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Age-stratified seroprevalence of vaccine-preventable infectious disease in Saravan, Southern Lao People's Democratic Republic

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ABSTRACT

Background: Lao People's Democratic Republic has frequent outbreaks of vaccine-preventable diseases (VPD). This study aimed to determine susceptibility and exposure to VPD in Saravan, a rural province with high ethnic diversity and some of the poorest health indicators nationwide.
Methods: Patients from three district hospitals and one provincial hospital were enrolled. Serum was tested by ELISA for IgG against hepatitis B virus (HBV), tetanus, diphtheria, measles, and rubella.
Results: The study enrolled 2463 participants aged 5–90 years. Exposure to HBV was 33.2% and increased with age up to 62.4% of those aged >50 years. HBV surface antigen prevalence was 5.7% in males and 2.4% in females; 9.9% had serology compatible with vaccination. Seroprevalence of protective anti-tetanus antibodies was 46.3% overall. Protective anti-diphtheria seroprevalence was 40.5%. Anti-measles seroprevalence increased from 16.7% in those aged 5–10 years to 97.7% in those aged >50 years. Anti-rubella seroprevalence was 93.0% overall. There were differences in seroprevalences between sex, districts and ethnicity.
Conclusions: Routine infant vaccination needs strengthening in order to close the immunity gaps. High burden of HBV infection should be addressed by increasing birth dose vaccine coverage. Regional and ethnic differences need to be investigated to enable a targeted approach to vaccination.

Introduction

Lao People's Democratic Republic (Lao PDR) is a landlocked lower-middle-income country in South East Asia with a population of around 7 million. It shares borders with China, Myanmar, Thailand, Cambodia, and Vietnam, and has a large ethnic diversity.

Routine infant vaccination within Lao PDR was formalized in the 1970s with the introduction of the Expanded Programme of Immunisation (EPI). This initially consisted of six vaccines but it was gradually expanded and now routine childhood vaccination includes: Bacillus Calmette–Guérin (BCG) and hepatitis B virus (HBV) vaccine at birth; pentavalent vaccine (Diphtheria-Tetanus-Pertussis- Hepatitis B-Haemophilus influenzae b; DTP-HepB-Hib), polio vaccine, pneumococcal vaccine at 6, 10 and 14 weeks; and measles/rubella combined vaccine at 9–11 and 12–18 months. Adult vaccination focuses on diphtheria/tetanus vaccination of pregnant women and women of childbearing age, in addition to various supplementary immunization activities (SIA) (e.g. against polio, measles and rubella). Despite the comprehensive vaccination programme, outbreaks of vaccine-preventable diseases (VPD) continue to occur, such as polio (Pauly et al., 2018), measles (Sengkeopraseuth et al., 2018), diphtheria (Nanthavong et al., 2015), and pertussis (Kleine et al., 2020). Outbreaks near to the borders with neighboring countries such as Vietnam have also raised fear of cross-border spread of disease. Vaccination coverage of the HBV birth dose also remains low due to the high number of unattended home births (Lao Statistics Bureau and UNICEF, 2018). As mother-to-child HBV transmission is believed to be the main route of infection in Lao PDR, HBV birth dose is key to reducing the burden of chronic infection, which is as high as 9% in adult males, and the consequent high levels of cirrhosis and liver cancer (Bray et al., 2018). Disease outbreaks often affect particular subgroups of the Lao population or remote regions of the country where vaccination coverage and immunogenicity are challenging (Evdokimov et al., 2017; Hefele et al., 2019; Nanthavong et al., 2015;

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Phimmasane et al., 2010; Sengkeopraseuth et al., 2018; WHO, 2012).

