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国立研究開発法人国立国際医療研究センター
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ラオス人民民主共和国における B 型肝炎有病率調査

Hepatitis B Prevalence Survey in Lao PDR

NCGM

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研究の遂行にあたりご指導、ご助言くださった皆様にお礼申し上げます。

ラオス人民民主共和国 保健省
　予防接種課 (National Immunization Program)
　国立検査疫学センター (National Center for Laboratory & Epidemiology)

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　母子保健統合サービス強化プロジェクト
　母子保健人材開発プロジェクト

世界保健機関
　西太平洋地域事務所
　ラオス事務所

略語

C.I.	Confidence interval　信頼区間
DPT-HepB	Diphtheria, pertussis, tetanus, hepatitis B vaccine シフテリア、百日咳、破傷風、B 型肝炎ワクチン
EPI	Expanded Programme on Immunization 拡大予防接種計画
HB	Hepatitis B　B 型肝炎
HBs 抗原	Hepatitis B surface antigen　B 型肝炎ウイルス表面抗原
ID	Identification number　識別番号
JICA	Japan International Cooperation Agency　国際協力機構
NCGM	National Center for Global Health and Medicine 国立国際医療研究センター
NCLE	National Center for Laboratory and Epidemiology 国立検査疫学センター
NIP	National immunization Program　国家予防接種課
PPS	Probability proportionate to size　確率比例抽出
SBA	Skilled birth attendance　熟練助産師
VPD	Vaccine preventable diseases　ワクチン予防可能疾患
WHO	World Health Organization　世界保健機関

サマリー

◘ 背景

ラオス人民民主共和国 (以下、ラオス) の一般人口における B 型肝炎の有病率を調べ、B 型肝炎ワクチン導入の効果を評価するため、ラオス保健省と国立国際医療研究センター (以 下、NCGM) は血清診断および質問票による横断調査を実施することになった。調査は 2012 年 2 月に全国規模で行われた。本報告書は調査結果をまとめたものである。

◘ 目的

調査目的は以下の通りである。

1. 小児における B 型肝炎ウイルス表面抗原 (HBs 抗原) 陽性率を推定する
2. 母親における HBs 抗原陽性率を推定する
3. 可能性のある危険因子を評価する

◘ 対象年齢、地域、調査時期

年齢　小児　5~9 歳
　　母親　15~45 歳
地域　全国
時期　2012 年 1~2 月

◘ 調査方法

全国 143 郡を 2 つの層に分けた ;2010 年の接種率データを用い、3 回目のジフテリア、百日咳、破傷風、B 型肝炎ワクチン (DPT-HepB) の接種率が高い郡と低い郡である。次に確率比例抽出により各層から無作為に 12 郡を選択し、さらに確率比例抽出により各郡から 2 村を選択した。それぞれの村 (クラスター) において、調査員が住民台帳から無作為に 21 の母子ペアを選んだ。選ばれた母子に対し調査の目的と方法、個人情報保持について説明し、同意が得られたのち、質問票および血液採取を実施した。血液は Determine® 迅速 診断キット（日本名：ダイナスクリーン・HBsAg II、アリーアメディカル社）を使用して HBs 抗原の診断を行った。

◘ 結果

計 2,016 の血液検体 (小児 1,008 例、および母親 1,008 例) を収集し、HBs 抗原陽性率
は 5~9 歳の小児において 1.7% (95%C.I.=0.8, 2.6)、妊娠可能年齢の母親において 2.9% (95%C.I.=1.6, 4.2) と推定された。子どもの HBs 抗原陽性は母親の HB 感染と有意に相関していたが、他の可能性のある危険因子 (母親の年齢、人種、最寄りの保健センターまでの 時間、母親の教育レベル、既往歴) とは相関していなかった。

◘ 考察および推奨

本研究はラオスにおける初めての全国 B 型肝炎有病率調査である。調査結果から推定される有病率は、周辺国から報告されている値より低かったが、その理由は不明である。B 型肝炎ワクチンの出生時接種と乳児期の 3 回接種は依然として重要と考えられる ; それゆえ、国家予防接種事業を注意深く監視し、繰り返し調査をすることが必要であろう。

1. はじめに

◘ 背景

B 型肝炎 (HB) ウイルス感染者は世界中で 20 億人を超え、毎年約 100 万人が HB ウイルス 感染により死亡している[1, 2]。そのうち 33% は肝細胞癌、残りは肝疾患の末期合併症による。世界保健機関 (WHO) の西太平洋地域事務局は、2003 年に決議案 WPR/RC54.R3 を、2005 年に決議案 WPR/RC56.R8 を採択し、5 歳児の慢性 B 型肝炎感染を 1% 未満に減らすという最終的な地域目標に向けた中間目標として、2012 年までにその感染率を 2% 未満に低下させることを提唱した。B 型肝炎コントロール戦略の地域での進展は、小児の B 型肝炎感染の劇的な低下をもたらし、上記の中間目標は 27 の国と地域で達成が期待されている (WPR/RC61/10)。

ラオス人民民主共和国 (以下 , ラオス) は、1984 年に特定の地域において拡大予防接種 計画 (EPI) を開始した。このプログラムは、1994 年に規模が全国に拡大された。B 型肝炎 ワクチンは 2002 年に EPI に加えられ、徐々に拡大された (表 1)。カンボジア、中国、タイ、ベトナムなどの近隣諸国の研究により B 型肝炎ウイルス表面抗原 (HBs 抗原) 陽性率が高いことが明らかになったが[3, 4, 5, 6]、これまでラオスの一般住民における慢性 B 型肝炎ウイルス有病率に関する報告はなされていない。したがって、現状を理解するため、そして母子感染予防を目標とする予防接種普及政策を評価するために、一般住民を対象とした B 型肝炎の血清陽性率調査が必要である[7, 8]。

ラオス保健省と国立国際医療研究センター (NCGM) は、血清陽性率調査について合意した。チームは、2011 年の 1 月と 2 月にパイロット調査としてラオスの中央部に集中し、2012 年の 1 月 ~2 月には国全体を対象とした。

表 1. ラオスにおける B 型肝炎予防接種活動

2002 年	定期予防接種として DTP-HepB
2004 年	首都の病院に出生時接種を導入
2005 年	2 つの南部県の病院を追加
2007 年	8 つの県の病院を追加
2008 年	残りの県と全 123 郡の病院を追加
2009 年	9 つの県の 50 郡において、保健センター職員による B 型肝炎出生時接種後の家庭 訪問を開始
2010 年	施設での勤務および家庭分娩への立ち会いが可能な熟練した助産師 (専門の技 能を持つ分娩介助者) の訓練

◘ 調査の目的

調査の主目的は、以下の通りである。

1. 小児における HBs 抗原の陽性率を推定する
2. 母親における HBs 抗原の陽性率を推定する
3. 可能性のある危険因子を評価する

2. 方法

◘ 標本サイズ算出

望ましい信頼度レベル 1.96、許容誤差 0.02、HBs 抗原陽性率 0.05、デザイン効果 2.0、階 層数 2 および回答率 0.95 と想定して、必要な標本サイズを 961 と計算した。これは、961 組の母子 (1,922 例) を意味する。実施を容易にするため、母子 1,008 組 (2,016 例) の収集 を計画した。

◘ サンプリング法

多段階層化収束抽出法を用いて、妊娠可能年齢の母親とその子供を選出した。

ラオスは首都ビエンチャンと 16 の県からなる国である。2005 年の国勢調査によると、国 内には 143 の郡があり、10,000 を超える村がある。われわれは、ジフテリア、百日咳、破傷風、B 型肝炎ワクチン (DPT-HepB) 3 回目の接種率によって、全郡を高接種率 (≧ 76%, 72 郡) と低接種率 (<76%,71 郡) の 2 層に分けた。第 1 段階として、確率比例抽出法 (PPS) を用いて、各層から 12 郡を無作為に選択した。第 2 段階では、PPS により各郡から 2 つの村を無作為に選択した。48 村のそれぞれにおいて、貧困削減プログラムによる居住者リスト に基づいて、対象世帯リストを作成した。貧困削減プログラムのリストが利用不能の場合 は、選択された村を調査チームが訪れた際に、EPI リストまたは関連する居住者リストを使 用した。紙のくじ引き法を用いて、21 組の母子が無作為に選択された。

質問票と血液採取に要する時間は一組あたり 20 分と予測され、また村内および村間の移動は困難であることが多いので、1 調査チームあたり 1 日に 6 組が適当であると考えられた。各チーム 2 名の調査員からなる 24 の調査チームが組織された。

◘ データ収集

血液サンプルは、訓練を受けた調査員が、選択された村において指尖を穿刺し採取した。 血液採取には、安全ランセットとガラス毛細管を用いた。Determine® 簡易試験を行うには、約 50 μ l の血液が必要である。

調査では、参加者の性、年齢と生年月日、居住地、社会的要因、肝炎の家族歴、予防接 種歴および B 型肝炎感染の潜在的危険因子を確認する質問票を用いた。

選択されなかった村で予備調査を実施した後、本調査直前に調査員に対する 2 日間の研修が行われた。研修には国レベル・県レベルの監督者も参加した。研修講師は、国家予防接 種課 (NIP) と国立検査疫学センター (NCLE) から採用した。

◘ D**etermine® を用いた HBsAg 検出**

Determine®(Arlier, Japan) を用いて HBs 抗原を測定した。この検査は、血液を検体パッドに載せた後に追跡液を加えると、15 分 ~24 時間後に、結果を読むことができる、というものである。

◘ データ入力と分析

全情報をエクセルファイルシートに入力し、データに誤りが無いように二重チェックを行った。STATA ver. 12.0 (Stata Corp., College Station, Tx, USA) により、結果を分析し た。

◘ 倫理

リスクを最小にするために、新品の使い捨て安全ランセットを用いて血液サンプルを採 取した。監督者と調査員は、研修会で訓練を受けた。調査員は製造者の説明書に従い、各 参加者に対してゴム手袋を使用した。使用の直後に、すべての安全ランセットと綿球をセーフティボックスに回収した。

調査を実施する前に、地元当局 (村のリーダーと地域の女性同盟の会長) および選出さ れた小児の両親または保護者は、書面または口頭による説明を受けた。ラオスの農村

部で は母親の 70% 以上は読み書きができないので、説明に際しては特に注意が払われた。

参加者の情報は匿名とし、機密を保持した。各参加者には識別番号 (ID) を付け、質問 票と血液検体同一の識別番号を記載した。

HBs 抗原の測定結果は、要求があれば母親に知らせた。調査チームは、結果を知らせる 前に以下の点を考慮した :1) ラオスではウイルス性 B 型肝炎は治療可能ではないこと、2) Determine® は個別の診断目的に使用されるのではなく、疫学的研究のために使用されるキ ットであること、3) 調査チームが B 型肝炎について詳細に説明しても、地域の信条により HBs 抗原陽性者は村民から差別を受ける可能性があること。

◘ 調査の実施

B 型肝炎有病率調査は、国、県、郡および村のレベルで準備・組織され、遂行・実施さ れた。調査の実施における各段階について以下にまとめる。

◘ 倫理的承認

NIP、NCLE および NCGM は、世界保健機関 (WHO) 西太平洋地域事務所およびラオ ス事務所からの支援を得て、調査の理論的根拠、方法論、必要な情報、財政的支援、必要な人的資源について検討した。研究グループは調査プロトコルを作成し、ラオス保健省お よび日本の NCGM の倫理委員会に提出した。ラオス保健省の倫理委員会は調査プロトコル を 2011 年 1 月 20 に承認し、NCGM の倫理委員会は 2011 年 1 月 6 日 (NCGM-950) および 2012 年 1 月 10 日 (NCGM-G-001130-00) に調査を承認した。

◘ 調査地への公式の要請状と候補者リストの収集

村の選定後、保健省の職員は、関連する県、郡および村へ公式の要請状を送った。事 前に完全な居住者リストは入手できなかったため、紙のくじ引き法を用いた選出が考慮さ れた。

◘ 調査参加者の選出

各村から提供されたリストに基づき、参加する 21 例の小児を NIP のスタッフが無作為に選出した。具体的には 1~250 の番号が付けられた 20 × 2cm の細長い長方形の紙片 21 枚を無作為に封筒から引いて 21 組の母子を選ぶという、紙のくじ引き法を用いて母子のペアを選出 した。各調査チームは、250 枚の紙片を入れた封筒を 1 つずつ保有した。

選定された村に充分な数の小児がいない場合は、調査チームは郡のセンターへ戻る途中 の最も近い村を選び、居住者の合同リストを作成して、同じ選出過程を取った。

◘ 監督者と調査員の研修

調査実施にあたり、国レベルの監督者 11 名 (NIP から 6 名、NCLE から 5 名)、県レベルの 監督者 13 名および現地の調査員 48 名を採用した。調査員の職種は、主に検査技師・疫学スタッフであった。国レベルの監督者は、1 つまたは 2 つの県を監督し、調査員からの説明 や質問に対応した。

監督者と調査員に対する 2 日間の研修において、安全ランセットを用いた指尖穿刺、血液採取と Determine® の結果の読み方、質問票調査の方法を訓練した。研修には、B 型肝炎に対する国の政策、ID システム、倫理的問題、および機密保持も含まれた。村における無作 為選出を確実にするために、紙のくじ引き法の使用が重視された。

◘ 材料の準備

調査を実施する前に、NIP スタッフは各参加者の ID システムを作成した。識別番号にはクラスターコード (県番号 - 郡番号 - 村名) と個人コード [01 から 21 までの 2 桁の数字 +C (小児の場合) または M(母親の場合)] が使われた。各 ID は、質問表用紙に記入され、Determine® 簡易検査でも使

用された。すべての調査物品はクラスターごとにまとめて包装され、調査地へ出発する前に各調査チームに配られた。

◘　現地での検査の実施

血液採取と質問票調査ののち、お礼の粗品 (菓子の包み、文房具など) を手渡した。参 加者やその妹・弟がのどに詰まらせる可能性があるので、あめ玉は避けた。監督者と調査 員は、毎日最後に質問票と血液検査結果を検証した。

データ収集は、移動時間を除き 1 月 25 日から 2 月 4 日まで行い、2 週間以内に完了した。デ ータ入力はラオスと日本で行われた。

3. 結果

調査チームは、道路事情の為に到達できなかった一村を除いて、選別された 48 村全てを 訪問する事が出来た。訪問できなかった一村の代替として、既定の選別基準に従い別の村 が選別された。調査は順調に進み、1008 名の小児と 1008 名の母親の検体とデータを収集 する事が出来た。最終的な回答率は 100% であった。しかし、43 組の母子は年齢基準に当 てはまらず分析から除外された。43 組の内訳は、4 名の小児が 9 歳以上、30 名が 5 歳未満、5 名の母親が 45 歳以上、4 名の母親が 15 歳未満であった。その結果、965 組の母子のデー タが HBs 抗原陽性率の計算に用いられた。

