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Seroprevalence of measles and rubella antibodies in vaccinated and unvaccinated infants in the Lao People's Democratic Republic

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ABSTRACT

Background: Even though measles vaccination was introduced in the Lao PDR in 1984, coverage rates remain consistently low and outbreaks continue to occur frequently. This study was performed to investigate the seroprevalence of measles and rubella antibodies in vaccinated and unvaccinated children from Central Lao PDR.

Methods: Antibody titres of 1090 children aged 8–29 months who were vaccinated at different levels of the health care system were assessed by ELISA. Bivariate and multivariable analyses were performed to identify factors affecting seropositivity against measles and rubella.

Results: Among the vaccinated children, 67.5% in Vientiane Province and 76.4% in Bolikhamxay Province were double positive/borderline for measles and rubella IgG. A high proportion of unvaccinated children at both study sites (24.4% and 38.4%) were positive/borderline for measles and/or rubella. Time since vaccination <180 days, more than two siblings, and a mother who is a farmer/labourer were negatively associated with seropositivity.

Conclusions: A high prevalence of measles and rubella antibodies was found in unvaccinated children, indicating widespread circulation of both viruses and underreporting of cases. The high proportion of vaccinated children still susceptible to measles suggests problems with vaccine immunogenicity, emphasizing the need for regular evaluations of vaccine efficacy and management.

Introduction

With large, recurrent outbreaks throughout many parts of the world, measles vaccination is as important as ever. In 2018, measles accounted for more than 140 000 deaths, most of them children <5 years of age, despite an efficient and safe vaccine available worldwide and for more than 50 years. In November 2019, 413 308 cases were reported globally for that year (World Health Organization, 2019a). Even though most individuals are able to eventually clear measles virus infection and establish life-long immunity, the infection causes complications such as pneumonia, encephalitis, brain damage, blindness, hearing loss, and death in some patients (World Health Organization, 2019b). Vaccination against measles is normally administered in combination with rubella and with or without mumps vaccination as bivalent (MR) or trivalent vaccine (MMR). Rubella virus infection causes only mild disease, especially in children. Infection during early pregnancy, however, can cause severe foetal defects known as congenital rubella syndrome (CRS), miscarriage, or stillbirth (World Health Organization, 2016).

The Lao People's Democratic Republic (PDR) is a land-locked country in Southeast Asia with a population of about 7 million people (Worldometer, 2020). Measles vaccination was introduced in Lao PDR in 1984 as part of the National Immunisation Programme (NIP) and rubella vaccination was added in 2011 (Phoummalaysith et al., 2018; Sengkeopraseuth et al., 2018). Currently both vaccinations are given in combination as MR vaccine to children between 9 and 11 months of age. A second MR dose was introduced in 2017 for children between 12 and 18 months of age (World Health Organization, 2017). In 2019, the

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estimated coverage rates of vaccination with the first dose of MR reached 69% nationwide, as estimated by WHO/UNICEF (World Health Organization and United Nations Children's Fund, 2019a), which is far below the vaccination coverage of 90–95% required for measles herd immunity (Nokes and Anderson, 1988).

This study was performed to assess the seroprevalence of measles and rubella antibodies in vaccinated and unvaccinated children, factors associated with antibody prevalence after vaccination, and the timeliness of routine MR vaccination in children from Vientiane Capital and Bolikhamxay, a central province.

Methods

Study participants

Serum samples collected in the framework of two non-randomized vaccine immunogenicity studies conducted in 2013/14 (Evdokimov et al., 2017) and 2017/18 (Hefele et al., 2019) were utilized for this study.

In 2017/18, 1174 children from Bolikhamxay Province and Vientiane Capital, all of whom had received the three doses of the DTPw–HepB–Hib vaccination, which was documented in either the hospital record or yellow cards, were included. In Vientiane, the parents of children aged 8–23 months attending the Children's Hospital for the MR vaccination or for unrelated health reasons were recruited. In Bolikhamxay, children aged 8–29 months were recruited at the village level, based on vaccination status with DTPw–HepB–Hib (Hefele et al., 2019). Due to low serum volumes, not all serum samples could be tested for measles and rubella antibodies. Two hundred and eighty-eight children were included from the 324 participants enrolled in Vientiane Capital. From the 850 participants recruited in Bolikhamxay province, 802 were included.

Results from children recruited in Bolikhamxay in 2017/18 were age-matched and compared to results from children recruited in Bolikhamxay during a study from this research group performed in 2013/14 (Evdokimov et al., 2017). In that study, participants between 9 and 50 months of age with three documented DTPw–HepB–Hib and a documented MR vaccination were recruited from Bolikhamxay, Vientiane, and Khammouane. The study sites were selected after consulting with health care workers, according to expected levels of vaccination coverage.

