoraval F, Hézode C, Pawlotsky J, Njouom R. Performance of rapid diagnostic tests for hepatitis B surface antigen detection in serum or plasma. *Diagn Microbiol Infect Dis*. 2021;100:115353.
  - 20 Bermúdez-Aza E, Shetty S, Ousley J, et al. Long-term clinical, immunological and virological outcomes of patients on antiretroviral therapy in southern Myanmar. *PLoS One*. 2018;13:e0191695.
  - 21 Keokenchanh S, Kounnavong S, Tokinobu A, et al. Prevalence of anemia and its associate factors among women of reproductive age in Lao PDR: evidence from a nationally representative survey. *Anemia*. 2021;2021:8823030.
  - 22 Jutavijittum P, Yousukh A, Samountry B, et al. Seroprevalence of hepatitis B and C virus infections among Lao blood donors. *Southeast Asian J Trop Med Public Health*. 2007;38:674–679.
  - 23 Blumberg B, Sutnick A, Londo W, McLartin L. Sex distribution of Australia antigen. *Arch Intern Med*. 1972;130:227–231.
  - 24 London W, Drew J. Sex differences in response to hepatitis B infection among patients receiving chronic dialysis treatment. *Proc Natl Acad Sci USA*. 1977;74:2561–2563.
  - 25 Wang S, Yeh S, Lin W, Wang H, Chen D, Chen P. Identification of androgen response elements in the enhancer I of hepatitis B virus: a mechanism for sex disparity in chronic hepatitis B. *Hepatology*. 2009;50:1392–1402.
  - 26 Klein S, Flanagan K. Sex differences in immune responses. *Nat Rev Immunol*. 2016;16:626–638.
  - 27 Jutavijittum P, Andernach I, Yousukh A, et al. Occult hepatitis B infections among blood donors in Lao PDR. *Vox Sang*. 2014;106:31–37.
  - 28 Xeuatvongsa A, Komada K, Kitamura T, et al. Chronic hepatitis B prevalence among children and mothers: results from a nationwide, population-based survey in Lao People's Democratic Republic. *PLoS One*. 2014;9:e88829.
  - 29 Komada K, Sugiyama M, Vongphrachanh P, et al. 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. *Int J Infect Dis*. 2015;36:21–26.
  - 30 Kaydalasouk K, Sayasinh K, Hübschen J, et al. Age-stratified seroprevalence of vaccine-preventable infectious disease in Saravan, Southern Lao People's Democratic Republic. *Int J Infect Dis*. 2021;107:25–30.
  - 31 Nguyen C, Ishizaki A, Chung P, et al. Prevalence of HBV infection among different HIV-risk groups in Hai Phong, Vietnam. *J Med Virol*. 2011;83:399–404.
  - 32 Tsuchiya N, Pathipvanich P, Rojanawiwat A, et al. Chronic hepatitis B and C co-infection increased all-cause mortality in HAART-naive HIV patients in northern Thailand. *Epidemiol Infect*. 2013;141:1840–1848.
  - 33 van Griensven J, Phirum L, Choun K, Thai S, De Weggheleire A, Lynen L. Hepatitis B and C co-infection among HIV-infected adults while on antiretroviral treatment: long-term survival, CD4 cell count recovery and antiretroviral toxicity in Cambodia. *PLoS One*. 2014;9:e88552.
  - 34 Zaw S, Tun S, Thida A, et al. Prevalence of hepatitis C and B virus among patients infected with HIV: a cross-sectional analysis of a large HIV care programme in Myanmar. *Trop Doct*. 2013;43:113–115.
  - 35 Sheridan S, Phimpachanh C, Chanlivong N, et al. HIV prevalence and risk behaviour among men who have sex with men in Vientiane Capital, Lao People's Democratic Republic, 2007. *AIDS*. 2009;23:409–414.
  - 36 Schlemmer G. Ethnic belonging in Laos: a politico-historical perspective. eds. In: Bouté V, Pholsena V, eds. *Changing Lives in Laos: Society, Politics and Culture in a Post-Socialist State*. Singapore: NUS Press; 2017:251–280.
  - 37 Arankalle V, Gandhe S, Borkakoty B, Walimbe A, Biswas D, Mahanta J. A novel HBV recombinant (genotype I) similar to Vietnam/Laos in a primitive tribe in eastern India. *J Viral Hepat*. 2010;17:501–510.
  - 38 Olinger C, Jutavijittum P, Hübschen J, et al. Possible new hepatitis B virus genotype, southeast Asia. *Emerg Infect Dis*. 2008;14:1777–1780.