965 名の内、17 名の小児 (1.8%) と 28 名の母親 (2.9%) が HBs 抗原陽性であった。下記の表は、サンプリング・デザインと標本荷重を加味した、母子の HBs 抗原陽性率であ る。3 回目の DPT-HepB ワクチン接種率による階層 (高接種率、低接種率) によって分け た陽性率も下表に示す。

母児の B 型肝炎抗原陽性率 (小児 ;5-9 歳、母親 ;15-45 歳)

表 . 全国母児 B 型肝炎抗原陽性

年齢	B 型肝炎抗原陽性	95% C.I.	デザイン効果
小児 (n=965)	1.7%	0.8-2.6%	1.1
母親 (n=965)	2.9%	1.7-4.2%	1.3

表 . 3 回目の DPT-HepB 接種率による階層別 HBs 抗原陽性率

年齢	B 型肝炎抗原陽性率	95% C.I.
小児、低接種率 (n=479)	2.3%	1.0-3.6
小児、高接種率 (n=486)	1.2%	0.2-2.2
母親、低接種率 (n=479)	1.9	0.7-3.1
母親、高接種率 (n=486)	3.7	2.0-5.4

表 . 背景因子による HBs 抗原

因子	内訳	小児		母親	
		HBs 抗原陰性	HBs 抗原陽性	HBs 抗原陰性	HBs 抗原陽性
母親の年齢	<=19	4	0	4	0
	20-24	85	1	82	3
	25-29	294	7	286	8
	30-34	275	6	266	9
	35-39	176	3	173	3
	40-45	131	0	127	4
人種	低地ラオ族	642	9	632	19
	中地ラオ族	242	6	243	5
	高地ラオ族	62	2	61	3
最寄りの保健センターまでの交通手段	徒歩	297	1	292	6
	自転車	14	0	14	0
	バイク	357	7	354	10
	車	178	5	177	6
	トラクター	63	3	62	4
	その他	14	0	14	0

因子	内訳	小児		母親	
		HBs 抗原陰性	HBs 抗原陽性	HBs 抗原陰性	HBs 抗原陽性
最寄りの保健センターまでの所要時間 (分)	0-4	31	0	30	1
	5-14	271	3	268	6
	15-29	226	5	220	11
	30-59	204	5	205	4
	60-480	153	3	152	4
母親の教育レベル	小学校修了せず	300	7	295	12
	小学校修了	369	5	364	10
	中学校修了	182	3	183	2
	高校修了	73	0	72	1
	大学修了	19	1	18	2
世帯主の職業	農夫	670	13	664	19
	漁夫	5	0	5	0
	労働者	91	1	87	5
	公務員	87	1	85	3
	工場勤務	8	0	8	0
	一般勤務	15	1	16	0
	商人	62	1	63	0
	その他	8	0	8	0
母親の手術歴の有無	有り	93	2	93	3
	無し	852	15	843	24
小児の性別	男児	479	7		
	女児	465	9		
分娩場所	県病院	203	4	201	6
	郡病院	103	2	100	5
	保健センター	10	0	10	0
	プライベート診療	11	0	10	1
	自宅	561	8	555	14
	森	53	3	55	1
	その他 (施設)	3	0	3	0
小児の手術歴の有無	有り	22	0		
	無し	922	16		

4. 考察と結論

◘ 考察

1. 調査の実施

血清 B 型肝炎有病率調査が成功裏に終了したのには下記の理由があった。

1) ラオス保健省が調査実施に強力にコミットしてくれた。
2) 全ての行政レベルにおいて、コミュニケーションと協力体制が円滑であった。
3) 村長をはじめとする地域の重要人物、保健ボランティアが調査に関与してくれた。
4) 2011 年の中部地域におけるパイロット調査を参考にする事が出来、調査チームが非常に よく準備されていた。

2. 母子の HBs 抗原陽性率

調査から得られた一般人口の推定 HBs 抗原陽性率は、小児においても、母親においても、近隣諸国およびラオスで報告されているよりもかなり低値であった [5, 6, 7, 8]。その原因と して、幾つかの理由が考えられた。

1) ラオスの人口密度は、近隣諸国と比較して低く、その為人と人との接触の機会が少ない。 それに加え、道路・鉄道・航空等の設備が整っておらず、ウイルスの伝播の機会が少ない。文化や人の行動の違いも、低い陽性率に影響を与えていると考えられる。
2) ラオスから以前発表された報告は、全人口を正確に代表していると云えないものが多か った。例えば、2 つの先行研究 (献血者 [9] と入院患者 [10]) では、都市部における高い罹患 率を示していたが、標本が一般人口を代表しているとは考えにくい。

3. 可能性のある危険因子

今回の調査は、母親の B 型肝炎感染状態以外には、児の感染状態に関する有意差のある危 険因子を同定できなかった。歯ブラシの共有、手術歴、母親の教育レベル、そして、民族 が、それぞれ独立して、感染状態と関連していたという報告がある [11, 12, 13, 14]。児の B 型肝炎感染状態と有意差のあるリスク因子を検出できなかった理由は明確ではないが、恐 らく、陽性例が少なすぎるせいではないかと思われた。今回の調査の第一の目的は、B 型肝 炎抗原陽性率を見る為であり、リスク因子の抽出は、一義的なものではない。また、HIV 陽性例に関して、B 型肝炎罹患が陽性に関連しているとする報告があるが、今回の調査では、予算も限られており、HIV を検索することはできなかった。

4. 世界保健機構 (WHO) 西太平洋地域の目標

WHO の B 型肝炎罹患に関する暫定目標は、2012 年までに 5 歳以上児の罹患率を 2% 未満 にするというものである (WPR/RC56.R8)。B 型肝炎コントロールの評価の為に点推定が 使われている。この基準に照らしてみると、ラオスは既に目標に達している事になる [7, 8]。 しかし、ラオスは WHO 西太平洋地域でワクチン接種率が最も低い国であるであることか ら、予防接種プログラムのみで目標を達成したとは考えにくい。以前の報告と比較して母
親の HBs 抗原血清陽性率が比較的低い事から、B 型肝炎予防接種プログラム導入前から既 に有病率は低かった事が示唆される。それゆえ現行の予防接種政策を単純に続けるだけで は、最終目標である 5 歳以上児の HBs 抗原陽性率を 1% 以下に抑えるのは難しいのではな いかと思われる。

◘ 推奨

1. 全国予防接種プログラムを評価する為には、HBs 抗原陽性率調査を繰り返す必要がある。 次回の調査時は、最新の人口データを使用する必要がある。
2. 次回調査時は、調査対象者として、下記を網羅することを推奨する
   1) 母親 ; 垂直感染の原因となる為

2) 父親 ; 児への水平感染の原因となる可能性がある為
3) 社会的弱者 (移民、コマーシャル・セックスワーカー); 彼らは非登録人口であるが、一般人口よりも高い有病率を示す可能性がある為。データ収集の際にはサンプリン グ法に特別な注意を払う必要がある (oversampling methodology の利用等)。

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資料

資料は巻末に収録（英語版に共通）

資料 1.　調査の為に選別された郡と村
資料 2.　質問票
資料 3.　インフォームド・コンセント・フォーム
資料 4.　研修内容
資料 5.　監督者と調査員
資料 6.　掲載論文

Hepatitis B Prevalence Survey in Lao PDR

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Department of Virology II

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Capacity Development for Sector-wide Coordination in Health-Phase2
Strengthening Integrated Maternal, Neonatal and Child Health Services
Sustainable Development of Human Resource for Health to Improve Maternal, Neonatal and Child Health Services

World Health Organization
Regional Office for the Western Pacific
Lao PDR office

Acronyms

C.I.	Confidence interval
DPT-HepB 3	Diphtheria, pertussis, tetanus, hepatitis B vaccine the third dose
EPI	Expanded Programme on Immunization
HB	Hepatitis B
HBsAg	Hepatitis B surface antigen
JICA	Japan International Cooperation Agency
MoH	Ministry of Health, Lao PDR
NCGM	National Center for Global Health and Medicine
NCLE	National Center for Laboratory and Epidemiology, MoH
NIP	National immunization Program, Maternal and Child Health, MoH
PPS	Probability proportionate to size
SBA	Skilled birth attendance
VPD	Vaccine preventable diseases
WHO	World Health Organization

Executive Summary

To assess current hepatitis B prevalence among general population and the impact of hepatitis B vaccine introduction in Lao PDR, Ministry of Health, Lao PDR, and NCGM planned a serology and questionnaire survey. The survey, which covers the whole country, was completed in February 2012. This report summarizes the results of the survey.

Objectives

Primary objectives of the survey are summarized below.

1. To estimate hepatitis B surface antigen sero-prevalence among children
2. To estimate hepatitis B surface antigen sero-prevalence among mothers
3. To evaluate the potential risk factors

Target ages, areas, and time

Ages	Children	Five to nine year-old
	Mothers	15 to 45 year-old
Areas	Whole country	
Time	January and February, 2012	

Survey methodologies

All 143 districts in Lao PDR were stratified into two categories; high and low third DPT-hepatitis B immunization coverage reported in 2010. 12 districts from each stratum were selected by probability proportionate to size (PPS) sampling, and then two villages were selected from each district by PPS. In each village (cluster), surveyors randomly selected 21 child-mother pairs from resident lists. After explanation of survey purposes, methods, confidentiality policy, and obtaining informed consent, questionnaire and blood sampling were conducted. Blood samples were tested HBsAg using Determine® rapid test.

Results

A total of 2,016 blood samples (1,008 children and 1,008 their mothers) were collected and found to have hepatitis B virus prevalence of 1.7% (95%C.I.=0.8, 2.6) for children between five to nine years old and 2.9% (95%C.I.=1.6, 4.2) for their mothers with child bearing age. Mother's infection status was positively associated with their children's HBsAg positivity, while other potential risk factors (mother's age, ethnicity, time to the nearest health center, level of mother's education, and medical history) were not.

Discussion and recommendations

This is the first nationwide population-based hepatitis B sero-prevalence survey in Lao PDR. The prevalence results of the survey were lower than the results from surrounding countries. The major reasons of differences are unclear. Hepatitis B birth dose and three vaccinations during infancy remain important preventive strategies; therefore, careful monitoring and repeated evaluation for national immunization program are needed.

1. Introduction

Background

More than two billion people have been infected with hepatitis B (HB) virus worldwide and every year approximately one million people die of HB virus infection, 33 % caused by hepatocellular carcinoma, and the rest with terminal complications of liver diseases [1, 2]. The Regional Committee for the World Health Organization (WHO) Western Pacific adopted the resolution WPR/RC54.R3 in 2003 and resolution WPR/RC56.R8 in 2005, calling for the reduction of 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. Regional progress in hepatitis B control strategies has resulted in a dramatic decline in hepatitis B infection among children, and 27 countries and areas are expected to achieve the milestone (WPR/RC61/10).

Lao People's Democratic Republic (Lao PDR) has initiated its expanded programme on immunization (EPI) in selected regions in 1984. The program has scaled up to nationwide in 1994. Hepatitis B has included in EPI in 2002, and gradually expanded (**Table 1**). Study from neighboring countries, such as Cambodia, China, Myanmar, Thailand, and Vietnam, revealed high HB antigen prevalence [3, 4, 5, 6], but there have been no previous reports of chronic HB virus infection rates among general population of Lao PDR. Therefore, population-based sero-prevalence survey for HB is necessary to understand the current situation and to evaluate the progress of immunization policy targeting prevention for mother to child transmission [7, 8].

The Ministry of Health (MoH), Lao PDR and National Center for Global Health and Medicine (NCGM) have agreed that sero-prevalence is investigated. In January and February 2011, the team focuses on central region of the country as the pilot survey, and the whole country was covered in January to February 2012.

Table 1. Hepatitis B vaccination activities in Lao PDR

2002	DTP-HepBDTP-HepB as routine immunization
2004	Birth dose introduced in Capital hospitals
2005	2 southern province hospitals added
2007	8 more provincial hospitals added
2008	Remaining provincial and all 123 district hospitals added
2009	Started Hepatitis B birth dose home visits using health center workers in 50 districts from 9 provinces
2010	Training of skilled birth attendants (SBA) who can work both in facilities and attend births at home

Survey objectives

Primary objectives of the survey are;

1. To estimate hepatitis B surface antigen sero-prevalence among children
2. To estimate hepatitis B surface antigen sero-prevalence among mothers
3. To evaluate the potential risk factors

2. Methods

Sample size calculation

Considering desired level of confidence measure of 1.96, margin of error of 0.02, expected HBsAg positive rate of 0.05, design effect of 2.0, with two strata, and response rate of 0.95, we calculated required sample size of 961, which indicates 961 pairs of mothers and children (1,922 individuals). For practical purposes, 1,008 pairs of children and mothers (2,016 individuals) are planned to be collected.

Sampling strategies

Stratified multi-stage random cluster sampling was used to select pairs of children and mothers with child bearing age.

Lao PDR comprises Vientiane capital and 16 administrative provinces in the country. The country includes 143 districts and more than 10,000 villages according to census 2005. We divided all districts into two strata in terms of DPT-HepB3 (diphtheria, pertussis, tetanus, and hepatitis B third dose) immunization coverage; high (72 districts >=76%) and low (71 districts <76%). Twelve districts were randomly selected from each stratum, with applying probability proportionate to size (PPS) sampling as the first stage. For the second stage, two villages were randomly selected from each district by PPS. In each of 48 villages, household list was made based on residents list by poverty reduction program. When poverty reduction program's list was not available, EPI list, or relevant residents list was used when survey team visits selected village. 21 child and mother pairs were randomly selected using a paper-based lottery system.

Since questionnaire and blood collection time is expected as 20 minutes per pair, and transportation is often difficult in and among villages, six pairs per day per survey team were considered to be appropriate. Twenty four survey teams were organized, and each team had two surveyors.

Data collection

Blood samples were taken by trained surveyors using finger-prick method in selected villages. Safety lancet® and glass capillary tube was used for taking blood. Approximately 50 micro liter of blood are needed to apply Determine rapid test.

The survey included a brief questionnaire to verify the participants' sex, age and date of birth, place of residence, sociodenographic characteristics, family history of hepatitis, vaccination history, and potential risk factors of getting hepatitis B.

After pre-testing in a non-selected village, two-day training session for supervisors and surveyors was held just before the survey. The national advisors attended the session, too. The lecturers were recruited from NIP and NCLE, MoH, Lao PDR.

HBsAg detection using Determine®

HBsAg was assayed using **Determine® (Arlier, Japan)**. Briefly explaining, the blood apply onto the sample pad, followed by the chase liquid. Fifteen

minutes to 24 hours later, the results can be read.