All samples from both studies were tested with the same ELISA kits in the same laboratory. During recruitment, the parents/guardians were informed about the vaccine immunogenicity study by a health care worker and gave informed consent. They could withdraw their participation at any time.

Vaccination dates

The vaccination history of the participants was recorded from the hospital records and/or yellow cards. The age of the participants in weeks at the time of the vaccination with measles and rubella was calculated. Vaccination dates on the yellow cards were considered more reliable since they stay with the mothers. Thus, priority was given to the yellow card to calculate the median age at vaccination. Whenever the yellow card was not available, the date in the hospital record was used. In Lao PDR, the MR vaccine is scheduled at 9–11 months of age. For the purpose of this study, vaccination between 9 and 11 months was considered 'timely'.

Serology

In 2017, venous blood samples (5 ml) were collected from participating children by a health care worker. Serum was separated by centrifugation on the day of collection and stored at 4 °C for a maximum of 5 days and then at −20 °C for a maximum of 2 months. Samples were stored afterwards at −80 °C at Institut Pasteur du Laos until testing. Commercial ELISA kits (Euroimmun IgG ELISA) were used to determine IgG antibody levels against measles and rubella virus.

The cut-off values for the antibody levels were based on the manufacturer's instructions: for anti-measles IgG, an antibody titre <200 IU/l was considered negative, a titre between ≥200 and <275 IU/l as borderline, and a titre ≥275 IU/l as positive. An anti-rubella antibody titre <8 IU/ml was considered negative, a titre between ≥8 and <11 IU/ml as borderline, and a titre ≥11 IU/ml as positive for anti-rubella IgG.

In the logistic regression analysis, borderline samples were considered as positive for anti-measles and for anti-rubella IgG.

Data analysis

Data analyses were conducted using R software (R Core Team, 2019) with the following packages: epitools (Aragon, 2017), car (Fox et al., 2010), MASS (Venables and Ripley, 2002), tidyverse (Wickham, 2017), rcompanion (Mangiafico, 2016), and broom (Robinson and Hayes, 2019).

In order to determine factors affecting seropositivity, bivariate analyses were performed. The Chi-square test or Fisher's exact test was used as appropriate. The odds ratio (OR), 95% confidence interval (CI), and *P*-value were calculated. The Shapiro–Wilk goodness-of-fit test was used to assess the normality of the data, and the correlation between two numerical variables was assessed by calculating the Spearman rank correlation coefficient. In the logistic regression, only variables with a *P*-value <0.2 in the bivariate analyses were included in the binomial generalized linear models (GLMs). Correlation or multicollinearity (variance inflation factor >2–5) between variables was tested. Variables not associated with the response variable were removed during the backward stepwise regression, considering both the *P*-value and the Akaike information criterion (AIC) of the model. A *P*-value <0.05 was considered statistically significant.

Results

Participant characteristics

In Vientiane Capital (n = 288), nearly all participants were accompanied by their mother (97.6%) and were of Tai-Kadai ethnicity (97.6%) (**Supplementary Material** Table S1). About half of the mothers (52.4%) had completed college or university training, 40% were government employees, 25.4% traders, and 26.7% housewives. More than half of the children (59.7%) were less than 12 months old (median age 9.7 months, range 8–23 months). Overall, 41.7% of the participants had been vaccinated with MR before enrolment; 58.3% of the participants had not received any MR vaccination or had been vaccinated on the day of enrolment into the study. For the purpose of this study, the participants who received MR vaccination on the day of enrolment into the study (n = 100; 34.7% of participants in Vientiane) were considered as unvaccinated for their serology but as vaccinated with respect to timeliness of the MR vaccination.

In Bolikhamxay (n = 802), 80.5% of the participants were accompanied by their mothers. The majority of the participants belonged to the Tai-Kadai ethnicity and 17.2% belonged to another ethnicity (**Supplementary Material** Table S1). Most participants were older than 12 months (75.5%) (median age 16 months, range 8–29 months). The majority of the participants from Bolikhamxay had been vaccinated with at least one dose of MR according to their vaccination documents (81.3%). Three participants received MR

vaccination on the day of recruitment and were considered as unvaccinated for serology but as vaccinated regarding the timeliness of the MR vaccination. Vaccination dates of 17 (2.1%) participants could not be verified (due to unreadable date, or when it was unclear which date belonged to which vaccination). Twenty-five participants (3.1%) had received a second dose of MR according to their immunization cards.

In the entire cohort, 761 participants were older than 11 months and should already have received the MR vaccination (excluding those participants whose vaccination status was unknown). Of those, 696 (91.5%) had received the MR vaccine.