Saravan province is a largely rural province situated in the south of Lao PDR. The provincial population was 396,942 in 2015. It has eight districts, two of which border with Vietnam to the West and two bordering Thailand to the East. The main languages are Lao-Tai and Mone-Khmer and there are 14 main ethnic groups (Lao Statistics Bureau, 2016). Vaccination coverage is low: 44.4% received the HBV birth dose vaccination in 2017 (ranking fourth lowest of 18 Lao provinces) and 10.4% of women received at least two doses of tetanus vaccine during their last pregnancy (second lowest nationwide) (Ministry of Health and Lao Statistics Bureau 2012, 2012). Due to its large rural population, ethnic diversity, poor health indicators, and its borders with two neighboring countries this study aimed to determine the exposure and susceptibility of the population to VPD in Saravan province.

Methods

Participants

This cross-sectional study was conducted in Saravan province between May 2017 and March 2019. Participants aged ≥5 years were recruited whilst attending the provincial hospital in Saravan town and three district hospitals for unrelated reasons. Sample size was chosen according to feasibility. Thus, a maximum target size of 2500 male and female participants was chosen. Both inpatients and outpatients were asked by the healthcare staff to participate in the study and sign the informed consent after having the study explained and reading the information sheet. In the case of participants unable to read Lao language, the healthcare workers read the consent form, and for participants unable to sign, a fingerprint was taken with the signature of a witness. Children aged <18 years were asked if they were willing to participate, and consent was taken from their parents. After collecting demographic information (age, sex, home-town, place of birth, religion, ethnicity, occupation, and marital status) the participants donated 5 mL of blood. Hepatitis B surface antigen (HBsAg) rapid testing (Standard Diagnostics) was performed by the participating hospital staff and results/counselling were given to the participants. The remaining blood was allowed to clot and serum separated by centrifugation. Serum was stored at −20 °C at the hospital and then sent to Institut Pasteur du Laos, where it was stored at −80 °C until use.

Laboratory assays

Serum was tested for the presence of antibodies or antigens by enzyme-linked immunosorbent assay (ELISA) (Diasorin, Italy for the Hepatitis B virus ELISAs and Euroimmun, Germany, for the other ELISAs). HBsAg-positive ELISA results were interpreted as "acute or chronic infection" and anti-HBs and anti-HBc antibody profiles were used to define "previously exposed" (anti-HBc positive) or "previously vaccinated" (anti-HBc negative, anti-HBs positive). For the purposes of this study, HBsAg ELISAs were only performed on anti-HBc-positive, anti-HBs negative samples, which accounted for the majority of HBsAg-positive participants (Jutavijittum et al., 2014).

Antibody titres of tetanus were classified according to manufacturer's instructions as follows: <0.1 IU/ml, "insufficient immunity: immediate boost recommended"; 0.1–0.5 IU/ml, "low immunity: immediate boost recommended"; >0.5–1.1 IU/ml "sufficient immunity: booster recommended after 2–5 years"; >1.1–5 IU/ml, "sufficient immunity: booster recommended after 5–10 years"; >5 IU/ml, "sufficient immunity: booster recommended after 10 years". Titres >0.5 IU/ml were classified as "protective". The interpretation of test results for antibody titres for diphtheria was taken from the manufacturer's recommendations: <0.01 IU/ml, "No protection"; 0.01–0.099 IU/ml, "Uncertain protection"; ≥0.1–1.0 IU/ml, "Immunisation protection present"; >1.0 IU/ml, "Long-term immunisation protection". Titres ≥0.1 IU/ml were classified as protective. For anti-measles immunoglobulin G (IgG), antibody titres <200 IU/L were considered negative, titres between 200 and 275 IU/L as borderline, and titres ≥275 IU/L as positive, as indicated by the kit recommendations. The cut-off values for the anti-rubella IgG antibody levels were based on the manufacturer's instructions: titres <8 IU/ml were considered negative, titres between 8 and <11 IU/ml as borderline, and titres ≥11 IU/ml as positive for anti-rubella IgG.

Data analysis

The data were described by descriptive and analytical biostatistics: mean, median, standard deviations, etc. The mean comparisons were made by the Student's test (t-test) and percentage comparisons were made by the Chi-squared test or the Fisher exact test, as appropriate. After monovariate analysis, any comparisons with $p < 0.2$ were included into logistical regression analysis. The final model was chosen using a likelihood ratio test, with $p < 0.05$ considered as statistically significant. Statistical analyses were performed using STATA version 14. During data analysis, "borderline" measles and rubella antibody titres were classed as "negative". Ethics approval for the study was provided by the Lao National Ethics Committee for Health Research (reference 018/NECHR/2017).