Data entry and analysis

All the information was input into the excel file sheets. Data was validated by testing double-entry checking.

The results were analyzed by STATA ver. 12.0 (Stata Corp., College Station, Tx, USA).

Ethical considerations

To minimize risks, each blood sample collection used a new, disposable lancet. Supervisors and surveyors were trained during training session. Surveyors followed manufacturer instructions and used a pair of latex gloves for each child. Immediately after use, all lancets and cotton balls were placed in safety boxes.

Before conducting the survey, local authorities (village leaders and the presidents of local Women's Union) and the parents or guardians of selected children received oral and written information. Special attention was paid for this explanation, as more than 70% of mothers would be illiterate in rural Lao PDR.

Participant information was remained anonymous and confidential. Each participant has identification number, which is common between questionnaire and blood sample.

The HBsAg results were informed to mothers on request. Survey teams considered following issues before giving results; 1) Viral hepatitis is not curable in Lao PDR, 2) Determine® is not used for individual diagnostic purposes, but for epidemiological research, 3) HBsAg positive persons may be discriminated by villagers according to local beliefes, even survey teams give detailed explanation about hepatitis B.

Survey implementation

The hepatitis B survey was prepared, organized, executed and implemented at national, provincial, district, and village levels. The steps involved in survey implementation are summarized as follows.

Ethical approval

NIP, NCLE, and NCGM had discussion of rationale, methodology, necessary information, funding, and human resources required for the survey with assistance from WHO (WPRO and Lao PDR) offices. The study group developed the survey protocol and submitted to ethical committees in MoH, Lao PDR and in NCGM, Japan. The ethical committee in MoH, Lao PDR approved the survey protocol in January 20, 2011, and the NCGM ethical committee approved the survey in January 6, 2011 (NCGM-950) and January 10, 2012 (NCGM-G-001130-00).

Official request letter to survey sites and collecting lists of eligible subjects

After selection of villages, the MoH cabinet sent an official letter to the relevant provinces, districts and villages. Complete lists of the residents were not

available beforehand, therefore, selection using paper-based lottery system was considered.

Survey subjects selection

Based on the lists provided by each village, NIP staff randomly selected 21 children for participation. Child and mother pairs were randomly selected using a paper-based lottery system in which 21 rectangular strips of paper, measuring 20 x 2 cm and numbered from 1 to 250, were randomly drawn from an envelope to select 21 child and mother pairs. Each survey team had one envelope containing 250 strips of paper.

When a selected village lacked sufficient number of children, the survey team selected the nearest village on the way back to district center, made combined list of residents, and followed the same selection process.

Training supervisors and surveyors

The study team recruited 11 national advisors (six from NIP and five from NCLE), 13 provincial supervisors, and 48 surveyors in the field. The surveyors' background were mainly laboratory and epidemiology staff. National advisors supervised one or two provinces, and responded to clarifications and questions from the surveyors.

Two-day training for both supervisor and surveyor provided finger-prick using safety lancet, blood collection and Determine® result reading, and taking questionnaire. The training also included national policy against hepatitis B, coding system, ethical issues, and confidentiality.To ensure random selection in villages, using a paper-made lottery sysytem was emphasized.

Preparing materials

Before conducting the survey, NIP staff prepared the coding system for each participant. Coding included a cluster code (province number-district number-village name) and a personal code (two digits ranging from 01 to 21 plus 'C' for children and 'M' for their mothers). Each code was written on the questionnaire sheets, and Determine® rapid test. All survey materials specific to each cluster were packaged together with. Each survey team received these packages of materials before departing to survey sites.

Conducting serology in the field

After the blood taking and questionnaire, a small gift was handed (a packet of confectionary, stationery materials, and so on), and hard candy was avoided, as participants' younger sibrings may have possibility of suffocation. At the end of each day, the supervisors and surveyors verified the questionnaire and blood test results.

The data collection was carried out from January 25th to February 4th excluding transportation time, and completed within two weeks. Data input was conducted in Lao PDR and in Japan.

3. Results

The survey teams successfully visited all 48 selected villages except one village, which could not be approached because of difficult road condition. An alternative village was chosen according to predetermined selection criteria. Data collection was successfully carried out and sampled 1,008 children and 1,008 their mothers. The overall response rate was 100%; however, 43 pairs were excluded from the analysis due to age ineligibility. That is, four children were over 9 years of age and 30 were less than 5 years of age.Moreover, five mothers were over 45 years of age and four were less than 15 years of age. A total of 965 pairs were included for the prevalence calculation.

Seventeen out of 965 children (1.8%) and 28 out of 965 mothers (2.9%) showed positive for HBsAg. The table below shows the overall HBsAg prevalence among children and their mothers after taking the sampling design and sampling weight for each individual into account. HBsAg prevalence in each age gourp by strata (high and low DPT-HepB 3 coverage) is also presented.

HBsAg prevalence among children (5 to 9 years old) and their mothers (15 to 45 years old)

Table. National HBsAg prevalence among children and their mothers

Ages	HBsAg prevalence	95% C.I.	Design effect
Children (n=965)	1.7%	0.8-2.6%	1.1
Mothers (n=965)	2.9%	1.7-4.2%	1.3

Table. HBsAg prevalence among children and their mothers by DPT-HepB 3 coverage

Ages	HBsAg positive rate	95% C.I.
Children, low coverage (n=479)	2.3%	1.0-3.6
Children, high coverage (n=486)	1.2%	0.2-2.2
Mothers, low coverage (n=479)	1.9	0.7-3.1
Mothers, high coverage (n=486)	3.7	2.0-5.4

Table . HBsAg distribution by background characteristics

Factors	Value	Children		Mothers	
		HBsAg negative	HBsAg positive	HBsAg negative	HBsAg positive
Mother's age	<=19	4	0	4	0
	20-24	85	1	82	3
	25-29	294	7	286	8
	30-34	275	6	266	9
	35-39	176	3	173	3
	40-45	131	0	127	4
Ethnicity	Lowland Lao	642	9	632	19
	Midland Lao	242	6	243	5
	Highland Lao	62	2	61	3
Transportation to the nearest health facility	Walk	297	1	292	6
	Bicycle	14	0	14	0
	Bike	357	7	354	10
	Car	178	5	177	6
	Tractor	63	3	62	4
	Others	14	0	14	0

Factors	Value	Children		Mothers	
		HBsAg negative	HBsAg positive	HBsAg negative	HBsAg positive
Time to the nearest health facility(min)	0-4	31	0	30	1
	5-14	271	3	268	6
	15-29	226	5	220	11
	30-59	204	5	205	4
	60-480	153	3	152	4
Mother's education level	Primary school not finished	300	7	295	12
	Primary school finished	369	5	364	10
	Junior high school finished	182	3	183	2
	High school finished	73	0	72	1
	College or university finished	19	1	18	2
Family head occupation	Farmer	670	13	664	19
	Fisherman	5	0	5	0
	Labour	91	1	87	5
	Public officer	87	1	85	3
	Factory employee	8	0	8	0
	General Employer	15	1	16	0
	Merchant	62	1	63	0
	Others	8	0	8	0
Mother's surgical operation	Yes	93	2	93	3
	No	852	15	843	24
Child's sex	Male	479	7		
	Female	465	9		
Place of delivery	Province hospital	203	4	201	6
	District hospital	103	2	100	5
	Health center	10	0	10	0
	Private clinic	11	0	10	1
	House	561	8	555	14
	In bush	53	3	55	1
	Others(facility)	3	0	3	0
Child's surgical operation	Yes	22	0		
	No	922	16		

4. Discussion and Recommendations

Discussion

1. Implementation of the survey

HBsAg sero-prevalence survey was successfully completed because of variety of reasons.

1) The MoH, Lao PDR strongly committed to conduct the survey.
2) Communication and coordination was well established in all levels of work.
3) Local authorities and health volunteers are well involved.
4) Survey team was very well prepared, because they learned a lot from the pilot study targeting central region of the country in 2011.

2. HBsAg prevalence among children and their mothers in child bearing ages

The estimated HBsAg prevalence of the general population was much lower in both children and adults than that of previous reports from neighboring countries and Lao PDR [3, 4, 5, 6]. There are several potential explanations for these observations.

1) Lao PDR's population density is lower than that of surrounding countries, and thus, human contact is less frequent. Additionally, road, railway, aviation, and related infrastructure are less developed in Lao PDR, and thus, there is less chance for spread of viruses. Cultural, and behavioral differences may contribute to the lower prevalence.

2) The majority of the previous surveys did not adequately represent the entire population of the country. For example, previous two studies (blood donors [9] and the hospitalized patients [10]) revealed high prevalence of hepatitis B in urban areas of Lao PDR, however, the sampled individuals did not represent the general population.

3. Potential risk factors

The survey revealed that no potential risk factors were significantly associated with the children's infection status, with the exception of the mothers' hepatitis B infection status. Previous HBsAg prevalence studieys revealed that toothbrush sharing, history of surgery, level of mother's education, and ethnicity were independently associated with hepatitis B infection [11, 12, 13, 14]. The reason why we could not find any potential risk factors having a positive association with hepatitis B infection among children is not clear, but may be due to the small number of positive cases. However, it should be noted that the primary objective of the present study was to assess HBsAg prevalence, and not its risk factors. Additionally, some reports found that HIV positive individuals are positively associated with hepatitis B virus infection; however, we did not investigate HIV due to limited budget.

4. WHO's regional target

The interim target of the WHO is to reduce HBsAg prevalence to less than 2% in children aged at least 5 years old by 2012 (WPR/RC56.R8). The point prevalence is used for monitoring the control

of hepatitis B. Following these criteria, Lao PDR had already achieved its goal [7, 8]. However, it is unlikely that Lao PDR had achieved the target through the immunization program alone because the country has the lowest immunization coverage of all countries in the region. Considering the relatively lower HBsAg seroprevalence among the mothers compare to those reported in previous studies suggests that the country has a lower prevalence even before the introduction of the hepatitis B immunization program. Therefore, the final target of reducing HBsAg prevalence to less than 1% in children aged at least 5 years could be difficult to achieve if the country simply continues its current immunization policy.

Recommendations

1. To evaluate the progress of national immunization programs, the HBsAg prevalence survey should be repeated. The next survey should utilize the latest census data.

2. When conducting the next survey, we recommend that the target population include;
   1) mothers, because they are the source of vertical transmission
   2) 2) fathers, because they may be the source of horizontal transmission of hepatitis B virus to children
   3) marginalized population, such as floating immigrants or commercial sex workers, because they are often not registered, and may have higher prevalence of hepatitis B than general population. Special attention should be paid to collect data from them, such as oversampling methodology.

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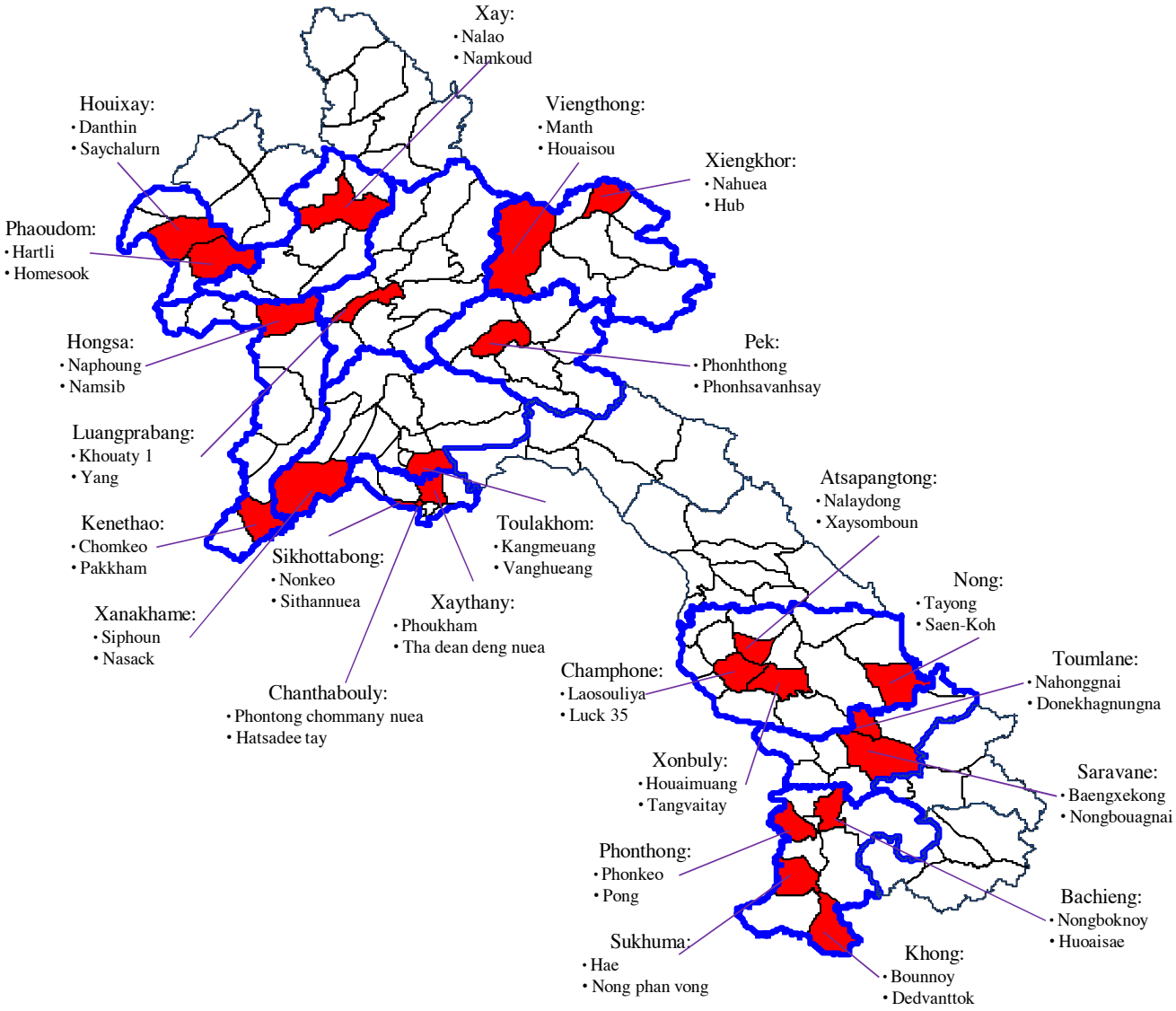
資料

Annexes

Selected Districts and villages



Questionnaire for mother and child to evaluate the result of giving service for preventing the spread of Hepatitis B

Questionnaire ID	Date (D/M/Y)	Interviewer

Remarks: The objective of this questionnaire is to collect the information on improving health care services giving to mother and child for prevention of Hepatitis B. This is confidential, so we will not record your name. This questionnaire will be destroyed after data entry into the computor. To investigate the information, please answer correctly acccording to your knowledge.