Most participants (69%) recruited during the study in 2013/14 (Evdokimov et al., 2017) were older than 24 months and 53.6% were male (**Supplementary Material**Table S2). In the 2013/14 study, the participants were on average about 3 months older (mean 19.5 months, median 19 months) than the participants included in the 2017 study (mean 16.2 months, median 16 months). About half the participants were born in a district hospital and 20% were born at home.

Prevalence of measles and rubella antibodies

Serological profile by study site

A total of 1090 samples were tested for both measles and rubella antibodies. Overall, 45.4% were positive for measles antibodies and 74.3% were positive for rubella antibodies; 15.5% and 1.3% of the participants were 'borderline'.

In Vientiane Capital, 28.5% and 43.1% of the participants were positive for measles and rubella IgG (Figure 1). In Bolikhamxay, only half of the participants (51.5%) were positive for measles antibodies and nearly 86% were positive for rubella antibodies.

Serological profile by vaccination status

Among all children at both study sites, 73.1% and 80.9% of the vaccinated children aged 12–16 months and >16 months, respectively, were positive/borderline for anti-measles IgG (**Supplementary Material**Figure S1). Nearly all (>98%) of the vaccinated children at both study sites aged >12 months were positive/borderline for anti-rubella IgG (**Supplementary Material**Figure S1).

In Vientiane Capital, 120 of the participants had documented vaccination against both measles and rubella, excluding those who were vaccinated on the day of enrolment. Among these, only two thirds (67.5%) were positive/borderline for both measles and rubella antibodies (Table 1). An additional 29 (24.2%) were positive/borderline for only rubella and one (0.8%) was positive/borderline for only measles antibodies. The 168 (58.3%) unvaccinated participants included the 100 participants who were enrolled on the day of their MR vaccination. The majority (75.6%) of the unvaccinated participants were double negative for both antibodies and 15 (8.9%) were double positive/borderline. An additional 17 (10.1%) were single positive/borderline for measles IgG and nine (5.4%) were single positive/borderline for rubella IgG. Of the 100 participants who were enrolled on the day of their MR vaccination, 29% were positive/borderline for either measles or rubella antibodies or for both.

In Bolikhamxay, 83.1% of the participants were vaccinated, but only 498 (76.4%) of them were positive/borderline for both anti-measles and anti-rubella IgG (Table 1). An additional 125 (19.2%) were only positive/borderline for anti-rubella IgG and only one (0.2%) was positive/borderline for anti-measles IgG (Table 1). Among the unvaccinated participants, 82 (61.7%) were double negative. A high proportion were double positive/borderline (25.6%) or only single positive for rubella IgG (12%) or measles IgG (0.8%).

In Bolikhamxay, slightly more unvaccinated children were positive for measles IgG compared to Vientiane Capital (26.3% vs 19.0%). More unvaccinated participants in Bolikhamxay were positive for rubella antibodies than for measles antibodies (37.6% vs 26.3%).

Serological profiles in Bolikhamxay in 2017 and 2013/2014

The serologies of the 652 vaccinated participants from 2017/18 were compared to those of vaccinated participants in the same age range (8–29 months, n = 155) who were enrolled in a vaccine immunogenicity study in 2013/14 (Evdokimov et al., 2017). In both studies, the proportion of male and female participants was similar (53.6% and 53.1% male participants). The time since MR vaccination was 2 months longer (range 0–22.9, mean 9.2, median 8.9 months) for participants in the 2013/14 study as compared to the 2017 study (range 0–19, mean 7.4, median 7.2 months).

In 2017, the anti-rubella seroprevalence was higher following vaccination compared to 2013/14 (95.5% vs 82%) (Table 2), while the anti-measles seroprevalence also increased from 66% in 2013/14 to 76.5% in 2017. Among vaccinated children in both studies, only 59.6% in 2013/14 and 76.4% in 2017 were seropositive for both anti-measles and anti-rubella.

Factors associated with measles and rubella antibody prevalence in vaccinated participants from Bolikhamxay Province

Six hundred and fifty-two participants in Bolikhamxay had received the MR vaccination. Factors associated with seroprevalence of double positive/borderline measles and rubella antibodies after vaccination were investigated by bivariate and multivariable