Results

Population characteristics

Two of the selected participants refused to participate due to fear of needles and blood and of stigmatisation depending on the blood test results. Therefore, a total of 2463 participants were enrolled (Table 1). The median age was 28 years (range 5–90) and 57.0% were females. A total of 40.2% of participants were from Lao Ngam district and 31.0%, 11.7% and 10.3% were from Saravan, Taoiy and Samoui districts, respectively; <10% of the participants were recruited from the remaining districts. Of participants, 59.8% were married and the main occupation was farming (60.8%). There were 10 ethnic minority groups represented in the study, with 52.9% following animism and 46.5% Buddhism. Almost half of participants did not know their place of birth; however, 46.7% stated that they had been born at home. One serum sample had only enough volume to test for HBV markers, and therefore all other markers had a denominator of n = 2462.

Serology

Hepatitis B virus (HBV)

Overall past exposure to HBV (anti-HBc positive) was 817/2463 (33.2%) (Table 2, Figure 1 and Supplementary Table S1). After multivariate analysis, exposure significantly increased with age; those aged 5–10 years had 1.5% anti-HBc seropositivity compared with 62.4% in those aged >50 years (OR 72.0 [24.5–211.7], $p < 0.0001$). Males had significantly higher exposure than females (37.4% and 30.0%; OR 1.7 [1.3–2.0], $p < 0.0001$). Ethnicity had a small effect on exposure: the Katou group had slightly higher exposure compared with the Inh group, who had lowest exposure (OR 2.6 [1.3–5.2], $p = 0.003$). The prevalence of chronic (or acute) infection, as defined by HBsAg detection by ELISA, was 3.8% overall (Table 2, Figure 1 and Supplementary Table S2). Prevalence was 0% in those aged 5–10 years, increased until the age of about 20 years

Table 1
Demographics of study population (or their parents).

Variable		n (%)
Sex	Male	1058 (43.0)
	Female	1405 (57.0)
Age	Median (range)	28 years (5–90)
District	Lao Ngam	990 (40.2)
	Saravan	764 (31.0)
	Taoiy	289 (11.7)
	Samoui	254 (10.3)
	Toomlarn	118 (4.8)
	Vapy	31 (1.3)
	Khongxedone	9 (0.4)
	Lakhonepheng	8 (0.3)
Ethnicity	Taoiy	356 (14.5)
	Lao	350 (14.2)
	Laven	324 (13.2)
	Suay	300 (12.2)
	Pako	279 (11.3)
	Katang	252 (10.2)
	Phouthai	168 (6.8)
	Katou	162 (6.6)
	Other (Alux/Gnae/Kadeau/Tang)	155 (6.3)
	Inh	117 (4.8)
Place of birth	No information	1213 (49.2)
	Home	1151 (46.7)
	Hospital	99 (4.0)
Marital status	Married	1472 (59.8)
	Single	938 (38.1)
	Other (widowed/divorced)	53 (2.2)
Occupation	Farmer	1498 (60.8)
	Student	692 (28.1)
	Government staff	161 (6.5)
	Unemployed	45 (1.8)
	Unknown	32 (1.3)
	Business person	22 (0.9)
	Other (monk, teacher, retired, soldier)	13 (0.5)
Belief	Animist	1304 (52.9)
	Buddhist	1145 (46.5)
	Christian	14 (0.6)

and then stabilised at around 5%. Males had a significantly higher HBsAg prevalence (5.7%) than females (2.4%; OR 2.5 [1.6–3.9], p < 0.0001). The serological profile for vaccination (anti-HBs positive, anti-HBc negative) was 9.9% overall and highest in those aged 5–10 years (39.5%) (Table 2). None of the hepatitis B markers were associated with district or place of birth.