I. General information (for mother*)
*age 15-45 years old

No.	question	answer	remark	code
Q101	Address	village_____ district_____ province_____		
Q102	Date of birth and age (mother)	(D/M/Y) ____/____/____ ___years old		[ ]
Q103	Ethnicity:	(select only one) 1. Lowland lao 2. Midland lao 3. Highland lao		[ ]
Q104	Do you use vehicle when you go to the nearest health facility? (provincial hospital, district hospital, clinic, health center). If yes, what kind of vehicle do you use?	(Select only one) 1. walk 2. bicycle 3. motor bike 4. car 5. hand tracter 6. others: specify_____		[ ]
Q105	How long does it take to the nearest health facility?	_____/_____ hours / minutes		
Q106	Which level did you finish your study? (for mother)	(Select only one) 1. no education 2. finished primary school 3. finished junior high school 4. finished high school 5. finished college / university 6. others specify_____		[ ]

Questionnaire ID

Q107	What is the occupation of the head of the family ?	(Select only one) 1. farmer (dry field or paddy) 2. fisherman 3. labor 4. public officer 5. factory employee 6. general employer 7. merchant 8. others specify _____		[ ]
Q108	Have you ever received blood transfusion?	(Select only one) 1. yes 2. no 3. do not know		[ ]
Q109	How many times have you had surgical operation? (including minor surgery, cesarean section, etc)	1. never 2. once 3. twice or more 4. do not know		[ ]
Q110	Is there anyone in your family who has liver disease or died from liver disease? (ex:jaundice of eye and body)	1. none 2. I have 3. Husband has or died from a liver disease 4. parent has or died from a liver disease 5. brother or sister has or died from a liver disease 6. do not know		Yes=1, No=0 [ ] [ ] [ ] [ ] [ ] [ ]
Q111	How many children do you have?	Number of children [ ]		[ ]

Questionnaire ID

I. Questions for a child
Choose the youngest child in the group of age between 5 to 9 years old

No.	question	answer	remark	code
Q201	Date of birth and age (child)	(D/M/Y) ____/____/____/ years old		
Q202	Sex of the child	(Select one) 1. male 2. female		[ ]
Q203	Where was this child born?	(Select only one) 1. provincial hospital 2. district hospital 3. health center 4. private clinic 5. house 6. in bush near house 7. other place specify: _____		[ ]
Q204	Why did you select the place to give birth?	(Choose all it apply) 1. feel safe 2. more convinient 3. more economical 4. family suggested 5. traditional birth attendant suggested 6. health center or hospital staff suggested 7. could not go to the hospital because the child was born too quickly 8. that has been the custom of the community 9. others specify _____		Yes=1, No=0 [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]

Questionnaire ID

Q205	Who attended or helped the delivery of this baby?	(Choose all it apply) 1. medical staff 2. village health volunteer 3. TBA 4. family member 5. no one 6. others specify _____		Yes=1, No=0 [ ] [ ] [ ] [ ] [ ] [ ]
Q206	Where did your child recieve vaccination?	(Select only one) 1. hospital 2. health center 3. in the village with medical staff 4. private doctor 5. did not recieve 6. do not remember 7. others specify _____		[ ]
Q207	How do you know about vaccine? Through what media or people do you get information about vaccine?	(Choose all it apply) 1. medical staff told you 2. it is wirtten on the vaccination note 3. brothers/sisters or friend told you 4. Radio / TV 5. local authority told you 6.others specify _____ 7. do not know		Yes=1, No=0 [ ] [ ] [ ] [ ] [ ] [ ] [ ]
Q208	Has the child ever received blood transfusion?	(Select only one) 1. yes 2. never 3. do not know		[ ]
Q209	How many times has the child had surgical operation?	(Select only one) 1. never 2. once 3. twice or more 4. don't know		[ ]
Q210	Has the child ever shared toothbrush with family members?	(Select only one) 1. yes, often 2. yes, sometimes 3. yes, but very rare 4. never 5. don't know		[ ]

Questionnaire ID

II.Other information related to immunization

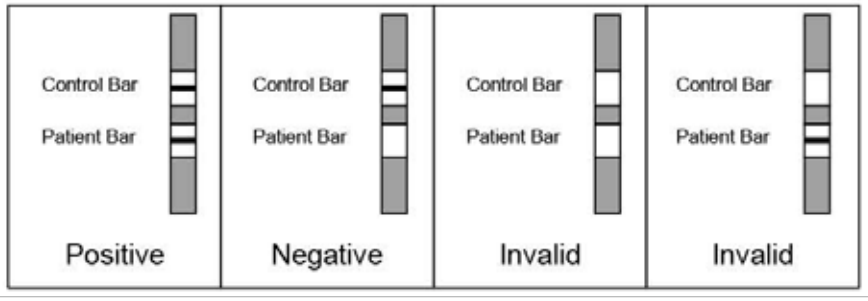
No.	question	answer	remark	code
Q301	Do you have vaccination card (yellow) or mother and child handbook? (for the child who was surveyed)	(Select only one) 1. have both 2. have only yellow card 3. have only mother and child handbook 4. have neither		[ ]
Q302	Usually who is the one to decide about giving vaccination?	(Select only one) 1. father 2. mother (yourself) 3.grandfather/ grandmother 4. village leader 5. others Specify_____		[ ]
Q303	How do you know the date that health center staffs come to give vaccination?	(Choose all it apply) 1. village leader 2. village health volunteer 3. woman's union 4. megaphone 5. advertisement poster 6. health center staff 7. official letter from the district governer 8. others specify _____		Yes=1, No=0 [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]

Check the child's Yellow Card, and record the dates of immunization below:

1.	BCG 0 – 11 months ...../...../..... D/M/Y	Hep B 0 – 24 hours ...../...../..... D/M/Y
2.	DPT-Hep B1 Minimum 6 weeks from 1 ...../...../..... D/M/Y	Polio1 Minimum 6 weeks from 1 ...../...../..... D/M/Y
3.	DPT-Hep B2 1 month after DPT-HepB1 ...../...../..... D/M/Y	Polio2 1 month after Polio1 ...../...../..... D/M/Y
4.	DPT-Hep B3 1 month after DPT-HepB2 ...../...../..... D/M/Y	Polio3 1 month after Polio2 ...../...../..... D/M/Y
5.	Measles	9 – 11 months ...../...../..... D/M/Y 12 – 23 months ...../...../..... D/M/Y

Record Determine results (Read Instruction carefully. Repeat test when 'Invalid')

	Determine test results	remark	code
Mother	1. positive 2. negative 3. not done		[ ]
Child	1. positive 2. negative 3. not done		[ ]



<Informed consent form for parents>

Dear parent,

1. Introduction

This research is conducted by Mother and Child Health Center, Ministry of Public Health, Lao PDR, in collaboration and agreement with NCGM (National Center for Global Health and Medicine).

2. Purpose of this research

Hepatitis B is caused by a virus called hepatitis B virus. If a virus stays in your body for a long period, virus can cause liver diseases later on. It seems that there are many people with this virus in Laos (possibly one person per 5-6 persons). It is important to prevent this virus to enter your body since it is difficult to treat once you get infected. Most people with this virus are considered to get infected from their mothers when they were born. You can avoid this disease for 95% if you get vaccinated immediately after you were born. Ministry of Health, Lao PDR has already started a vaccination programme to prevent mother to child transmission of this virus.

Ministry of Health needs information how many mothers and children have this virus in order to utilise this information to improve this programme in the future.

3. Participant selection of this research

We are inviting children (5-9 years of age) and their mothers (15-45 years of age).

4. Method of this research

We take a blood sample from your fingertip by using a safety lancet. We draw your blood from a small wound in your fingertip and put it on a diagnostic kit and a filter paper. The amount of blood we need is approximately between 0.05mL and 02 mL. All the blood taking process is done by a technician who has been trained for this research. They put a clean tape on your wound to prevent germs to get inside afterwards. We use a new safety lancet needle for each individual.

5. Confidentiality

We will ensure that your information and your child's information are kept safe and anonymously. No one except the staff involved in this research will have access to information.

6. Right to refuse or withdraw

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Refusing to participate will not give you any disadvantage. You may stop participating in the research at any time you wish. Stop to participate will not give you any disadvantage either.

The above is information about this research and we inviting you to be a part of this research. Please contact any of the members of staff if you have any further questions or queries.

The person in charge of the study

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Director
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Mother and Child Health Center
Ministry of Public Health, Lao PDR

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<Informed consent for parents>

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study and understand that I have the right to withdraw from the study at any time without in any way affecting my medical care.

Print name of participant	Signature of participant	Date(Day/Month/Year)
_____	_____	_____

If illiterate

A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participant who is illiterate should include their thumb print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of participant	Signature of participant	Date(Day/Month/Year)
_____	_____	_____

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of participant	Signature of participant	Date(Day/Month/Year)
_____	_____	_____

A copy of this informed consent form has been provided to the participant

Training agenda 2012

23rd Jan		
08:00	Registration	
08:30	Opening	Dr. Phengta, Dr. Hachiya
09:00-10:00	Purpose of WS	Dr. Anonh
10:00-10:15	Break	
10:15-10:30	Study design	Dr. Hachiya, Dr. Anonh
10:30-12:00	Household listing (Lecture and practice)	Dr. Tongchanh
12:00-13:30	Lunch	
13:30-15:00	Questionnaire (Lecture and practice)	Dr. Tongchanh
15:00-15:15	Break	
15:15-16:15	TOR of surveyors, supervisors, advisors	Dr. Phengta, Dr. Anonh
16:15-16:30	Q & A	
24th Jan		
08:30-10:30	Blood sampling (Lecture and practice)	Dr. Tongchanh
10:30-10:40	Break	
10:40-11:20	Ethical consideration	Dr Anonh
11:20-12:00	Writing the identification numbers (Items; questionnaires)	Dr Anonh
	Reporting using SMS and collecting data (Lecture and practice)	Dr Anonh
12:00-13:00	Financial and logistic issues	
13:00	Closing	Dr Phengta, Dr Hachiya

Number	Name	Roles during Survey	Position
1	Ms. Vilaphenh Yengmala	Vientiane C Supervisor	Technical Officer
2	Ms. Kham Taune Kouangvanh	Vientiane C Surveyor	Epidemiology
3	Ms. Bounteing Phommavongsa	Vientiane C Surveyor	Deputy Chief of Labo.
4	Mr. Khamsen Phanouvong	Vientiane C Surveyor	Epidemiology
5	Ms. Sengphet Douangsavay	Vientiane C Surveyor	Laboratory
6	Pengthong Keomahavong	Vientiane C Surveyor	Epidemiology
7	Ms. Lamthong Pamisay	Vientiane C Surveyor	Laboratory
8	Mr. Houmpheng Thionkeo	Oudomxay Supervisor	Epidemiology
9	Phonepaseuth	Oudomxay Surveyor	Epidemiology
10	Thongkham Keobouachanh	Oudomxay Surveyor	Laboratory
11	Ms. Ketchanh Sysavath	Bokeo Supervisor	Epidemiology
12	Mr. Khamsim	Bokeo Surveyor	Epidemiology
13	Ms. Souchitta Heuanmisavath	Bokeo Surveyor	Laboratory
14	Mr. Bounleune Sitdavanh	Bokeo Surveyor	Epidemiology
15	Thongphet	Bokeo Surveyor	Laboratory
16	Mr. Phanthaly	Luangprabang Supervisor	Epidemiology
17	Ms. Somdy	Luangprabang Surveyor	Epidemiology
18	Ms. Manisouk Phonpadid	Luangprabang Surveyor	Laboratory
19	Mr. Aiew Thong	Huaphan Supervisor	Surveillance staff
20	Mr. Phanthong Souvannaly	Huaphan Surveyor	Surveillance staff
21	Mr. Bountienne Souphanthong	Huaphan Surveyor	Epidemiology
22	Ms. Pom Keohomdy	Huaphan Surveyor	Laboratory
23	Mr. Khamphanh Keobounta	Huaphan Surveyor	Laboratory
24	Ms. Siamphone Vannithorn	Xayabouly Supervisor	Epidemiology
25	Mr. Touk Souvannasing	Xayabouly Surveyor	Laboratory
26	Dr. Souvanxay Phetthanaxay	Xayabouly Surveyor	Epidemiology
27	Mr. Thongdy Phouangkeo	Xayabouly Surveyor	Laboratory
28	Mr. Songkharm Masouvanh	Xayabouly Surveyor	Epidemiology
29	Ms. Somsanith Ounthavong	Xiengkuang Supervisor	Surveillance staff
30	Mr. Thoumphone Bounlieng	Xiengkuang Surveyor	Surveillance staff
31	Ms. Khamla Yoysaykhem	Xiengkuang Surveyor	Laboratory
32	Mr. Khounlavanh Keolakotphosy	Vientiane P Supervisor	Epidemiology
33	Ms. Manisong Vikayhong	Vientiane P Surveyor	Epidemiology
34	Ms. Bounvang Phinith	Vientiane P Surveyor	Laboratory
35	Mr. Sisouphan Davanh	Vientiane P Surveyor	Epidemiology
36	Ms. Bouasone Vilailoth	Vientiane P Surveyor	Laboratory
37	Ms. Orlathay Phongphoun	Savannakhet Supervisor	Epidemiology
38	Mr. Inpeng Nanthanontry	Savannakhet Surveyor	Epidemiology
39	Mr. Salika Kietsatit	Savannakhet Surveyor	Laboratory
40	Mr. Khampha Senviseth	Savannakhet Surveyor	Laboratory
41	Ms. Phoukhao	Savannakhet Surveyor	Epidemiology
42	Ms. Sypaseulk	Savannakhet Surveyor	Laboratory
43	Ms. Bounthan Souvannavong	Savannakhet Surveyor	Epidemiology
44	Ms. Bounta Xayavong	Savannakhet Surveyor	Laboratory
45	Dr. Laycham Chamsina	Savannakhet Surveyor	Epidemiology
46	Dr. Viengsayphone Mylounsa	Saravane Supervisor	Epidemiology
47	Ms. Souphalack Keoounheuane	Saravane Surveyor	Laboratory
48	Dad Samkham	Saravane Surveyor	Epidemiology
49	Mr. Sengdavy Syonesa	Saravane Surveyor	Epidemiology
50	Mr. Vilayvong	Saravane Surveyor	Laboratory
51	Ms. Viengsavanh Phimpiseng	Champasak Supervisor	Epidemiology
52	Mr. Khamla Souphavady	Champasak Surveyor	Epidemiology
53	Mr. Visay Xounthay	Champasak Surveyor	Epidemiology
54	Ms. Khaysy Vonvilay	Champasak Surveyor	Epidemiology
55	Ms. Phimmasone Duangvilay	Champasak Surveyor	Laboratory
56	Mr. Maly Thoubthong	Champasak Surveyor	Epidemiology