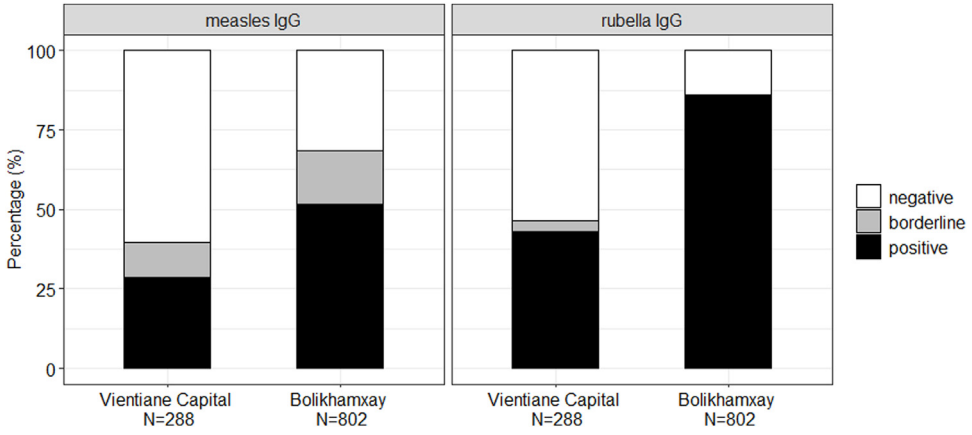

Figure 1. Serological anti-measles and anti-rubella IgG profiles of all participants by recruitment site. N = number of participants.

Table 1
Seroprevalence of measles and rubella IgG antibodies in vaccinated and unvaccinated children by recruitment site (participants with unclear vaccination status were not included; n = 17).

				Rubella IgG, n (%)		
				Positive and borderline	Negative	Total
VTN	Vaccinated (n = 120)	Measles IgG, n (%)	Positive and borderline	81 (67.5)	1 (0.8)	82 (68.3)
			Negative	29 (24.2)	9 (7.5)	38 (31.7)
			Total	110 (91.7)	10 (8.3)	120
	Unvaccinated (n = 168)	Measles IgG, n (%)	Positive and borderline	15 (8.9)	17 (10.1)	32 (19.0)
			Negative	9 (5.4)	127 (75.6)	136 (81.0)
			Total	24 (14.3)	144 (85.7)	168
BLX	Vaccinated (n = 652)	Measles IgG, n (%)	Positive and borderline	498 (76.4)	1 (0.2)	499 (76.5)
			Negative	125 (19.2)	28 (4.3)	153 (23.5)
			Total	623 (95.6)	29 (4.5)	652
	Unvaccinated (n = 133)	Measles IgG, n (%)	Positive and borderline	34 (25.6)	1 (0.8)	35 (26.3)
			Negative	16 (12.0)	82 (61.7)	98 (73.7)
			Total	50 (37.6)	83 (62.4)	133

VTN, Vientiane Capital; BLX, Bolikhamxay.

Table 2
Seroprevalence of measles and rubella IgG antibodies in vaccinated participants included in the studies in 2013/14 and 2017 in Bolikhamxay Province.

				Rubella IgG, n (%)		
				Positive and borderline	Negative	Total
BLX2013/14	Vaccinated (n = 155)	Measles IgG, n (%)	Positive and borderline	92 (59.6)	10 (6.5)	102 (65.8)
			Negative	36 (23.2)	17 (11.0)	53 (34.2)
			Total	128 (82.6)	27 (17.4)	155
BLX2017	Vaccinated (n = 652)	Measles IgG, n (%)	Positive and borderline	498 (76.4)	1 (0.2)	499 (76.5)
			Negative	125 (19.2)	28 (4.3)	153 (23.5)
			Total	623 (95.6)	29 (4.5)	652

BLX, Bolikhamxay.

analysis (Table 3). All positives for measles were also positive for rubella, except for one participant. In the bivariate analysis, participants were more likely to be positive/borderline for measles and rubella antibodies if their mothers had a higher socio-economic status (i.e., being traders or employees or had received a higher education) and if the children had been born at a district hospital or provincial hospital as compared to at home or at a health centre. Surprisingly, longer time since vaccination (>180 days ago) was also associated with higher seroprevalence. Mon-Khmer or Hmong-Mien ethnicity, having more than two siblings, living more than 10 km from the nearest health care facility (HCF), or having been vaccinated at a health centre were negatively associated with being double positive/borderline for both anti-rubella and anti-measles IgG.

After logistic regression, participants with more than two siblings, whose mothers were farmers or labourers, or who were vaccinated <180 days ago were less likely to be seropositive. In addition, having been vaccinated at a health centre or living more than 10 km from the nearest HCF were also retained in the final model, but were not significant. The fit of the overall model in comparison to the null model was significant ($P < 0.0001$, area under the receiver operating characteristic curve (AUC) 68.3%, pseudo-R^2 = 15.2%).

Timeliness of MR vaccination

The majority of participants in Vientiane Capital (76.4%) and in Bolikhamxay (81.7%) had been vaccinated as documented in the vaccination records (including those participants who were vaccinated on the day of recruitment).

