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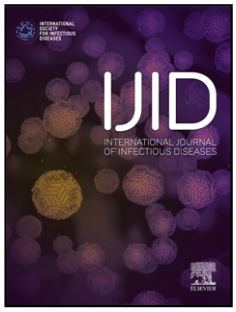

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Seroprevalence of measles and rubella antibodies in vaccinated and unvaccinated infants in the Lao People´s Democratic Republic

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Highlights

- Measles and rubella seroprevalence in vaccinated and unvaccinated Lao children
- Proportion of vaccinated children double positive for measles/rubella increased
- High prevalence of anti-measles/anti-rubella antibodies in unvaccinated children
- Results indicate widespread circulation of viruses and/or underreporting of cases
- Findings show need to strengthen measles and rubella surveillance in Laos

Article Type: Major Article

Abstract

Introduction

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

Methods

Antibody titers 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% and 76.4% were double positive/borderline for measles and rubella IgG in Vientiane and Bolikhamxay province respectively. 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 ago, more than two siblings and a mother who is a farmer/labourer were negatively associated with seropositivity.

Discussion

We found a high prevalence of measles and rubella antibodies in unvaccinated children, indicating wide-spread 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.

Keywords: Measles; Rubella; Immunogenicity; Vaccination; Immunization; Child Health

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, 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, in some patients the infection causes complications such as pneumonia, encephalitis, brain damage, blindness, hearing loss and death (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 South-East Asia with a population of about 7 million people (Worldometer, n.d.). 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 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).

In this study, we 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, all of whom had received the three doses of the DTPw-HepB-Hib vaccination, documented in either the hospital record (HR) or yellow cards (YC), from Bolikhamxay province and Vientiane Capital were included. In Vientiane, the parents of children aged 8 to 23 months attending the Children's Hospital for the MR vaccination or for unrelated health reasons were recruited. In Bolikhamxay, children aged 8 to 29 months were recruited at 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. 288 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 in 2013/14 (Evdokimov et al., 2017). In that study, participants between 9-50 months 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 healthcare workers, according to expected levels of vaccination coverage rates.

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 HR and/or YC. The age of the participants in weeks at the time of the vaccination with measles and rubella was calculated. Vaccination dates in the YC were considered more reliable since they stay with the mothers. Thus, priority was given to the YC to calculate the median age at vaccination. Whenever the YC was not available, the date in the HR 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 two 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 titer <200 IU/L was considered negative, a titer between ≥200 to <275 IU/L as borderline and a titer ≥275 IU/L as positive. An anti-rubella antibody titer <8 IU/ml was considered negative, a titer between ≥8 to <11 IU/ml as borderline and a titer ≥11 IU/ml as positive for anti-rubella IgG.

In 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. Chi- square test and Fisher's exact test were used as appropriate. Odds ratio (OR), 95% confidence intervals (CI) and p-value were calculated. Shapiro-Wilks goodness-of-fit test was used to assess the normality of data and the correlation between two numerical variables was assessed by calculating the Spearman rank correlation coefficient. In logistic regression, only variables with a p-value<0.2 in 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

Participants' 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 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, ranging from 8-23 months). 41.7% of the participants were vaccinated with MR before enrolment. 58.3% of the participants did not have any MR vaccination or were vaccinated at the day of enrolment into the study. For the purpose of this study, the participants who received MR vaccination at the day of the enrolment in 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 Table S1). Most participants were older than 12 months (75.5%) (median age=16 months, 8-29 months). The majority of the participants from Bolikhamxay were vaccinated with at least one dose of MR according to their vaccination documents (81.3%). Three participants received MR vaccination at the day of the 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). 25 participants (3.1%) received a second dose of MR according to their immunization cards.

In the entire cohort, 761 participants were older than 11 months and should have already received the MR vaccination (excluding those participants whose vaccination status is unknown). From those, 696 (91.5%) 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 (Suplementary Table S2). In the 2013/14 study the participants were on average about 3 months older (mean=19.5 months, median=19 months) than 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 profiles by study site

1090 samples were tested for both measles and rubella antibodies. Overall, 45.4% and 74.3% were positive for measles and rubella antibodies, respectively. 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 or 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 profiles 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 (Figure S1). Nearly all (>98%) of the vaccinated children at both study sites aged >12 months were positive/borderline for anti-rubella IgG (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 1 (0.8%) was positive/borderline for only measles antibodies. The 168 (58.3%) unvaccinated participants included the 100 participants who were enrolled at 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 9 (5.4%) were single positive/borderline for rubella IgG. From the 100 participants who were enrolled at 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 1 (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 results from 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 (0-22.9, mean=9.2, median=8.9 months) for participants in the 2013/14 study as compared to the 2017 study (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 to both anti-measles and anti-rubella.