Tetanus and diphtheria

Overall, 42.4% had "insufficient immunity" against tetanus and 11.3% had "low immunity". Sufficient immunity (titres >0.5 IU/ml) was detected in 46.3%, with 14.2%, 27.9% and 4.2% needing future booster doses in 2-5, 5-10 and 10 years' time. After multivariate analysis, sex had a large impact on protection: males (14.5%) were significantly less protected against tetanus than females (70.2%; OR 0.05 [0.04–0.07], p < 0.0001). Overall levels of protection were highest in the age group 31–40 years (61.9%), compared with those aged 5–10 years (40.7%; OR 2.9 [1.9–4.4], p < 0.0001). This was mainly due to the increased titres and protection in older women. The level of protection in women aged >20 years was 78.5%. Ethnicity, district, occupation, and religion were not associated with level of tetanus protection (Figure 2 and Supplementary Table S3). Overall, 3.3% of participants had "no protection" against diphtheria and 56.2% had "uncertain protection". "Immunization protection" was present in 33.1% and 7.3% had "long-term protection". The highest protective levels were found in women aged 11–20 years (53.6%). After multivariate analysis, seroprevalence of protective antibodies (titres >0.1 IU/ml) was slightly lower in males (37.0%) than in females (43.0%; OR 0.7 [0.6-0.9], p = 0.001). No other factors were associated with diphtheria protection (Figure 3 and Supplementary Table S4).

Measles and rubella

Most participants had immunity against measles (1808/2462; 73.4%) and 6.6% were borderline. Measles seroprevalence significantly increased with age from 16.7% in those aged 5–10 years to 97.7% in those aged >50 years (OR 145.9 [65.8–323.5], p < 0.0001). Males had slightly lower prevalence of protective anti-measles antibodies (68.4%) than females (77.2%) at all ages (OR 0.6 [0.5–0.8], p < 0.001). There was a significant difference in anti-measles seroprevalence between some of the ethnic groups, namely: the Lao Tai group had lower seroprevalence (69.1%) than the Inh group (93.1%, OR 0.2 [0.08–0.5], p = 0.001). There was no significant association of protective anti-measles seroprevalence with any of the other parameters (Figure 4 and Supplementary Table S5). Anti-rubella IgG seroprevalence was high from an early age, with 93.0% seroprotected overall and 1.5% with borderline results. Seroprevalence of protective antibodies varied by district, the lowest being in Khongsedone (77.8%), which was significantly different from the highest prevalence in Toomlarn (98.3%; OR 21.5 [2.5–182.9], p = 0.005). There was no association of anti-rubella seroprevalence with ethnicity, sex or any other parameter (Figure 4 and Supplementary Table S6).

Discussion

In order to reduce the high burden of HBV infection in Lao PDR, the vaccine was introduced at 6, 10 and 14 weeks of age between 2002 and 2004 onwards and was complemented by the birth dose in 2003/2004. The current study in Saravan Province, Southern Lao PDR, showed that HBV exposure (anti-HBc positive) significantly increased with age – from 1.5% in those aged 5–10 years to >60% in those aged >50 years. HBsAg prevalence also increased with age from 0% in 5–10 years, to 2.8% in 11–20 years, and then stayed about 5% in those >20 years. The low levels in the youngest age group indicated a positive impact of the introduction of the routine HBV infant vaccine and are in agreement with previous data (Hefele et al., 2020). Conversely, high levels of exposure and HBsAg in adults born before vaccine introduction were comparable with other studies of Lao adults from central Lao PDR (Black et al., 2014) but lower than those in the north of the country (Nouanthong et al. submitted). As in previous studies (Black et al., 2015, 2014; Jutavijittum et al., 2014) males had a higher prevalence of HBsAg

Table 2
Hepatitis B serological markers.