At both study sites, the median age at vaccination with the first dose of MR was 10 months, ranging from 9 to 23 months in Vientiane and from 3 to 21 months in Bolikhamxay (**Supplementary Material**Table S3). In Bolikhamxay, the median age at vaccination was similar irrespective of the health facility level. At each study site, most participants had been vaccinated with MR between the ages of 9 and 11 months (Table 4), however the proportion of participants vaccinated after 11 months of age increased from 6% at the central hospitals to 33.7–46.7% at lower ranked HCFs.

Among the participants recruited in Vientiane at the central hospitals, the median time since vaccination was 83 days (approximately 3 months; ranging from 0 to 455 days), while it was 216 days (approximately 7 months; ranging from 0 to 570 days) among the participants in Bolikhamxay Province.

Discussion

This seroprevalence study included both unvaccinated children and children with documented MR vaccination. It was found that a very high percentage of unvaccinated children already had antibodies against measles in both the rural and urban locations, including children enrolled on the day of MR vaccination. In rural Bolikhamxay, slightly more unvaccinated children were already seropositive for anti-measles IgG by the time of enrolment than in Vientiane Capital (26.3% vs 19%). The high prevalence of measles is surprising, since only three and 10 measles cases were reported in Lao PDR in 2017 and in 2018, the years of the sample collections. In 2019, Lao PDR experienced a measles outbreak and reported 1119 cases (UNICEF, 2019; World Health Organization, 2020). The present study cohort included children aged 8–29 months. Some of the younger children, e.g. <10 months, may still have had persisting maternal antibodies. However, in an unpublished study, it was found that by 8 months of age virtually all children had lost their maternal antibodies. Thus, interference of maternal antibodies at the time of the vaccination cannot solely explain the

Table 3
Risk factor analysis for being double positive or borderline for both anti-measles and anti-rubella IgG in vaccinated participants in Bolikhamxay.

Variable	Categories	Number of rubella and measles IgG positive/borderline per total number (%)	Bivariate analysis		Multivariable analysis	
			OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Age of the mother (years)	≤20	31/45 (68.9)		NS		
	>20 to ≤30	297/397 (74.8)				
	>30	122/156 (78.2)				
	NA	48/54 (88.9)				
Ethnicity	Tai-Kadai	436/556 (78.4)	Ref.			
	Mon-Khmer and Hmong-Mien	62/96 (64.6)	0.5 (0.32–0.8)	0.006		
Occupation of mother	Farmer and labourer	213/311 (68.5)	Ref.			
	Trader, government employee, private employee	285/341 (83.6)	2.34 (1.61–3.4)	<0.0001	1.85 (1.2–2.88)	0.006
Level of education of mother	None and primary school	222/308 (72.1)	Ref.			
	Secondary school and university	276/344 (80.2)	1.57 (1.09–2.26)	0.016		
Antenatal care	No/unknown	45/63 (71.4)		NS		
	Yes	453/589 (76.9)				
Tetanus vaccination during pregnancy	Yes	412/543 (75.9)		NS		
	No	86/109 (78.9)				
Household members	<6	261/349 (74.8)		NS		
	≥6	237/303 (78.2)				
Household income	≤1 000 000 Kip	197/265 (74.3)		NS		
	>1 000 000 Kip	301/387 (77.8)				
Distance to nearest HCF	<10 km	379/479 (79.1)	Ref.			
	≥10 km	119/173 (68.8)	0.58 (0.39–0.86)	0.009	0.67 (0.44–1.02)	0.061
Age of child	≤12 months	66/103 (64.1)	Ref.		Correlated with time since MR vaccination	
	>12 months	432/549 (78.7)	2.07 (1.32–3.25)	0.002		
Sex of child	Male	272/350 (77.7)		NS		
	Female	226/302 (74.8)				
Duration of breastfeeding	≤6 months	339/446 (76)		NS		
	>6 months	159/206 (77.2)				
Number of siblings	<2	183/221 (82.8)	Ref.			
	≥2	315/431 (73.1)	0.56 (0.37–0.85)	0.006	0.52 (0.34–0.8)	0.003
Place of birth	At home or HC	170/249 (68.3)				
	PH, DH, CH	328/403 (81.4)	2.03 (1.41–2.93)	<0.001		
Hepatitis B birth dose	Yes	395/514 (76.8)		NS		
	No	103/138 (74.6)				
Time since vaccination	<180 days	171/246 (69.5)	Ref.			
	≥180 days	320/395 (81)	1.87 (1.29–2.71)	0.001	1.93 (1.32–2.83)	0.001
	NA	7/11 (63.6)				
Place of vaccination	PH/DH	263/314 (83.8)	Ref.			
	HC	235/335 (70.1)	0.46 (0.31–0.67)	<0.0001	0.66 (0.42–1.02)	0.061
	NA	0/3 (0.0)				

CH, central hospital; CI, confidence interval; DH, district hospital; HC, health centre; HCF, health care facility; MR, measles–rubella vaccine; NA, not available; NS, not significant; OR, odds ratio; PH, provincial hospital.