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

652 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 analysis (Table 3). All positives for measles were also positive for rubella, except for one participant. In bivariate analysis, participants were more likely to be positive/borderline for measles and rubella antibodies when their mothers had a higher socio-economic status (i.e. being traders or employees or had received a higher education) and when the

children were born at a district hospital or provincial hospital as compared to at home or at a health center. Surprisingly, longer time since vaccination (>180 days ago) was also associated with higher seroprevalence. Mon-Khmer or Hmong-Mien ethnicity, having more than 2 siblings, living more than 10 km from the nearest health care facility (HCF) or having been vaccinated at a health center 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 center 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-value<0.0001, 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%) were vaccinated as documented by vaccination records (including those participants who were vaccinated at the day of recruitment).

At both study sites, the median age at vaccination with the first does of MR was 10 months, ranging from 9 to 23 months in Vientiane and from 3 to 21 months in Bolikhamxay (Supplement Table S3). In Bolikhamxay the median age at vaccination was similar irrespective of the health facility level. At each study site most participants were vaccinated with MR between the age of 9 and 11 months of age (Table 4), however, the proportion of participants vaccinated after 11 months of age increased from 6% at the CHs to 33.7% - 46.7% in lower ranked HCFs.

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

DISCUSSION

The present seroprevalence study included both unvaccinated children as well as children with documented MR vaccination. We found that a very high percentage of unvaccinated children already had antibodies against measles in both the rural and urban location, including children enrolled at 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 3 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). Our cohort included children 8-29 months. Some of the younger children, e.g. <10 months may still have had persisting maternal antibodies. However, in an unpublished study, we 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 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 can again not 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 circulate much wider than the measles virus. With only 10 rubella cases reported during the year of this study in Lao PDR, rubella seems highly underreported (World Health Organization, 2020).

One reason for the persisting high incidence of measles and rubella could be 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. Both 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 is only slightly higher in Bolikhamxay than in Vientiane (measles: 76.5% vs 68.3%; rubella: 95.6% and 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 our study received only one dose of the MR vaccine, and receiving a second dose of the MR vaccine, which was introduced in 2017 and may also improve seropositivity rates in the future (World Health Organization, 2017).

In our study only children were included who had received all three doses of the pentavalent DTPw-HepB-Hib vaccine. 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 previously observed by us (Hefele et al., 2020) and others (Sychareun et al., 2014), we cannot exclude the possibility that some of the documented vaccinated children did not receive their vaccination or vice versa.

In 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 against both measles and rubella. The distance to the nearest HCF and vaccination at a health center also seemed to play a role and were retained in the best fitting logistic regression model, but these variables were not significant. In our 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, our 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 age 9-12 months (89.8%). However, the proportion of participants vaccinated after 12 months of age increased from 6% at the CHs to 33.7%-46.7% in lower ranked HCFs. We have previously observed vaccination delays on 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 our 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 the parents' recall since it is not recorded in the vaccination documents. We observed mismatches of vaccination dates in vaccination cards and HRs before (Hefele et al., 2020) and cannot exclude that not all participants with a vaccination date were truly vaccinated.

CONCLUSION

In this study, we found a high prevalence of anti-measles and anti-rubella antibodies in unvaccinated children at both study locations, which may be indicative of wide-spread circulation of both viruses and possibly underreporting of measles and rubella cases. We recommend to strengthen the surveillance of 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 increased, 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 a thorough evaluation of vaccine management is needed.