Serological markers			No. samples (% of age group)						
Anti-HBs	Anti-HBc	HBsAg	5–10	11–20	21–30	31–40	41–50	>50	Total
–	–	–	152 (58.9)	467 (73.1)	310 (65.3)	182 (54.2)	145 (46.3)	146 (33.0)	1402 (56.9)
+	–	–	102 (39.5)	73 (11.4)	22 (4.6)	15 (4.5)	12 (3.8)	20 (4.5)	244 (9.9)
+	+	–	2 (0.8)	54 (8.5)	77 (16.2)	65 (19.3)	59 (18.8)	131 (29.6)	388 (15.8)
–	+	–	2 (0.8)	27 (4.2)	44 (9.3)	56 (16.7)	83 (26.5)	123 (27.8)	335 (13.6)
–	+	+	0 (0)	18 (2.8)	22 (4.6)	18 (5.4)	14 (4.5)	22 (5.0)	94 (3.8)

Data show results from ELISA testing. All anti-HBs-positive samples or anti-HBc-negative samples were assumed to be HBsAg negative.

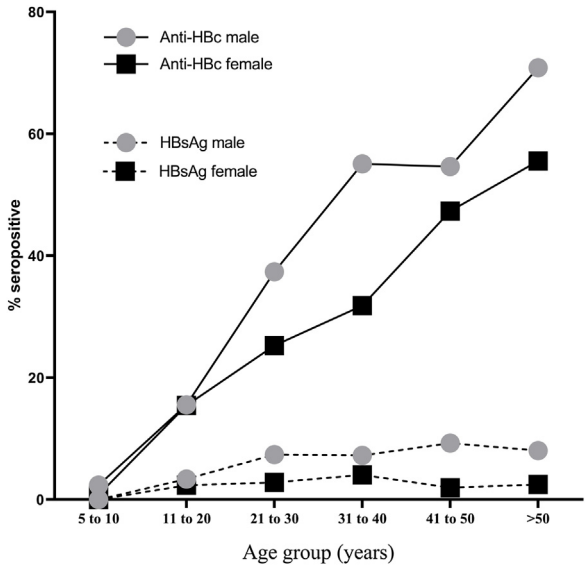

Figure 1. Age- and sex-stratified HBV exposure and HBsAg seroprevalence.

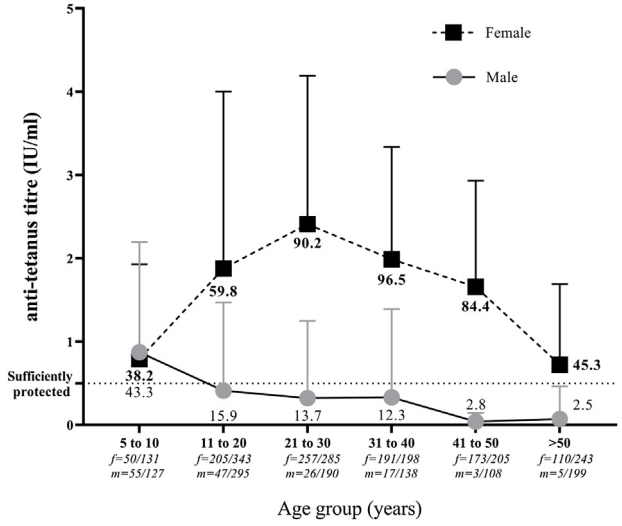

Figure 2. Age- and sex-stratified average titres for anti-tetanus antibodies. Numbers on graph represent percentages of protected males (standard font) and females (bold font). Numbers below the x-axis represent number of females (f) and males (m) "sufficiently protected"/"tested" per age group.

(5.7%) and exposure (37.4%) than females (2.4% and 30.0%, respectively). This disparity started from a young age, and in other settings has been suggested to be a result of a difference in sex hormones (Wang et al., 2015). Despite this sex difference, HBsAg seroprevalence in women aged 20–50 years was high at 2.9%. This indicates a significant risk of onward transmission via mother-to-child infection, which is thought to be the main route of infection in Lao PDR. Given that most chronic infections have been shown to be established during birth and the perinatal period, vaccination with the birth dose of HBV vaccine needs to be maintained in Saravan province. The vaccine serological profile (anti-HBs positive/anti-HBc negative) was 39.5% of children aged 5–10 years. According to the Lao Social Indicator study II (LSIS II), vaccination coverage with HBV birth dose in Saravan province was 44.4%, and 60.2% of infants received all three DTP-HepB-HiB doses in 2017 (Lao Statistics Bureau and UNICEF, 2018). Therefore, the low percentage of people with vaccination serological profiles in central Lao PDR (Evdokimov et al., 2017; Hefele et al., 2019) probably reflects low vaccination coverage and/or low vaccine immunogenicity and antibody waning.