Table 4
Age at vaccination by health care level.

Age at vaccination[a]	Health care level					
	CH (*n* = 217) *n* (%)	PH (*n* = 157) *n* (%)	DH facility (*n* = 92) *n* (%)	DH outreach (*n* = 61) *n* (%)	HC facility (*n* = 60) *n* (%)	HC outreach (*n* = 270) *n* (%)
<9 months	0 (0.0)	4 (2.6)	2 (2.2)	0 (0.0)	1 (1.7)	8 (3.0)
9–11 months	201 (92.6)	141 (89.8)	57 (62.0)	35 (57.4)	31 (51.7)	158 (58.5)
12–18 months	13 (6.0)	12 (7.6)	31 (33.7)	26 (42.6)	28 (46.7)	100 (37.0)
>18 months	3 (1.4)	0 (0.0)	2 (2.2)	0 (0)	0 (0.0)	4 (1.5)

CH, central hospital; PH, provincial hospital; DH, district hospital; HC, health centre.
[a] Participants were removed from the table when the place of vaccination was unknown or when the calculated time value was negative (since this indicates a mistake made in the vaccination records) (*n* = 15).

apparent high prevalence of measles in this cohort. If this reflects the true incidence of measles in these children in these two locations, the disease may still be circulating and may be underreported.

The prevalence of rubella antibodies was similar to that of measles in Vientiane (14.3% vs. 19%), but considerably higher in Bolikhamxay than in Vientiane (37.6% vs. 14.3%) and compared to measles in Bolikhamxay (37.6% vs. 26.3%). This high seroprevalence

of rubella in both locations again cannot solely be explained by persisting maternal antibodies, since these are lost much earlier (unpublished results). However, maternal anti-measles antibodies may persist longer than anti-rubella antibodies, causing a lower anti-measles response compared to the anti-rubella response. Since rubella vaccination was only introduced in 2011, i.e., only 6 years before this study, the rubella virus may still be circulating much more widely than the measles virus. With only 10 rubella cases reported during the year of this study in Lao PDR, rubella appears to be highly underreported (World Health Organization, 2020).

One reason for the persisting high incidence of measles and rubella could be a low efficacy of the MR vaccine and the weak response/seroconversion of children to the two components of the vaccine. Indeed, in our sub-cohort of children with documented MR vaccination, only 68.3–76.5% had antibodies against measles. In contrast, more than 90% were anti-rubella seropositive (91.7–95.6%). This may reflect the higher immunogenicity of the rubella component of the vaccine and/or a higher circulation of the rubella virus in this population. The two components may differ in terms of stability, as shown in a study in Lao PDR in 2018 (Hachiya et al., 2018) in which the measles component of the vaccine was found to be more heat-sensitive. The anti-measles and anti-rubella seroprevalence was only slightly higher in Bolikhamxay than in Vientiane (measles: 76.5% vs 68.3%; rubella: 95.6% vs 91.3%), suggesting either higher natural infection in Bolikhamxay or a higher immunogenicity of the vaccine.

The comparison of the anti-measles and anti-rubella seroprevalence between 2017 and 2013/2014 in rural Bolikhamxay showed a considerable difference. Between the two studies, the anti-rubella seroprevalence increased from only 82% to 95.5%. While the anti-measles seroprevalence also increased from 66% to 76.4%, it nevertheless remains below the 90–95% needed to ensure herd immunity (Nokes and Anderson, 1988). In 2017 and 2013/2014, only 59.6% (92/155) and 76.4% (498/652) were double seropositive for both anti-measles and anti-rubella, because of the low anti-measles seroprevalence. The increased seroprevalence over the years may be due to a better vaccine response through improved vaccine management and is in line with the approximately 20% increase in protection rates against diphtheria, tetanus, and hepatitis B in the current cohort compared to 2013/14 (Evdokimov et al., 2017; Hefele et al., 2019). It should be noted that the vast majority of the children in the present study had received only one dose of the MR vaccine, and receiving a second dose of the MR vaccine, which was introduced in 2017, may also improve seropositivity rates in the future (World Health Organization, 2017).