Number	Name	Roles during Survey	Position
57	Ms. Saovalith Simeuang	Champasak Surveyor	Laboratory
58	Ms. Phaiboun Chansavad	Champasak Surveyor	Laboratory
59	Ms. Manivone Bouathong	Champasak Surveyor	Laboratory
60	Dr. Darouny Phonekeo	Vientiane C National Advisor	NCLE staff
61	Dr. Anonh	Oudomxay National Advisor	EPI Director
62	Dr. Khansay Sengsaya	Bokeo National Advisor	NCLE staff
63	Dr. Virasack Somoulay	Luangprabang National Advisor	NCLE staff
64	Dr. Khamphet Louanglat	Hoaphan National Advisor	EPI staff
65	Dr. Khongxay	Xayabuly National Advisor	EPI staff
66	Dr. Souphatsone Houathougkham	Xiengkuang National Advisor	NCLE staff
67	Dr. Dasavanh Manivong	Vientiane P National Advisor	MCH staff
68	Dr. Chansay Pathammavong	Savannakhet National Advisor	EPI staff
69	Dr. Chanthavy Soulaphy	Saravane National Advisor	NCLE staff
70	Dr. Somvang Bouphaphanh	Champasak National Advisor	EPI staff
71	Dr. Phengta Vongphrachanh	Lecturer	NCLE Director
72	Dr. Bounthanom Sengkeopraseuth	Lecturer	NCLE staff
73	Mr. Khamphet	Driver	EPI staff
74	Mr. Bouavanh Boualivanh	Accounting	EPI staff
75	Ms. Bounsalong Xayasinh	Accounting	EPI staff
76	Ms. Bounphet Sisoumang	Accounting	EPI staff
77	Ms. Phailamphanh Manivong	Vientiane P National Advisor	Epidemiology
78	Ms. Vilaphanh Yengmala	Savannakhet National Advisor	Surveillance staff

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PLOS ONE

Chronic Hepatitis B Prevalence among Children and Mothers: Results from a Nationwide, Population-Based Survey in Lao People's Democratic Republic

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Abstract

Background: Hepatitis B is regarded as a serious public health issue in Lao People's Democratic Republic (Lao PDR), a Southeast Asian country. However, disease epidemiology among the general population is not well known, and thus a nationwide cross-sectional survey for hepatitis B surface antigen (HBsAg) prevalence in children and their mothers was conducted.

Methods and findings: We applied three-stage cluster sampling using probability proportionate to size. After randomly selecting child (5 to 9 years old) and mother (15 to 45 years old) pairs from the selected villages, questionnaires and HBsAg rapid tests were conducted. Data from 965 child and mother pairs were analyzed. Multivariate logistic regression analyses were used to investigate the independent association of individual background characteristics for the odds of being HBsAg positive. In total, 17 children and 27 mothers were HBsAg positive. HBsAg prevalence was estimated to be 1.7% (95% confidence interval: 0.8%-2.6%) in children, and 2.9% (95% confidence interval: 1.7%–4.2%) in their mothers after taking sampling design and weight of each sample into account. Mother's infection status was positively associated with HBsAg positivity in children ($p<0.001$), whereas other potential risk factors, such as ethnicity, proximity to health centers, and history of surgery, were not. There were no significant associations between mother's HBsAg status and history of surgery, and other sociodemographic factors.

Conclusions: Despite the slow implementation of the hepatitis B vaccination program, HBsAg prevalence among children and their mothers was not high in Lao PDR compared to reports from neighboring countries. The reasons for the differences in prevalence among these countries are unclear. We recommend that prevalence surveys be conducted in populations born before and after the implementation of a hepatitis B vaccination program to better understand the epidemiology of hepatitis B.

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Competing Interests: The authors have declared that no competing interests exist.

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Introduction

More than two billion people have been infected with hepatitis B worldwide, and among these individuals, more than 350 million suffer from chronic hepatitis B virus (HBV) infection [1,2,3]. Infection with HBV results in 600,000 to 1.2 million deaths per year due to chronic hepatitis, cirrhosis, and hepatocellular carcinoma [2,4]. HBV is responsible for 60% to 80% of the world's hepatocellular carcinoma cases, one of the major three causes of death in Africa, Asia, and the Pacific Rim, and accordingly, has been categorized as a Group 1 carcinogenic agent to humans by the International Agency for Research on Cancer [5].

The prevalence of hepatitis B differs throughout the world. Southeast Asian countries have been estimated to have a chronic HBV infection rate of more than 8% before the introduction of hepatitis B vaccination [6]. The Western Pacific region of the World Health Organization (WHO), to which most of the Southeast Asian countries belong, is assumed to have a high prevalence of hepatitis B [7]. Specifically, the prevalence is estimated to be 9% to 12% among women of childbearing age [8] and 8% to 10% among children in pre-vaccine era [9]. The WHO

estimates that the region has 28% of the global population, while it accounts for almost half of all chronic hepatitis B infections worldwide [10].

Hepatitis B vaccination, especially within 24 hours after childbirth, is considered the most effective and efficient preventive measure against hepatitis B infection [3,11]. Based on these assumptions, the WHO set goals to lower the prevalence of chronic hepatitis B among children over 5 years of age to 2% by 2012 and 1% by 2017. To achieve these goals, the WHO plans to increase immunization coverage to 65% for the birth dose and 80% for the third dose of the hepatitis B vaccine [7].

Lao People's Democratic Republic (Lao PDR) is a Southeast Asian country, located in the center of the Indochina peninsula. The country is landlocked and surrounded by China, Vietnam, Cambodia, Thailand, and Myanmar. The neighboring countries report relatively high hepatitis B prevalence compared to other parts of the world. For example, a survey from two provinces in Cambodia reported a hepatitis B surface antigen (HBsAg) prevalence of 7.7% (95% CI: 6.2%–9.3%) among healthy volunteer adults [12]. Another population-based survey in a province in rural Vietnam found that 18.8% (95% CI: 15.7%–21.9%) of adults and 12.5% (95% CI: 9.7%–15.3%) of infants were HBsAg positive at the time of the survey [13]. Thus, Lao PDR has been regarded as one of the hyperendemic countries for hepatitis B for quite some time and is ranked as a priority country by the WHO [7,9] despite a lack of data on the prevalence in a representative population. Pre-vaccine era prevalence was estimated as 11.8% [4], 8–10% [9], or 8% or more [6] for Lao PDR and Indochina countries. In response to this situation, Lao PDR has implemented the hepatitis B vaccine into the routine immunization program since 2002 (at 6, 10, and 14 weeks after birth), as well as birth dosing since 2004. The birth dosing was initiated at referral hospitals in the capital city, and then gradually expanded into rural hospitals (2006), and eventually home deliveries (2010). However, since then, no direct investigation has been conducted, and thus a nationwide survey is warranted [7,9]. The routine immunization coverage is reported as 56% for BCG, 50% for the third DPT, 50% for the third hepatitis B, 40% for measles, and 46% for oral polio vaccine in 2007, when a proportion of target children were born [14].

The primary objective of the present study was to estimate the chronic HBV infection rates by measuring the seroprevalence of HBsAg among children aged 5 to 9 years, and their mothers aged 15 to 45 years.

Methods

Ethical considerations

The survey protocol was reviewed and approved by the Ethical Committee of the Ministry of Health, Lao PDR, and the institutional review board of the National Center for Global Health and Medicine, Japan (NCGM-G-001130-00). Access to selected households was granted by the Ministry of Health, and the provincial and district government authorities.

After obtaining approval to conduct the survey from local authorities, surveyors explained the purpose of the survey to village leaders, selected participants, and their caregivers, assured them that all information would be strictly confidential and that no names would be gathered, and that there would be no benefit or penalties for agreeing or refusing to participate. Written informed consent was obtained from each mother on behalf of her child for each pair. Written informed consent was obtained from legal representatives (next of kin, caregivers, or guardians) when mothers were illiterate. The respondents' names were not recorded on the questionnaire sheets.

Study population

The target population was children aged 5 to 9 years (date of birth: January 2, 2002 to January 1, 2007) and their mothers aged 15 to 45 years (date of birth: January 2, 1966 to January 1, 1997) living in the selected cluster at the time of the survey. The reasons for this selection criteria are: 1) the national and regional hepatitis control policy target is to reduce chronic hepatitis B prevalence among children aged 5 years or older [7]; 2) Lao PDR does not have reliable HBsAg prevalence data among healthy adults, and mothers of childbearing age are considered the major source of hepatitis B infection for children; and 3) our pilot survey revealed that between 20 and 25 mother and child pairs can be practically sampled from each village.

Calculation of sample size

The equation used to calculate the required sample size is as follows [15,16]:

$$n = Z^2 \times p(1-p)DEFF \times 2/(d^2 \times RR)$$

where n = sample size
Z = significance level for 95% confidence
p = expected prevalence
$DEFF$ = design effect
d = precision
RR = response rate

The sample size (n) of 961 was calculated on the basis of an expected HBsAg seroprevalence (p) of 5%, a 5% level of significance (Z), precision (d) of ±2.0%, design effect ($DEFF$) of 2.0, two strata, and response rate (RR) of 95%. For field practicability, we requested 24 survey teams to sample 21 child and mother pairs from each cluster, with the aim of gathering 1,008 pairs in total.

Survey design and sampling

The survey applied a stratified three-stage random cluster sampling design, a type of probability sampling recommended by the WHO [15,17]. The survey was carried out by 24 survey teams (two members per team). Team members were recruited from the same districts that were under investigation to implement the survey more smoothly. The survey teams consisted of epidemiology, surveillance, or laboratory staff. The survey teams were supervised by 11 national personnel (six from the National Immunization Program and five from the National Center for Laboratory and Epidemiology, Ministry of Health) as well as 13 provincial officers.

For stratified multistage cluster sampling, immunization coverage by district and population data were obtained from the National Immunization Program, the Ministry of Health, and the Department of Statistics, Lao PDR. For post-survey weight adjustment, the survey teams obtained the latest population data from village leaders or health volunteers.

All 143 districts in Lao PDR were stratified into two strata, one having high (more than 76%) and the other having low (76% or less) immunization coverage for the third diphtheria, pertussis, tetanus, and hepatitis B (DPT-HepB)vaccines as reported in 2010. For the first stage, we selected 12 districts from each stratum using probability proportionate to size (PPS) sampling based on the population census of 2005. For the second stage, we selected two villages from each selected district by PPS sampling, and 48

villages were randomly sampled in total. In the instances in which the selected village lacked a sufficient number of children or the survey team could not approach the selected village due to safety or security reasons, the nearest village on the way back to the district center was selected. For each selected village, surveyors obtained a list of households, including age and sex, primarily from the poverty reduction program data with the assistance of the village leader, women's union, and/or healthcare volunteer. From these lists, 21 mothers aged 15 to 45 years old with children aged 5 to 9 years were randomly selected using a paper-based lottery system. When a mother had multiple children aged 5 to 9 years old, the youngest child was chosen for the survey. Special attention was paid to ensure that the child's biological mother was surveyed, as adoption is common in rural Lao PDR.

The survey was carried out from January 25th to February 4th, 2012. Each survey team successfully approached their assigned villages, with the exception of one village, which could not be visited because of road difficulties. An alternative village was chosen according to the predetermined selection criteria. In total, 1,008 children and 1,008 mothers were sampled. The overall response rate for HBsAg was 100%; however, 43 pairs were excluded from the analysis due to age ineligibility. That is, one child was over 9 years of age and 33 were less than 5 years of age. Furthermore, three mothers were over 45 years of age and six were less than 15 years of age. This happened as 43 mothers confused calendar age with traditional age. In rural areas, newborns start at one year old and a year is added to their age for each passing of a Lunar New Year. The surveyors asked participants for their age in years and their date of birth, and checked that they matched. A total of 965 pairs were included for analysis.

Questionnaires

A brief face-to-face questionnaire was administered to the sampled mother. The questionnaire consisted of 25 questions in four domains of inquiry: sociodemographic background of the family (i.e., ethnicity, family head's occupation, and mother's education level), family history of liver diseases, including mother, demographic characteristics of the child (i.e., age, sex, and place of birth), and immunization records. Additionally, questions were asked regarding exposure to potential risk factors for acquiring hepatitis B infection (e.g., history of blood transfusion, surgical operation, and sharing of toothbrush). The questionnaire was developed in English, translated into Lao, back-translated into English, and then compared and revised by bilingual staff members. A small pilot test was conducted prior to the data collection.

Testing for HBsAg

We used a simple and rapid test (Alere Determine HBsAg test card; Alere Medical Co. Ltd., Chiba, Japan) rather than the traditional ELISA test, as it was better suited to use in the field [14]. The sensitivity and specificity of the test were reported as high in two Asian countries [18,19]. In Vietnam, the Determine HBsAg test validity was measured based on comparison with HBsAg EIA. Results were 100% in both sensitivity and specificity in 328 samples [18]. In China, the Determine HBsAg performance was evaluated in comparison with HBsAg EIA for 671 samples. The sensitivity was reported to be 98.9% and specificity 100% [19]. The Determine HBsAg examination kit is one of the most reliable point-of-care HBsAg tests, and is recommended by the WHO [15]. HBsAg testing was performed according to the manufacturer's instructions. Blood was collected from a finger prick using a safety lancet (BD Safety Lancet, Becton Dickinson, NJ, USA) and glass capillary tube, and the blood was applied onto the sample pad of the rapid test kit. After applying the chase buffer, surveyors assessed the results after at least 15 minutes, but no longer than 24 hours. When no control bar appeared after 15 minutes, the test results were considered invalid, and the test was repeated. Blood spots were collected onto filter paper for further testing. A 2-day training session was organized for surveyors and supervisors on the use of the rapid test and the completion of the questionnaire. To ensure the safety of the blood collection procedure, surveyors always used a new pair of latex gloves. Surveyors were instructed to place all capillary tubes and lancets into safety boxes immediately after use.

Data entry and statistical analysis

All of the completed questionnaires were brought to a centralized location and the data were entered into a Microsoft Excel 2007 spreadsheet. Data were double-entered and cross-checked. Logistic regression tests and odds ratios were used to examine the relationship between the independent variables and HBsAg results. Multivariate logistic regression was used to investigate the independent association of different household and individual characteristics with the odds of being HBsAg positive. All estimates and standard errors were calculated by taking the multistage clustered sampling design and the weight of each sample into account to give representative, unbiased results. A p value <0.05 was considered statistically significant.

In our regression analyses, we adjusted for potential confounders by using the following variables: third DPT-HepB immunization coverage at the location of current residence, mother's age, ethnic group, mother's education level, family head's occupation, and mother's HBsAg status. For multivariate logistic regression analyses, multicollinearity was tested by calculating the variance inflation factors for each independent variable, and a value of more than 10 was considered as having multicollinearity.