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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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

Declaration of interests

☒ 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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FIGURES

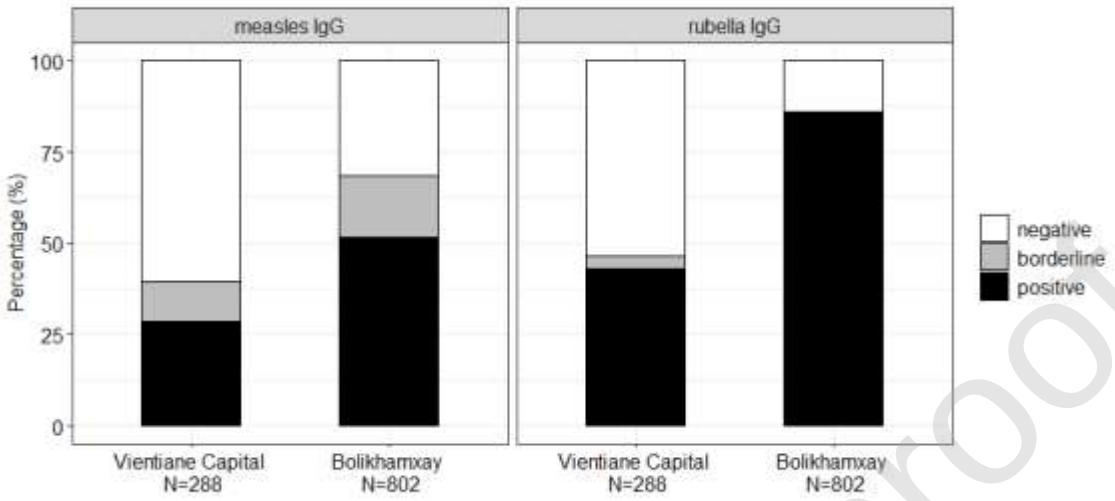

Figure 1 Serological anti-measles and anti-rubella IgG profiles of all participants by recruitment site. N = number of participants.

TABLES

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		
				positive & borderline n(%)	negative n(%)	total
VTN	vaccinated	Measles IgG	positive & borderline n(%)	81 (67.5)	1 (0.8)	82 (68.3)
	(N=120)		negative n(%)	29 (24.2)	9 (7.5)	38 (31.7)
			total	110 (91.7)	10 (8.3)	**120**
	unvaccinated	Measles IgG	positive & borderline n(%)	15 (8.9)	17 (10.1)	32 (19.0)
	(N=168)		negative n(%)	9 (5.4)	127 (75.6)	136 (81.0)
			total	24 (14.3)	144 (85.7)	**168**
BLX	vaccinated	Measles IgG	positive & borderline n(%)	498 (76.4)	1 (0.2)	499 (76.5)
	(N=652)		negative n(%)	125 (19.2)	28 (4.3)	153 (23.5)
			total	623 (95.6)	29 (4.5)	**652**
	unvaccinated	Measles IgG	positive & borderline n(%)	34 (25.6)	1 (0.8)	35 (26.3)
	(N=133)		negative n(%)	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 positive & borderline n(%)	negative n(%)	total
BLX - 2013/14	vaccinated (N=155)	Measles IgG	positive & borderline n(%)	92 (59.6)	10 (6.5)	102 (65.8)
			negative n(%)	36 (23.2)	17 (11.0)	53 (34.2)
			total	128 (82.6)	27 (17.4)	**155**
BLX - 2017	vaccinated (N=652)	Measles IgG	positive & borderline n(%)	498 (76.4)	1 (0.2)	499 (76.5)
			negative n(%)	125 (19.2)	28 (4.3)	153 (23.5)
			total	623 (95.6)	29 (4.5)	**652**

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 & measles IgG positive/borderline per total number (%)	Bivariate analysis		Multivariable analysis	
			OR [95% CI]	p-value	OR [95% CI]	p-value
Age of the mothers	≤ 20	31/45 (68.9)		NS		
	> 20 - ≤ 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 & Hmong-Mien	62/96 (64.6)	0.5 [0.32-0.8]	0.006		
Occupation of mother	Farmer & Labourer	213/311 (68.5)	ref			
	Trader, gov. employee, priv. 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 & primary school	222/308 (72.1)	ref			
	Secondary school & 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)				
Houshold 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	≤ 6months	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)				

NS = not significant; HC = health center;DH = district hospital; PH = provincial hospital; CH = central hospital; HCF = health care facility

Table 4 Age at vaccination by health care level

Age at vaccination[1]	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)

[1]Participants were removed from the table when the place of vaccination was unkown or when the calculated time value was negative (since it indicates a mistake made in the vaccination records) (n = 15)
CH = Central Hospital, PH = Provincial Hospital, DH = District Hospital, HC = Health Center.