The Katou group had 49.4% HBV exposure overall, which was significantly higher than the reference group (Inh) after multivariate analysis. This may reflect differences in vaccination coverage or risk practices, although there were no significant differences in vaccination serology or HBsAg prevalence. Unfortunately, further analysis was not possible due to small numbers of Katou participants; therefore, this warrants further investigation.

A positive impact of the Lao national immunisation programme was reflected in the elimination of maternal and neonatal tetanus in 2014. In the current study, more women were protected than males (70.2% and 14.5%, respectively) due to tetanus vaccination of all women aged between 15 and 49 years before and during pregnancy. The WHO recommends that at least 80% of women receive two or more doses of tetanus vaccine during pregnancy to maintain maternal neonatal tetanus elimination. Nevertheless, >20% of women of child-bearing age remain susceptible to tetanus. This corresponds with earlier findings of low levels of tetanus vaccination in Savannakhet province in Lao PDR, challenging tetanus elimination, particularly in remote areas (Ounnavong et al., 2020). It was also found that males and children were particularly susceptible, suggesting the need to improve routine infant vaccination coverage and monitor disease incidence in men.

This study detected a low level of protection against diphtheria in all age groups; 40.5% overall had antibody titres associated with sufficient immunity (>0.1 IU/ml). Although susceptibility was high in all age groups, this is particularly concerning in young children, who have highest risk of severe disease (Nanthavong et al., 2015). Since the introduction of three doses of diphtheria-containing vaccine in infants more than 40 years ago, there has been a significant reduction in diphtheria outbreaks. Nevertheless, cases still occur: for example, there were 73 nationwide in 2019 (World Health Organization, 2020), emphasizing the need for further strengthening of routine vaccination coverage and vaccine management (Evdokimov et al., 2017; Hefele et al., 2019; Nanthavong et al., 2015). The small but significant difference between seroprevalence in males (37.0%) compared with females (43.0%) is most likely due to the introduction of diphtheria vaccine to women of child-bearing age in the form of diphtheria-tetanus nationwide in 2012.

Seroprevalence of protective measles antibodies was <20% in children aged 5–10 years and 50% in those aged 11–20 years. This high susceptibility of the younger population is surprising, especially since Lao PDR introduced measles vaccines for children aged 9–11 months in 1984, and a second dose in 2017 for children aged 12–18 months. The significant increase of measles seroprevalence in the older age groups probably indicates cumulative wild-type exposure with age or possible inclusion in SIA such as that in 2011, which covered children aged 9 months to 19 years (approximately 9–30 years at the time of the study) (Hachiya et al., 2018; World Health Organization, 2020). Several recent measles outbreaks in Lao PDR, including 1119 reported cases in 2019 (World Health Organization, 2020), have likely resulted from low vaccination coverage and compromised vaccine immunogenicity (Vynnycky et al., 2019; Hefele et al., manuscript in preparation). Interestingly, seroprevalence was particularly high in the Inh ethnic group (93.1%), perhaps reflecting wild-type virus circulation. There were 117 participants from this ethnicity, and no data on measles cases could be found.