All statistical analyses were carried out using STATA version 12 (Stata Corp., College Station, TX). Means and proportions were calculated using STATA's 'svy' function, with each sample weighted according to estimated population size.

Results

Socioeconomic backgrounds

The baseline characteristics of the 965 mothers and their children are summarized in Table 1. The mean age of the mothers was 29.1 years (95% CI: 26.2–33.1), and the mean age of the children was 5.8 years (95% CI: 5.4–6.3). Of the sampled children, 474 (49.4%) were male and 486 (50.6%) were female (five were unknown).

HBsAg prevalence among the general population

Of the 965 pairs included in the study, 17 children and 27 mothers were positive for HBsAg. Six child and mother pairs were HBsAg positive. The estimated prevalence was 1.7% for children (95% CI: 0.8%–2.6%) and 2.9% for mothers (95% CI: 1.7%–4.2%) after taking the sampling design and weight of each sample into account. HBsAg prevalence did not change significantly between DPT-HepB3 high and low coverage districts in both children and mothers (Table 2).

Potential risk factors

To determine whether background characteristics affect HBsAg status, we conducted multivariate logistic regression analysis in children and their mothers. In children, the mother's HBsAg status was positively associated with hepatitis B infection (Table 3),

Table 1. HBsAg prevalence among children (5 to 9 years old) and mothers (15 to 45 years old) in Lao PDR by selected background characteristics.

		n	%	Children's HBsAg (+)	%	95% CI	Mothers' HBsAg (+)	%	95% CI
Mothers' age(n = 965)	15–19	4	0.41	0	0.00		0	0.00	
	20–24	85	8.80	1	1.18	0.00–3.52	3	3.53	0.00–7.53
	25–29	294	30.47	7	2.38	0.63–4.13	8	2.72	0.85–4.59
	30–34	275	28.50	6	2.18	0.44–3.92	9	3.27	1.16–5.39
	35–39	176	18.24	3	1.70	0.00–3.64	3	1.70	0.00–3.64
	40–45	131	13.58	0	0.00		4	3.05	0.07–6.04
Ethnicity (n = 963)	Low land Lao	651	67.60	9	1.38	0.48–2.28	19	2.92	1.62–4.22
	Mid land Lao	248	25.75	6	2.42	0.49–4.34	5	2.02	0.25–3.78
	High land Lao	64	6.65	2	3.13	0.00–7.51	3	4.69	0.00–10.01
[1]Transportation (n = 939)	on foot	298	31.74	1	0.34	0.00–1.00	6	2.01	0.41–3.62
	bicycle	14	1.49	0	0.00		0	0.00	
	motor bike	364	38.76	7	1.92	0.51–3.34	10	2.75	1.06–4.43
	car	183	19.49	5	2.73	0.35–5.12	6	3.28	0.67–5.88
	hand tractor	66	7.03	3	4.55	0.00–9.71	4	6.06	0.15–11.97
	other	14	1.49	0	0.00		0	0.00	
[2]Time (n = 901)	< 5 minutes	31	3.44	0	0.00		1	3.23	0.00–9.81
	5 to 15 minutes	274	30.41	3	1.09	0.15–2.33	6	2.19	0.45–3.93
	15 to 30 minutes	231	25.64	5	2.16	0.27–4.06	11	4.76	2.00–7.53
	30 to 60 minutes	209	23.20	5	2.39	0.30–4.48	4	1.91	0.04–3.79
	> 60 minutes	156	17.31	3	1.56	0.00–4.68	4	2.56	0.06–5.07
[3]Education (n = 962)	did not finish primary school	307	31.91	7	2.28	0.60–3.96	12	3.91	1.73–6.09
	primary school	374	38.88	5	1.34	0.17–2.51	10	2.67	1.03–4.32
	junior high	185	19.23	3	1.62	0.00–3.46	2	1.08	0.00–2.59
	high school	73	7.59	0	0.00		1	1.37	0.00–4.10
	college/univ	20	2.08	1	5.00	0.00–15.47	2	10.00	0.00–24.41
	other or unknown	3	0.31	1	33.33	0.00–100.00	0	0.00	
[4]Occupation (n = 963)	farmer	683	70.92	13	1.90	0.88–2.93	19	2.78	1.55–4.02
	fisherman	5	0.52	0	0.00		0	0.00	
	laborer	92	9.55	1	1.09	0.00–3.25	5	5.43	0.71–10.16
	public officer	88	9.14	1	1.14	0.00–3.40	3	6.25	1.70–10.80
	factory employee	8	0.83	0	0.00		0	0.00	
	general employee	16	1.66	1	6.25	0.00–19.57	0	0.00	
	merchant	63	6.54	1	1.59	0.00–4.76	0	0.00	
	others	8	0.83	0	0.00		0	0.00	
Mother's surgery (n = 962)	yes	95	9.88	2	2.11	0.00–5.05	3	3.16	0.00–6.74
	no	867	90.12	15	1.73	0.86–2.60	24	2.77	1.67–3.86
Child's sex (n = 960)	male	474	49.38	9	1.89	0.67–3.13			
	female	486	50.63	7	1.44	0.38–2.50			
Place of delivery (n = 961)	province hospital	207	21.54	4	1.93	0.04–3.82	6	2.90	0.59–5.20
	district hospital	105	10.93	2	1.90	0.00–4.56	5	4.76	0.62–8.90
	health center	10	1.04	0	0.00		0	0.00	
	private clinic	11	1.14	0	0.00		1	9.09	0.00–29.35
	at home	569	59.21	8	1.41	0.44–2.38	14	2.46	1.18–3.74
	in the forest	56	5.83	3	5.36	0.00–11.44	1	1.79	0.00–5.36
	other health facility	3	0.32	0	0.00		0	0.00	
Child's surgery (n = 960)	yes	22	2.29	0	0.00				

Table 1. Cont.

	n	%	Children's HBsAg (+)	%	95% CI	Mothers' HBsAg (+)	%	95% CI
no	938	97.71	16	1.71	0.88–2.54			

[1]Transportation to the nearest health facility, [2] Time to the nearest health facility, [3] Mothers' completed education, [4] Family head's occupation.
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whereas the other potential risk factors were not associated according to the adjusted odds ratio. We did not obtain information regarding the type of delivery, and we did not find significant differences in HBsAg prevalence associated with delivery settings. No independent factor was positively associated with HBsAg positivity in mothers, according to the adjusted odds ratio (Table 4).

Immunization status

Written immunization records were available for 213 out of 965 children (22.1%). One hundred ninety eight children were vaccinated with three doses of hepatitis B vaccine, and 34 children were immunized on the day of birth or the following day. Five out of 213 children with immunization records were HBsAg positive (2.35%; 95% CI: 0.30–4.40%), while 12 of 752 without immunization records were HBsAg positive (1.60%; 95% CI: 0.70–2.49%). The differences between the two groups were not significant ($p = 0.46$).

Discussion

HBsAg prevalence among the general population

The estimated HBsAg prevalence in the general population was much lower in both children and adults than that of previous reports from neighboring countries and Lao PDR. For example, HBsAg prevalence in adults in Cambodia, Thailand, and Vietnam was reported to be 7.7% (95% CI: 6.2%–9.3%) [12], 6 to 10% [15,20], and 18.8% (95% CI: 15.7%–21.9%) [13], respectively. Data on HBsAg prevalence amongst children was relatively scarce, and reported to be 3.5% (95% CI: 2.4%–4.8%) in Cambodia [21], and 18.4% (95% CI: 13.4%–23.4%) in Vietnam [13]. In Lao PDR, studies in blood donors, hospitalized patients, and Lao migrant workers tested in Thailand showed HBsAg prevalence of 8.73% (95% CI: 8.69%–8.77%) [22], 17.99% (95% CI: 17.81%–18.17%) [23], and 6.86% (95% CI: 6.80%–6.92%) [24] based on the given numerators and denominators in the articles, respectively.

Since the study objective was to estimate the nationwide HBsAg prevalence among the general population of Lao PDR, and thus the study design is a cross sectional survey, it is difficult to explain the reasons for the unexpectedly low prevalence. There are several potential explanations for this observation. The survey methodology used was very different from that used for blood donors, patients, and migrant workers. We used probability sampling and thus the results are representative of the whole population, whereas studies of blood donors, hospitalized patients, and migrant workers used non-probability sampling and therefore the results are restricted to these populations. The primary objective of our survey was to estimate HBsAg prevalence among the general population, so probability sampling was the most appropriate choice. Demographic conditions among the sampled population are determined by survey methodology, and therefore the results showed discrepancy. The WHO strongly recommends probability sampling for hepatitis B prevalence survey [7,15,17]. Although Lao PDR has the lowest population density of the Indochina peninsula countries [25], the precise effects on hepatitis B prevalence of the reduced frequency of human to human contact due to the country's relatively low population density and less developed infrastructure remain unclear.

The number of HBsAg positives varied from 0 to 4 per cluster. Since the sampling design of the survey aimed to estimate the prevalence in the whole country, it is difficult to determine whether these differences reflect the local endemic status.

Potential risk factors

Our survey revealed that no potential risk factors were significantly associated with the children's infection status, with the exception of the mothers' hepatitis B infection status. HBsAg prevalence surveys in other countries revealed that history of surgery [26,27], level of education [26], and ethnicity [28] were independently associated with hepatitis B infection. The reason why we could not find any potential risk factors positively associated with hepatitis B infection among children is not clear. However, it should be noted that the primary objective of the present study was to assess HBsAg prevalence, and not its risk factors. Additionally, some reports found that HIV positive individuals are positively associated with hepatitis B virus infection

Table 2. HBsAg prevalence among children (5 to 9 years old) and mothers (15 to 45 years old).

	Children's HBsAg (+)	%	95% CI	Standard error	Design effect	Mothers' HBsAg (+)	%	95% CI	Standard error	Design effect
High coverage districts (n = 486)	6	1.14	0.23–2.04	0.44	0.82	18	3.79	1.79–5.79	0.97	1.24
Low coverage districts (n = 479)	11	2.39	0.75–4.03	0.79	1.27	9	1.88	0.49–3.37	0.69	1.22
Total (n = 965)	17	1.72	0.81–2.63	0.44	1.10	27	2.93	1.65–4.20	0.61	1.28

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Table 3. Unadjusted and adjusted odds ratio for being HBsAg positive among children from five to nine years old in Lao PDR by selected background characteristics.

		Unadjusted odds ratio	95% CI	p	Adjusted odds ratio	95% CI	p
DPT3 coverage	high	1(reference)					
	low	2.13	0.73–6.21	0.16	3.47	0.77–15.64	0.10
Mothers' age	15 to 29	1(reference)					
	30 to 45	0.70	0.28–1.78	0.44	0.87	0.31–2.47	0.79
Ethnicity	Low land Lao	1(reference)					
	others	1.90	0.67–5.40	0.22	1.41	0.26–7.72	0.68
Education	none	1(reference)					
	finished primary school or upper	1.50	0.67–3.36	0.30	1.03	0.27–3.89	0.96
Occupation	white collar	1(reference)					
	blue collar	1.15	0.37–3.64	0.80	0.60	0.18–1.96	0.38
Sex	male	1(reference)					
	female	0.75	0.21–2.62	0.63	0.65	0.21–2.08	0.46
Birth place	health facility	1(reference)					
	non-health facility	0.98	0.39–2.49	0.97	0.79	0.28–2.21	0.64
Mothers' HBsAg	negative	1(reference)					
	positive	24.02	9.45–61.07	0.00	28.13	10.21–77.53	0.00

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[29,30]; however, we did not investigate HIV due to limited budget.

WHO's regional target

The interim target of the WHO is to reduce HBsAg prevalence to less than 2% in children aged at least 5 years old by 2012 [7,31]. The point prevalence is used for monitoring the control of hepatitis B. The Regional Office for the Western pacific recommended that the country conduct a national HBsAg prevalence survey to verify whether the country has reached the regional prevalence target [9]. Following these criteria, Lao PDR had already achieved its goal. However, it is unlikely that Lao PDR achieved the target through the immunization program alone because the country has the lowest immunization coverage of all countries in the region [7,9]. Considering the relatively lower HBsAg seroprevalence among the mothers compared to those reported in previous studies, it is likely that Lao PDR had a lower prevalence even before the introduction of the hepatitis B immunization program. Therefore, the final target of reducing HBsAg prevalence to less than 1% in children aged at least 5 years could be difficult to achieve if the country simply continues its current immunization policy.

A nationwide prevalence survey targeting the general population is ideally conducted before implementing the immunization

Table 4. Unadjusted and adjusted odds ratio for being HBsAg positive among mothers from 15 to 45 years old in Lao PDR by selected background characteristics.

		Unadjusted odds ratio	95% CI	p	Adjusted odds ratio	95% CI	p
DPT3 coverage	high	1(reference)					
	low	0.50	0.20–1.28	0.14	0.47	0.19–1.16	0.10
Mothers' age	15 to 29	1(reference)					
	30 to 45	1.03	0.43–2.51	0.94	0.94	0.39–2.25	0.88
Ethnicity	Low land Lao	1(reference)					
	others	0.80	0.30–2.17	0.65	0.68	0.25–1.85	0.44
Education	none	1(reference)					
	finished primary school or upper	1.68	0.70–4.01	0.23	2.04	0.89–4.68	0.09
Occupation	white collar	1(reference)					
	blue collar	1.71	0.53–5.55	0.35	1.93	0.68–5.50	0.21
History of surgery	no	1(reference)					
	yes	1.28	0.39–4.25	0.67	1.30	0.35–4.78	0.68

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strategy to evaluate hepatitis B epidemiology. However, we were able to understand the epidemiology to some degree, even after implementation of immunization policy, because adults usually represent the pre-vaccination era [15,17].

Strengths of the study

The present study is the first nationwide survey on the prevalence of hepatitis B in the general population both before and after the implementation of a hepatitis B immunization policy in Lao PDR and other Southeast Asian countries. We applied multistage stratified cluster sampling to better represent the general population. The design effect of prevalence was calculated between 0.8 and 1.3, which was acceptable as we set it around 2.0 before the survey.

The background characteristics of our sampled population were similar to those of another nationwide population-based study, the Lao PDR Reproductive Health Survey (LRHS) [32] conducted in 2005. For example, the locations of current residence (north, central, and south) were 33.3%, 41.7%, and 25.0% in our survey, and 38.6%, 38.9%, and 22.5% in the LRHS. The levels of mothers' completed education (none, primary school, secondary school or more) were 31.9%, 38.9%, and 29.2% in our survey, and 28.8%, 43.7%, and 27.5% in the LRHS. The LRHS applied the multistage stratified cluster sampling method and surveyed more than 13,000 women all over the country. A direct comparison of the populations sampled by the two different surveys is difficult to perform as the primary objectives were different. Despite this, our sampled population is considered to likely represent the general population in Lao PDR.