Only those children who had received all three doses of the pentavalent DTPw–HepB–Hib vaccine were included in this study. This represents a significant selection bias in favour of those with access to vaccination services. Among the participants older than 11 months, 91.5% had received the MR vaccine. Compared to the general population of children in Bolikhamxay, this is certainly an overestimation of vaccine coverage. In 2019, the nationwide vaccination coverage with MR was estimated to be 69% (World Health Organization and United Nations Children's Fund, 2019b). Since problems with health records and management in Lao PDR have been observed previously by us (Hefele et al., 2020) and others (Sychareun et al., 2014), we cannot exclude the possibility that some of the documented vaccinated children had not received their vaccination or vice versa.

In the logistic regression, having more than two siblings, a mother who is a farmer or labourer, and having received the vaccine <180 days ago were independently associated with being less likely to be seronegative for both measles and rubella. The distance to the nearest HCF and vaccination at a health centre also seemed to play a role and were retained in the best fitting logistic regression model, but these variables were not significant. In a previous study (Hefele et al., 2019), the place of vaccination was strongly associated with vaccine-induced seroprotection against diphtheria, tetanus, and hepatitis B. Interestingly, in the current study, participants were more likely to be positive if they had received their MR vaccination more than 180 days prior to sample collection. This finding could be indicative of continued exposure to circulating measles virus. Unfortunately, this study only covered a limited age range and we could not further investigate the antibody dynamics in this cohort.

The median age at vaccination was found to be similar at both study sites, with 10 months at the central hospitals in Vientiane and 10–11 months in Bolikhamxay. In Bolikhamxay, MR vaccination was mostly given between the ages of 9 and 12 months (89.8%). However, the proportion of participants vaccinated after 12 months of age increased from 6% at the central hospitals to 33.7–46.7% in lower ranked HCFs. We have previously observed vaccination delays in lower levels of the health care system for the pentavalent vaccine (Hefele et al., 2020). Vaccination coverage and vaccination timeliness are separate issues but they are connected: delays in routine vaccination increase the risk of missed opportunities. Delayed vaccination also increases the window of disease susceptibility, facilitating disease outbreaks.

Limitations

Besides the geographic limitations, it is not possible to differentiate between natural and vaccine-induced antibodies, which complicates the interpretation of the study findings. The serum samples were collected in the framework of two independent studies and represent a convenience sample. Only children with a full course of DTPw–HepB–Hib were recruited, which limits the representativeness of the prevalence and timeliness findings. The specific place of vaccination (by outreach service or on site) was based on parental recall since it is not recorded in the vaccination documents. We have observed mismatches of vaccination dates on the vaccination cards and in the hospital records before (Hefele et al., 2020) and cannot exclude that not all participants with a vaccination date were truly vaccinated.

Conclusions

In this study, a high prevalence of anti-measles and anti-rubella antibodies was found in unvaccinated children at both study locations, which may be indicative of widespread circulation of both viruses and possibly underreporting of measles and rubella cases. We recommend the strengthening of surveillance for rubella and measles cases by systematically using the case definition for identifying suspected cases and systematic laboratory testing for improved reporting. The difference in measles and rubella antibody prevalence in vaccinated children reflects higher immunogenicity of the rubella component of the vaccine and/or a more active circulation of rubella virus. Compared to a previous study, the percentage of double positive vaccinated children was higher, but the response to the measles component of the vaccine remains substantially lower than the 90–95% threshold required for establish herd immunity. These results suggest that a thorough evaluation of vaccine management is needed.

Author contributions

Lisa Hefele: project administration; data and sample collection; investigation; formal analysis; writing – original draft. Kinnaly Xaydalasouk: data and sample collection; investigation; review and editing of manuscript draft. Daria Kleine: data and sample

collection; investigation; review and editing of manuscript draft. Anousin Homsana: data and sample collection; investigation; review and editing of manuscript draft. Dalouny Xayavong: data and sample collection; investigation; review and editing of manuscript draft. Sengdavanh Syphan: data and sample collection; investigation; review and editing of manuscript draft. Judith M. Hübschen: investigation; review and editing of manuscript draft. Claude P. Muller: conceptualization; writing – original draft; review and editing of manuscript draft. Antony P. Black: conceptualization; project administration; review and editing of manuscript draft.