In contrast to measles, prevalence of protective anti-rubella antibodies was high in all age groups in this study. Given the lower measles seroprevalence in children and recent data showing wild-type rubella circulation in Lao children (Nouanthong et al., 2019), it is likely that the high rubella virus seroprevalence in children in

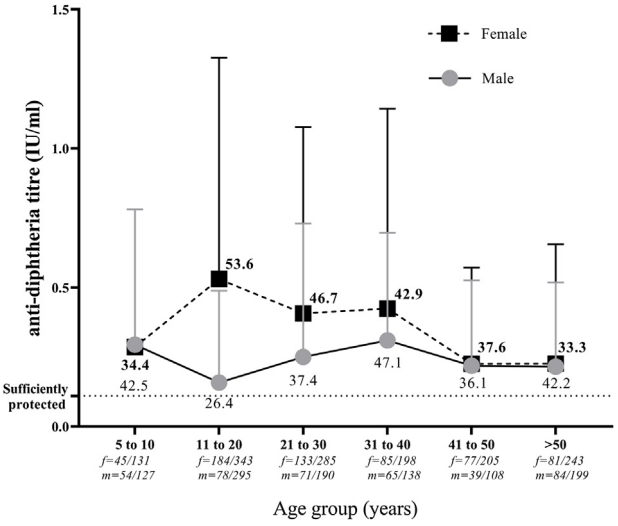

Figure 3. Age- and sex-stratified average titres for anti-diphtheria antibodies. Numbers on the graph represent percentages of protected males (standard font) and females (bold font). Numbers below the x-axis represent number of females (f) and males (m) "sufficiently protected"/"tested" per age group.

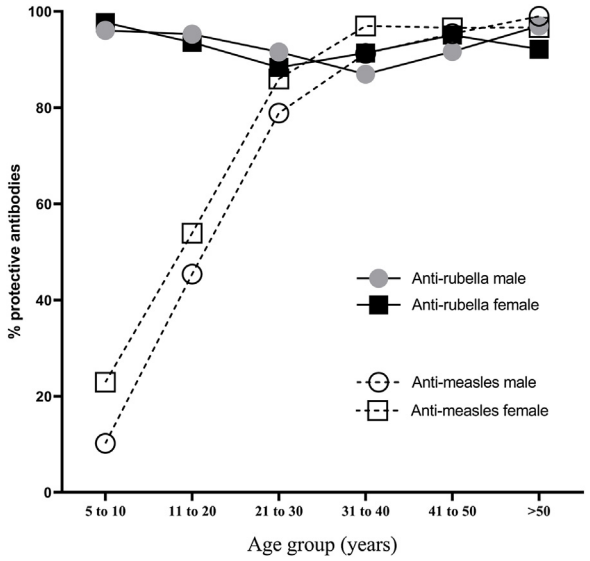

Figure 4. Sex- and age-stratified measles and rubella seroprotected (excluding borderline results).

Saravan was due to wild-type infection as well as vaccination. The different seroprevalences between districts may indeed reflect local outbreaks of the disease or different vaccine coverage. The high seroprevalence in adults is mostly due to natural virus infection, as the rubella containing M/R vaccine was only added to the childhood immunization schedule in 2011/2012, although there have been subsequent SIA, which may partially account for some of the seropositivity in the age groups up to 30 years of age. In women aged >20 years, the rubella seroprevalence was high (91.5%), indicating that the risk of infection during pregnancy and consequent Congenital Rubella Syndrome is low in this setting.

Overall, the data show that routine childhood vaccination in Saravan needs to be strengthened. Regarding measles and rubella, further investigations are needed into the apparent low immunogenicity of the measles component of the vaccine. Indications of disparate vaccine coverage and/or disease exposure between districts and ethnicities also warrant further investigation. A booster dose of a DT-containing vaccine is suggested worldwide by the WHO at the age of 12–23 months and later (World Health Organization, 2018). It is recommended that Lao PDR review this policy for the DTP-HepB-Hib vaccine, as indicated by the low diphtheria, tetanus and HBV protection found in this study. In addition, the coverage of the birth dose of HBV vaccine needs improving, in order to reduce the early life exposure to HBV (e.g. mother-to-child transmission).

Ethical approval

Ethics approval for the study was provided by the Lao National Ethics Committee for Health Research (reference 018/NECHR/2017).

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Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2021.04.033.

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