Limitations of the study

There are several limitations in our study that should be addressed. First, the population data is based on the census conducted in 2005. After 2005, the population distribution may have changed and some of the villages could have merged, thereby creating bias in the findings. Fortunately, we did not survey any villages that disappeared or merged.

Second, floating or marginal populations are likely to be missed from the residential lists, and these populations could be a source of HIV and hepatitis B virus infections [33]. In future seroprevalence surveys, these subpopulations should be accounted for by using specific approaches, such as oversampling.

Third, population immunity levels were difficult to measure or estimate. The possession of immunization certificates was low, because many participants had already finished their scheduled vaccinations before 12 months of age, and relevant documents were lost. In the present study, we did not have enough data from health centers due to time and budget limitations. Since we did not examine immunization markers, such as HBsAb, herd immunity levels are unknown.

Lastly, adult men were not included in the survey. Serological studies in the past indicated that men have higher HBsAg rates than women [8,21,28]. In Lao PDR, male blood donors presented with 9.7% HBsAg positive prevalence, while the prevalence in females was 6.2% [22]. When considering the disease burden of hepatitis B virus infections, it is better to include both sexes [26].

To the best of our knowledge, this is the first nationwide, population-based serological survey on chronic hepatitis B virus infections both before and after implementation of hepatitis B immunization in Southeast Asia, where disease burden is high. As such, our results provide valuable information on a hepatitis B immunization program and a useful baseline against which to compare future assessments in this region.

National immunization policy should be based on the disease epidemiology [3]. However, in Southeast Asia, understanding of the epidemiology of hepatitis B remains unsatisfactory. Even when a country implements a hepatitis B immunization program for children and the prevalence of disease reaches the target (i.e., less than 2% among children aged 5 years or older), we cannot conclude that the immunization program alone contributed to reduced disease prevalence without comparing it to the disease prevalence in the pre-vaccine generation, i.e., adults. Nationwide surveys assessing disease prevalence in the generations before and after the implementation of a vaccination program will provide valuable information for understanding hepatitis B epidemiology. Therefore, we recommend surveying hepatitis B seroprevalence in both generations.

Conclusions

We determined the nationwide HBsAg prevalence among children (1.7%; 95% CI: 0.8%–2.6%) and their mothers (2.9%; 95% CI: 1.6%–4.2%) in Lao PDR. This is the first report to estimate the nationwide prevalence of chronic hepatitis B in pre- and post-hepatitis B immunization generations in Southeast Asia, where hepatitis B infections are a substantial burden. The estimated prevalence was below that of previous studies, suggesting that our understanding of this disease's epidemiology is lacking and warrants further investigation. We recommend that the prevalence among the pre- and post-vaccine eras should be investigated when conducting hepatitis B seroprevalence surveys.

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Author Contributions

Conceived and designed the experiments: AX MH KI TW MS. Performed the experiments: KK TK PV CP KP DP BS VSO TS. Analyzed the data: KK TK MH. Contributed reagents/materials/analysis tools: KI TW MS. Wrote the paper: AX PV MH. Revised the manuscript: KK TK PV CP KP DP BS VSO KI TW MS. Arranged laboratory for diagnosis: PV KI TW MS.

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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.

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 ±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 μl 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

			Children (5–9 years old)			Mothers (15–45 years old)		
	n	(%)	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% 4	(0.05–4.25)	3.76% 7	(1.00–6.52)
Vientiane Province ($n=182$)	2.75% 5	(0.35–5.14)	6.59% 12	(2.95–10.23)
Borikhamxay Province ($n=179$)	3.91% 7	(1.04–6.78)	5.59% 10	(2.19–8.98)
Khammuane Province ($n=180$)	1.11% 2	(0.00–2.66)	2.78% 5	(0.35–5.20)
Savannakhet Province ($n=184$)	1.63% 3	(0.00–3.48)	3.26% 6	(0.67–5.85)
Total ($n=911$)	2.09% 21	(0.79–3.38)	4.06% 40	(2.58–5.54)

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 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).

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.

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 smoking[23] 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 donors[25] 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 Vietnam[13] 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 Vietnam[32] 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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Original Article

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Factors affecting childhood immunization in Lao People's Democratic Republic: A cross-sectional study from nationwide, population-based, multistage cluster sampling

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Summary **Vaccines are one of the most important achievements in public health, and a major contributor to this success is the Expanded Programme on Immunization (EPI). The effective vaccination series of the EPI should be used by its target population. Various factors influence the utilization of the EPI vaccination series. In Lao People's Democratic Republic (Lao PDR), immunization coverage was lower than the regional average. This study evaluates risk factors affecting immunization underutilization among children five to nine years of age. It is a cross-sectional study from nationwide, population-based, multistage cluster sampling. The children who have received 'standard six' antigens and those who have been partially immunized are compared. In a bivariate analysis, household occupation, maternal age, means of transportation, time to the nearest health facilities, the child's birthplace, birth attended by medical staff, and notification of vaccination date by medical staff, village authority, or megaphone were associated with vaccination status. The final multivariate logistic regression model revealed that maternal age and notification of vaccination date by the village authority increased the odds of full vaccination, while notification of vaccination date by megaphone had decreased those odds. Further detailed qualitative research may be needed to discover how maternal sociodemographic factors influence the utilization of these services. Future research needs to target younger children and must include health care provider factors related to vaccination services.**

Keywords: Expanded programme on immunization (EPI), full vaccination, childhood vaccination

1. Introduction

Vaccines have substantially reduced the global burden of infectious diseases. They are considered one of the most important achievements in public health and one of the most cost-effective preventive services for children (*1-7*). The major contributor to this success is the Expanded Programme on Immunization (EPI) of the World Health Organization (WHO), United Nations Children's Fund (UNICEF) and Global Alliance Vaccine Initiative (GAVI) (*6*). The EPI was launched in 1974 as a worldwide alliance of collaborating nations whose goal was to expand immunization services and coverage (*6*).

The success of EPI does not only depend on effective vaccination series, but also on achieving optimal use by its target population and high immunization coverage (*4,8*). Pinpointing non-vaccination factors is important for achieving the EPI targets (*9,10*). The utilization of vaccination services depends on numerous factors such as provision of EPI services including outreach services, accessibility of these services, number of health workers, availability of safe needles, syringes, and cold chain, as well as health education and knowledge and attitude of mothers (*5,11,12*). Once a child enters the vaccination system, completion of the series is determined by the mother's educational level, employment status, experience with vaccination services, adequate schedule information, immigration status, and overall socioeconomic status (*13-15*). Various factors

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are important for the initiation and completion of the vaccination series.

The EPI was initiated in Lao People's Democratic Republic (Lao PDR) in 1979 (*16*). This programme greatly contributed to the progress of basic immunization coverage through the support of international partners and the government of Japan (*17*). However, immunization coverage in Laos PDR became stagnant after the WHO Western Pacific Region achieved regional polio eradication in 2000 (*17*). Immunization coverage was lower than regional average: measles immunization coverage among 1-year-olds was 64%, diphtheria tetanus toxoid and pertussis (DTP3) immunization coverage among 1-year-olds was 74%, hepatitis B (HepB) immunization coverage among 1-year-olds was 74%, tuberculosis (Bacille Calmette-Guérin vaccine: BCG) immunization coverage among 1-year-olds was 72% and polio (OPV3) immunization coverage among 1-year-olds was 76% (*18,22*). The risk factors for non-vaccination need to be studied to achieve optimal use of the vaccination services in Lao PDR. Therefore, the aim of this study is to evaluate risk factors affecting underutilization of childhood immunization among children five to nine years of age.

2. Material and Methods

2.1. *Study location and population*

Lao PDR is located in Southeast Asia and bordered by five countries: Burma, China, Vietnam, Cambodia, and Thailand. In 2010, its population was approximately 6.2 million and its under-five mortality rate was 54 out of 1,000 live births (*19*).

2.2. *Sampling and sampling frame*

This analysis has been done in the sub-population of the nation-wide survey for Hepatitis B sero-prevalence. All 143 districts in Lao PDR were stratified into two strata according to their immunization coverage. Twelve districts were randomly selected from each stratum, and two villages were selected from each district *via* probability of population proportional to size sampling. After randomly selecting 21 children (five to nine years old) and their mothers (15 to 45 years old) from the selected villages, questionnaires were administered.

One thousand and eight pairs of mothers and children were recruited and assessed for eligibility for this study. Forty-three pairs were excluded either because mothers were younger than 15 years of age or older than 45 years of age, or children were younger than five years of age or older than nine years of age. Four hundred and sixty-five pairs were excluded because they did not have vaccination certifications such as yellow cards or mother and child handbooks. Three pairs were excluded because possession of the vaccination certification was unknown. Among the 497 pairs with the vaccination certification, 284 pairs were excluded because they could not show their certification on the day of the survey. From this, 213 pairs were included in this study.

In Lao PDR, the yellow cards or mother and child handbooks are provided to children who received any vaccination regulated by the Laos PDR Ministry of Health. Vaccination dates were transcribed from those cards, and any vaccination documentation was considered sufficient evidence (*20*). Each child's immunization record was checked against the EPI immunization schedule recommended by the WHO (*21,22*). The following categories were used: fully immunized, if the "standard six" antigens – BCG, DTP3 (3 doses), OPV3 (3 doses), and measles vaccines – have been received on the day of interview; and partially immunized, if at least one recommended vaccine dose was not given (*23,24)*. This study compared factors between children who completed their standard vaccinations (full vaccination) and children who had not completed their vaccinations at the time of the survey (partial vaccination).

2.3. *Collection of data*

The survey, which used a face-to-face, interview-based questionnaire, was conducted from 25 January 2012 to 4 February 2012 by a survey team. A pilot study was conducted prior to the survey to check for clarity and consistency of the questionnaire. The survey team collected demographic information, vaccination status, and other relevant information. Each survey team had two surveyors. These surveyors received two days of training, which consisted of an overview of research methods, interview strategies, and ethical considerations. Before each interview, written consent was obtained from the child's mothers and vaccination dates were transcribed from yellow cards or mother and child handbooks.

2.4. *Statistical analysis*

The proportions of children who received all the recommended routine vaccination according to the policy of the Expanded Programme of Immunization (EPI) in Lao PDR and its 95% confidence interval (CI) were calculated. Bivariate analysis was performed to assess the relationship between vaccination status and its risk factors. A chi-square test was used for categorical variables and Student's *t* test was used for continuous variables. Crude odds ratios (ORs) and 95% CIs were calculated. Based on the results of the bivariate analysis), all significant factors and ethnicity were entered into the multivariate logistic regression analysis. Ethnicity was added to the final multivariate model because its diversity presented a major challenge in health service delivery due to cultural and language barriers. However, the variable "birthplace of the children" was excluded

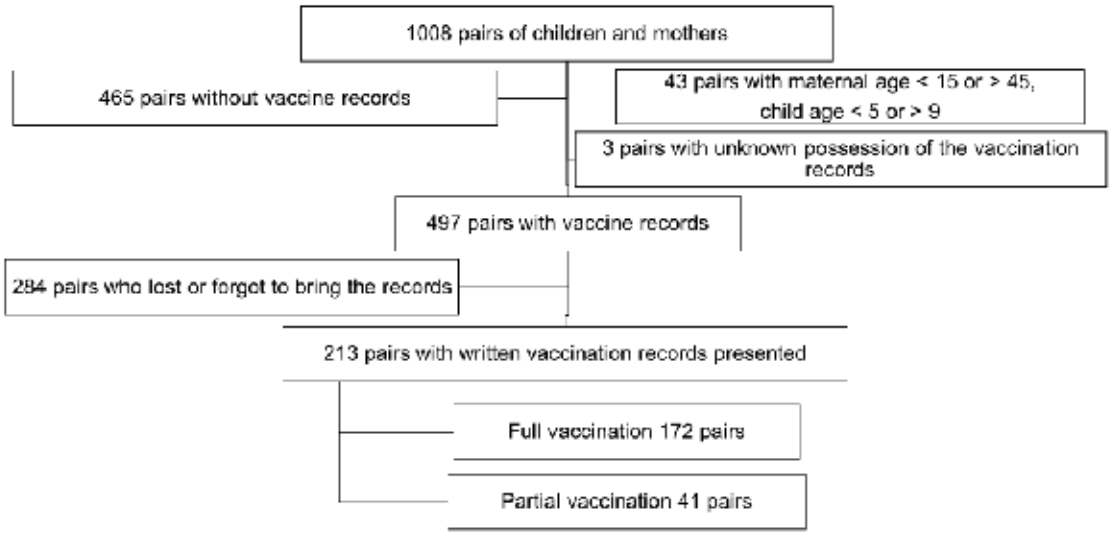

Figure 1. Study profile.

from the final multivariate logistic regression analysis since it showed the highest multicollinearity among the variables in the model. Multicollinearity was detected by using the variance inflation factor (VIF). Stata version 11.0 was used to perform all statistical analyses.

2.5. *Ethical considerations*

The survey was reviewed and approved by the Ethical Committee of the Ministry of Health, Lao PDR, and the institutional review board of the National Center for Global Health and Medicine, Japan (NCGM-G-001130-00). Access to selected households was granted by the Ministry of Health and the provincial and district government authorities.

3. Results

3.1. *Study profile of all subjects*

Figure 1 shows the profile of research subjects. 213 pairs were eligible. The case (full vaccination) group had 172 children and the control (partial vaccination) group had 41 children. The proportion of children, aged five to nine, who hold certificates of vaccination (either a yellow card, a mother and child handbook, or both) was 49.3% of the sample, while the proportion of children with an eligible record of vaccination was 21.1% of the sample (data not shown).

3.2. *Proportion of children vaccinated by antigen*

The statistics of children vaccinated with each antigen are presented in Table 1. All vaccination rates were over 80% except for the HepB birth dose, which was 19.2%. Among those 19.2%, only five children received the birth dose on the day of birth or the next day. The dates for the HepB birth dose for the rest of the children (80.8%) were not accurate. This low rate and record confusion may be due to the fact that the HepB vaccination for newborns was integrated into the EPI in 2004, which gradually expanded from central hospitals to rural areas. Most (80.8%) of the children were fully immunized between the ages of five and nine, according to the recommended EPI schedule.