Ethical approval

The study was approved by the Lao National Ethics Committee (reference numbers 033/2017/NECHR, 032/2017/NECHR, 031/2017/NECHR, 056/2017/NECHR) and by the internal ethics review board of the Institut Pasteur du Laos.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Aragon Tomas J. Epitools: epidemiology tools. 2017.
Evdokimov K, Sayasinh K, Nouanthong P, Vilivong K, Samountry B, Phonekeo D, et al. Low and disparate seroprotection after pentavalent childhood vaccination in the Lao People's Democratic Republic: a cross-sectional study. Clin Microbiol Infect 2017;23:197–202, doi:http://dx.doi.org/10.1016/j.cmi.2016.10.007.
Fox J, Weisberg S, Fox J. An R companion to applied regression. 2010.
Hachiya M, Miyano S, Mori Y, Vynnycky E, Keungsaneth P, Vongphrachanh P, et al. Evaluation of nationwide supplementary immunization in Lao People's Democratic Republic: population-based seroprevalence survey of anti-measles and anti-rubella IgG in children and adults, mathematical modelling and a stability testing of the vaccine. PLoS One 2018;13:e0194931, doi:http://dx.doi.org/10.1371/journal.pone.0194931.
Hefele L, Kleine D, Chanthavilay P, Nouanthong P, Xaydalasouk K, Phathammavong O, et al. Timeliness of immunisation with the pentavalent vaccine at different levels of the health care system in the Lao People's Democratic Republic: a cross-sectional study. PLoS One 2020;1–15, doi:http://dx.doi.org/10.1371/journal.pone.0242502.
Hefele L, Syphan S, Xayavong D, Homsana A, Kleine D, Chanthavilay P, et al. Seroprotection at different levels of the healthcare system after routine vaccination with diphtheria-tetanus-pertussis whole cell–hepatitis B–haemophilus influenzae type B in Lao People's Democratic Republic. Clin Infect Dis 2019;69(12):2136–44, doi:http://dx.doi.org/10.1093/cid/ciz143.
Mangiafico S. rcompanion: Functions to support extension education program evaluation [R statistical package]. 2016.
Nokes DJ, Anderson RM. The use of mathematical models in the epidemiological study of infectious diseases and in the design of mass immunization programmes. Epidemiol Infect 1988;101:1–20, doi:http://dx.doi.org/10.1017/S0950268800029186.
Phoummalaysith B, Yamamoto E, Xeuatvongsa A, Louangpradith V, Keohavong B, Saw YM, et al. Factors associated with routine immunization coverage of children under one year old in Lao People's Democratic Republic. Vaccine 2018;36:2666–72, doi:http://dx.doi.org/10.1016/j.vaccine.2018.03.051.
R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, Vienna, Austria; 2019.
Robinson D, Hayes A. broom: Convert Statistical Analysis Objects into Tidy Tibbles. 2019.
Sengkeopraseuth B, Khamphaphongphane B, Vongphrachanh P, Xeuatvongsa A, Norasingh S, Pathammvong C, et al. Analysing the characteristics of a measles outbreak in Houaphanh province to guide measles elimination in the Lao People's Democratic Republic. West Pac Surveill Response J 2018;9:9–15, doi:http://dx.doi.org/10.5365/wpsar.2017.8.1.012.
Sychareun V, Hansana V, Phengsavanh A, Chaleunvong K, Eunyoung K, Durham J. Data verification at health centers and district health offices in Xiengkhouang and Houaphanh Provinces, Lao PDR. BMC Health Serv Res 2014;14:1–10, doi:http://dx.doi.org/10.1186/1472-6963-14-255.
UNICEF. Over 20 million children worldwide missed out on measles vaccine annually in past 8 years, creating a pathway to current global outbreaks - UNICEF. 2019. https://www.unicef.org/laos/press-releases/over-20-million-children-worldwide-missed-out-measles-vaccine-annually-past-8-years.
Venables WN, Ripley BD. Modern applied statistics with S. Fourth. New York: Springer US; 2002.
Wickham H. tidyverse: easily install and load the "Tidyverse". 2017.
World Health Organization. WHO vaccine-preventable diseases: monitoring system. 2020 global summary. 2020. https://apps.who.int/immunization_monitoring/globalsummary/countries?countrycriteria%5Bcountry%5D%5B%5D=LAO.
World Health Organization. WHO | Measles – global situation. Emergencies Prep Response. 2019. https://www.who.int/csr/don/26-november-2019-measles-global_situation/en/.
World Health Organization. Fact sheet measles. 2019. https://www.who.int/news-room/fact-sheets/detail/measles.
World Health Organization. Laos introduced the measles rubella second dose to protect children aged 12 to 18 months. 2017. https://www.who.int/laos/news/detail/22-11-2017-laos-introduced-the-measles-rubella-second-dose-to-protect-children-aged-12-to-18-months.
World Health Organization. WHO | Rubella. Immunization, Vaccines Biol. 2016. https://www.who.int/immunization/diseases/rubella/en/.
World Health Organization, United Nations Children's Fund. Lao PDR's Democratic Republic: WHO and UNICEF estimates of immunization coverage: 2019 revision. 2019.
World Health Organization, United Nations Children's Fund. Lao People's Democratic Republic: WHO and UNICEF estimates of immunization coverage: 2018 revision. 2019.
Worldometer. Laos population. Worldometer; 2020. https://www.worldometers.info/world-population/laos-population/.