Table 1. Proportion of children having received the various EPI antigens according to vaccination cards

EPI antigen	Total (%), (n = 213)	95%CI
HepB0	19.2	13.9-24.6
BCG	97.2	94.9-99.4
OPV1	97.7	95.6-99.7
OPV2	94.8	91.8-97.8
OPV3	93.4	90.1-96.8
DPT1	98.6	97.0-100
DPT2	95.8	93.1-98.5
DPT3	93.4	90.1-96.8
MV1	83.6	78.6-88.6
Full vaccination***	80.8	75.4-86.1

***Full vaccination – BCG, diphtheria-tetanus perttusis (DTP) (3 doses), polio (3 doses), and measles vaccines

3.3. *Risk factors of childhood immunization*

Among the sociodemographic factors of the family, household occupation, maternal age, means of transportation, and time to the nearest health facilities were associated with full vaccination of the children (Table 2). Birthplace of the children and birth attended by medical staff were associated with vaccination status (Table 3). Notification of vaccination date by

Table 2. Risk factors of childhood immunization (sociodemographic factors)

Items	Full vaccination *n* = 172, (%)	Partial vaccination *n* = 41, (%)	Crude OR (95%CI)	*p*-value
Ethnicity				
Lowland Lao	102 (59.3)	25 (61.0)	Reference	
Midland Lao	61 (35.5)	14 (34.2)	1.07 (0.52-2.21)	0.86
Highland Lao	9 (5.2)	32 (4.9)	1.10 (0.22-5.43)	0.90
Household occupation			Reference	
farmer	126 (73.3)	35 (89.7)	0.31 (0.10-0.94)	0.03
not farmers	46 (26.7)	4 (10.3)		
Mean maternal age (years)	32.7 (CI; 31.8-33.7)	29.3 (CI; 27.5-31.2)		0.00
Maternal education				
no education	68 (39.5)	16 (39.0)	Reference	0.32
primary school	55 (32.0)	19 (46.3)	0.68 (0.32-1.45)	0.25
junior high school	34 (19.8)	4 (9.8)	2.00 (0.62-6.45)	0.43
high school	10 (5.8)	1 (2.4)	2.35 (0.28-19.73)	0.89
university	5 (2.9)	1 (2.4)	1.18 (0.13-10.78)	
Sex of children				
boy	74 (43.0)	21 (51.2)	0.69 (0.34-1.38)	0.29
girl	97 (56.4)	41 (46.3)	Reference	
unknown	1 (0.6)	1 (2.4)		
Mean number of children	3.4 (CI; 3.1-3.7)	3.2 (CI; 2.6-3.9)		0.71
Transportation				
walk	65 (37.8)	13 (31.7)	Reference	
bicycle	3 (1.7)	0 (0)		
bike	44 (25.6)	18 (43.9)	0.49 (0.22-1.10)	0.08
car	27 (15.7)	0 (0)		
tractor	33 (19.2)	5 (12.2)	1.32 (0.43-4.02)	0.63
boat	0 (0)	3 (7.3)		
N/A	0 (0)	2 (4.9)		
Mean time to the nearest health facilities (minutes)	29.5 (CI; 25.0-33.9), *n* = 152	48.1 (CI; 20.5-75.7), *n* = 37		0.02
Decision-maker for vaccination				
Father	36 (20.9)	9 (22.0)	Reference	
Mother	129 (75.0)	29 (70.7)	1.11 (0.48-2.56)	0.80
Grandparents	4 (2.3)	3 (7.3)	0.33 (0.06-1.76)	0.20
Village leader	2 (1.2)	0 (0)		
Others	1 (0.6)	0 (0)		

N/A; Non applicable, CI; 95% confidence interval.

Table 3. Risk factors of childhood immunization (delivery history)

Items	Full vaccination *n* = 172, (%)	Partial vaccination *n* = 41, (%)	Crude OR (95%CI)	*p*-value
Birth place of children				
provincial hospital	38 (22.1)	3 (7.3)	Reference	
district hospital	24 (14.0)	9 (16.9)	0.21 (0.05-0.86)	0.03
health centre	4 (2.3)	1 (2.4)	0.32 (0.03-3.80)	0.36
private clinic	2 (1.2)	1 (2.4)	0.16 (0.01-2.29)	018
home	72 (41.9)	26 (63.4)	0.22 (0.06-0.77)	0.02
bush near house	31 (18.0)	0 (0)		
N/A	1 (0.6)	1 (2.4)		
Birth attended by the medical staff				
yes	94 (54.7)	14 (34.2)	2.27 (1.09-4.69)	
no	77 (44.8)	26 (63.4)	Reference	0.02
N/A	1 (0.6)	1 (2.4)		
Birth attended by the village health volunteer				
yes	32 (18.6)	3 (7.3)	2.88 (0.83-10.10)	0.08
no	137 (79.7)	37 (90.2)	Reference	
N/A	3 (1.7)	1 (2.4)		
Birth attended by the traditional birth attendant				
yes	33 (19.2)	9 (22.0)	0.81 (0.35-1.87)	0.62
no	136 (79.1)	30 (73.2)	Reference	
N/A	3 (1.7)	2 (4.9)		
Birth attended by the family member				
yes	75 (43.6)	19 (46.3)	0.79 (0.39-1.60)	0.51
no	95 (55.2)	19 (46.3)	Reference	
N/A	2 (1.2)	3 (7.3)		
Birth attended by nobody				
yes	4 (2.3)	2 (4.8)	0.46 (0.08-2.60)	0.36
no	167 (97.1)	38 (92.7)	Reference	
N/A	1 (0.6)	1 (2.4)		

N/A; Non applicable, CI; 95% confidence interval.

Table 4. Risk factors of childhood immunization (source of information of vaccination)

Items	Full vaccination *n* = 172, (%)	Partial vaccination *n* = 41, (%)	Crude OR (95%CI)	*p*-value
Source of information of vaccination (medical staff)				
yes	129 (75.0)	32 (78.1)	0.66 (0.27-1.60)	0.35
no	43 (25.0)	7 (17.1)	Reference	
N/A	0 (0)	2 (4.9)		
Source of information of vaccination (information on the vaccination cards)				
yes	51 (24.8)	9 (22.0)	1.45 (0.64-3.29)	0.37
no	117 (68.0)	30 (73.2)	Reference	
N/A	4 (1.3)	2 (4.9)		
Source of information of vaccination (family member or friends)				
yes	40 (23.3)	14 (34.2)	0.58 (0.27-1.21)	0.14
no	129 (75.0)	26 (63.4)	Reference	
N/A	3 (1.7)	1 (2.4)		
Source of information of vaccination (TV)				
yes	67 (39.0)	13 (31.7)	1.36 (0.66-2.84)	0.40
no	102 (59.3)	27 (65.9)	Reference	
N/A	3 (1.7)	1 (2.4)		
Source of information of vaccination (local authority)				
yes	97 (56.4)	28 (68.3)	0.58 (0.27-1.22)	0.14
no	72 (41.9)	12 (29.3)	Reference	
N/A	3 (1.7)	1 (2.4)		
Source of information of vaccination date (medical staff)				
1. yes	33 (19.2)	18 (43.9)	0.31 (0.14- 0.64)	0.00
2. no	138 (80.2)	23 (56.1)	Reference	
N/A	1 (0.6)	0		
Source of information of vaccination date (village health volunteer)				
yes	62 (36.1)	24 (58.5)	1.16 (0.57-2.36)	0.68
no	108 (62.8)	16 (39.0)	Reference	
N/A	2 (1.2)	1 (2.4)		
Source of information of vaccination date (local authority)				
yes	170 (98.8)	38 (92.7)	6.71 (1.05-42.7)	0.02
no	2 (1.2)	3 (7.3)	Reference	
Source of information of vaccination date (woman's union)				
yes	78 (45.4)	19 (46.3)	0.96 (0.48-1.90)	0.91
no	94 (54.7)	22 (53.7)	Reference	
Source of information of vaccination date (official letter from district governor)				
yes	27 (15.7)	6 (14.6)	1.09 (0.42-2.86)	0.85
no	144 (83.7)	35 (85.4)	Reference	
N/A	1 (0.6)	0		
Source of information of vaccination date (megaphone)				
yes	40 (23.3)	20 (48.8)	0.32 (0.15-0.66)	0.00
no	132 (76.7)	21 (51.2)	Reference	
Source of information of vaccination date (poster)				
yes	27 (15.7)	5 (12.2)	0.57 (0.48-3.74)	0.57
no	145 (84.3)	36 (87.8)	Reference	

N/A; Non applicable, CI; 95% confidence interval.

medical staff, village authority, or megaphone was also associated with full vaccination (Table 4).

Based on the bivariate analysis all variables significantly associated with full vaccination status and ethnicity were included in the multivariate logistic regression model. The results revealed that maternal age and obtaining information of the vaccination date by the village authority increased the odds of full vaccination. However, obtaining information on the vaccination date by megaphone had decreased the odds of full vaccination (Table 5).

4. Discussion

This study highlights the factors associated with the vaccination status of children aged five to nine in Lao PDR. Multivariate analysis of the risk factors for childhood immunization showed that maternal age and notification of the vaccination date by the village authority were positively associated with full

Table 5. Factors associated with full vaccination of children (logistic regression); occupation of household head-grouped

Factors	Adjusted odds ratio	95%CI	*p*-value
Sociodemographic factors			
Ethnicity	1.321	0.575-3.036	0.512
Household occupation	0.269	0.067-1.085	0.065
Maternal age	1.087	1.008-1.172	0.031
Transportation to the health facility	1.174	0.852-1.618	0.327
Time to the nearest health facility	0.989	0.977-1.001	0.076
Delivery history			
Birth attended by medical staff	2.617	0.936-7.317	0.067
Source of EPI information			
Source of information of vaccination date by medical staff	0.422	0.150-1.185	0.102
Source of information of vaccination date by local authority	17.430	1.827-166.280	0.013
Source of information of vaccination date by megaphone	0.204	0.065-0.637	0.006

immunization. Notification of the vaccination date by megaphone was negatively associated with full immunization.

4.1. *Sociodemographic factors*

According to the bivariate analysis, maternal age was associated with vaccination status. Furthermore, there was a linear trend that showed that full vaccination increases with maternal age. However, the relationship between childhood immunization and maternal age varies in the literature. There are some studies that show that maternal age was not associated with childhood immunization (*6*). One study from Africa showed the influence of younger maternal age on the utilisation of medical care (*8*).

This study did not show that maternal education was associated with vaccination status, which may be due to the contextual effects of maternal education on children's immunization in Lao PDR (*25*). Many studies have shown that maternal education or literacy is positively associated with the vaccination status of the children (*1,6,8,9,13,25*). In addition, some studies showed that the mother's knowledge of specific immunizations was associated with full vaccination (*3,5,17*). However, in Mali, Koumaré *et al*. reported no difference associated with parental knowledge about EPI diseases and full vaccination (*3*). In India, Parashar showed that literate women in a village may influence other women's capacity to seek and take advantage of state-provided healthcare, and even children of uneducated mothers may have better health knowledge due to residential or employment proximity to literate women through social influence (*25*). Further detailed research is needed to determine why maternal education was not associated with childhood immunization in this context.

Maekawa *et al*. showed that distance to health facilities in a rural region of Lao PDR was associated with vaccination status (*17*). This study focused on time rather than distance to health facilities since access depends on not only the distance but also the accessibility of the road and the availability of transportation. Therefore, mean time to the nearest health facilities was associated with full vaccination status in the bivariate analysis; however, it was not significant in the logistic regression model, which may be due to the established nationwide outreach service of EPI (*26*). Further research is needed to explore the association of vaccination status and the actual accessibility to the vaccination sites, combining precise information on distance, time to the vaccination sites, and availability of transportation.

4.2. *Delivery history*

Birth attended by medical staff was associated with full vaccination of the children; however, it was not significant in the multivariate analysis. According to Maekawa e*t al.*, bivariate analysis showed that immunization status was associated with whether mothers obtained information on immunisation before delivery; however, this factor was not significant in the multivariate analysis (*17*). Maekawa *et al.* also revealed that household visits and receipt of information before delivery influenced the number of fully immunized children . Further analysis revealed that household visits contributed to higher full-vaccination rates, especially among illiterate mothers. Therefore, it may not be sufficient to give information only once before or after the delivery; it might be better to provide information over several household visits.

4.3. *Source of EPI information*

The notification of the vaccination date by village authority had higher odds of full vaccination. On the other hand, notification of the vaccination date by megaphone was negatively associated with vaccination status. These results show that comprehensive and appropriate information dissemination may be key for vaccinating children against the EPI diseases (*3,6*).

There are several strengths in this study. This information has been collected through a nationwide survey using multistage cluster sampling. Geographically, the survey dissemination covered a wide portion of

Lao PDR and the ethnic minorities. A sampling frame allowed sampling bias to be a minimum. Only written records were used to avoid inaccuracies in vaccination history. Yellow cards and mother and child handbooks were the sole source of immunization information in this study. Parent's recall was not included in the interview. Rodewald *et al*. stated that the gold standard for measuring vaccination status, other than serological testing to detect immunity, is a parent-linked and provider-validated immunization status measure (*2*). Parent-linked means that parents name all immunization providers and provider-validated means obtaining the immunization records for each child and combining those data into a single record. Multiple studies have documented the inaccuracy of parents as the sole source of immunization status. The single-provider record check method, used in this study, lies between the parental recall and the parent-linked, provider-verified measure. The validity of this method depends on the frequency and reliability with which immunizations are included in the medical records (*2*). However, this study has several limitations. Firstly, this analysis has been done in the sub-population of the nation-wide survey for Hepatitis B sero-prevalence and only included the children and mothers who possessed any certifications of vaccination; therefore, the number of children in full and partial vaccination is skewed and no immunization or risk factor information was included from the children without the vaccination certifications. Second, the target of this study was children five to nine years old, which is older than the normal target population for a risk factor-based study. Thirdly, this study did not focus on health care provider factors, which may influence the vaccination status. Utilization of vaccination services is dependent on sociodemographic factors of the target population, as well as the number of health workers and availability of safe needles and syringes (*5*). Two studies show that children missed immunizations due to the provider's reluctance to vaccinate children while sick or when visiting health care centres for other purposes (*9,28*). This study did not focus on these provider risk factors. Lastly, this is a cross-sectional study; therefore, it is difficult to generate cause-and-effect relationships of childhood immunization and risk factors due to the study's design.

In conclusion, this study reinforces the importance of appropriate means of notification or provision of information on vaccination services in order to ensure full immunization (*13*). In the policy and programme level in Lao PDR, it may be necessary to implement appropriate information system on vaccination services at the community level such as mobilizing the village authorities. Older mothers showed higher odds of having fully immunized children, however, other sociodemographic factors such as maternal education was not associated with vaccination status. Further detailed qualitative research may be needed to discover how this factor influences vaccination services, along with other maternal sociodemographic factors such as education. Future research needs to target younger children to accurately collect information on vaccination records and other sociodemographic factors. Future studies also have to include health care provider factors that affect vaccination services.

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ラオス人民民主共和国における B 型肝炎有病率調査

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Hepatitis B Prevalence Survey in Lao PDR

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