

SHORT COMMUNICATION

Low seroprotection against diphtheria and tetanus in Lao adolescents

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Abstract

Objectives: There is currently no booster diphtheria or tetanus vaccine for Lao children before adolescence, despite international recommendations. We investigated seroprotection against diphtheria and tetanus among Lao adolescents.

Methods: Seven hundred seventy-nine serum samples were tested for anti-diphtheria and anti-tetanus antibodies.

Results: Overall, 25.8% of the adolescents had antibody titers corresponding to protection against diphtheria and 30.9% to sufficient immunity against tetanus. Female participants >16 years were more likely to be protected against diphtheria ($p < 0.001$) and tetanus ($p < 0.029$).

Conclusion: Low protection against diphtheria and tetanus, possibly due to low vaccination coverage or antibody waning, suggests booster doses are warranted before adolescence.

KEYWORDS

diphtheria, Laos, seroprevalence, tetanus, vaccination

INTRODUCTION

Diphtheria, caused by *Corynebacterium diphtheriae*, usually spreads person-to-person by droplets. The typical clinical manifestation is pseudo-membranous pharyngitis and the disease has a mortality rate of 5%–10% [1]. Tetanus occurs after infection with toxigenic strains of the bacterium *Clostridium tetani*, mainly via broken skin or in mothers and neonates during/shortly after childbirth due to unclean birth practices or cord care. The toxin causes motor neuron hyperactivity, hypertonia and muscle spasms resulting in high morbidity and mortality in the absence of medical intervention [2, 3].

Diphtheria and tetanus vaccines are important during childhood, typically given in combination and with or without other vaccines. WHO recommends three doses of diphtheria and tetanus vaccination in the first 12 months of life

and three further booster doses before adolescence [4]. Women of child-bearing age are also included in tetanus vaccination schedules in order to protect both the mother and newborn against Maternal and Neonatal Tetanus (MNT). WHO recommends to provide booster doses to women of reproductive age who have not yet received six doses [4].

The Lao People's Democratic Republic (PDR) is a landlocked country in Southeast Asia with approximately 7 million inhabitants. The Lao PDR is administratively divided into 17 provinces and 1 prefecture [5], which again are separated into 148 districts. The country has some of the lowest health indicators in the WHO Western Pacific Region, for example, high under-5 mortality rates (46 per 1000 children in 2017 [6]). The birth-rate in adolescence is also high at 101 per 1000 rural adolescent women in 2017 [6]. Nevertheless, significant health improvements have been achieved in the past 10 years, including MNT elimination—that is, less than one case per 1000 live births—in 2014 [7]. In the Lao PDR, diphtheria and tetanus vaccinations are part of the

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Sustainable Development Goal: Good Health and Wellbeing.

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pentavalent vaccine (diphtheria-tetanus-pertussis-hepatitis B-*Haemophilus influenzae*; DTP-HepB-Hib) scheduled at 6, 10 and 14 weeks of age. Yearly vaccination coverage estimates are available from 2010 onwards, but estimates for earlier years are less well documented. In the Multiple Indicator Cluster Survey from 2006, the first dose of the Diphtheria-Tetanus-Pertussis (DTP)-containing vaccine was given to 60.1% of the children aged 12 to 23 months. The proportion of children receiving the vaccine declined with subsequent doses and only 31.8% received the third dose [8]. WHO-recommended boosters for tetanus and diphtheria vaccines before the age of 15 are not routinely given. Tetanus toxoid-containing vaccines are offered to pregnant women during antenatal care (ANC) services, but the nationwide coverage was estimated at only 39.7% in 2021. Tetanus/diphtheria (Td) vaccinations may also be given to girls starting from age 15 in school-based campaigns, but there is no official reporting about this [9, 10].

Although reports of tetanus are rare, previous studies have suggested low immunity in male Lao adolescents [11] and missed opportunities to vaccinate pregnant women during ANC [12]. Furthermore, in 2017 less than half of Lao women of childbearing age were estimated to be protected against tetanus [6]. More than 100 cases of diphtheria were reported in 2012/2013 and in 2015 and 73 cases were reported in 2019 in the Lao PDR, likely due to low vaccination coverage and/or suboptimal vaccine immunogenicity [13–15].

The aim of this study was to determine seroprotection against diphtheria and tetanus among Lao adolescents in Vientiane Capital and Bolikhamxay province, thereby including urban and rural areas, and to provide evidence-based data to policy makers regarding the need for booster vaccinations.

METHODS

Study population

Serum samples from 779 adolescents collected in the framework of a randomised cross-sectional hepatitis B serology study in 2018 were utilised in this study. The sample collection has been described in detail previously [16]. In brief, randomly selected students aged between 11 and 18 years from schools in Vientiane Capital and Bolikhamxay province (Paksan and Pakkading districts) were asked to participate. All students and their guardians were asked for their consent and were informed that they could withdraw at any time. The study was approved by the Lao National Ethics Committee (Nr 022/NECHR) and the Institutional Review Board of the Institut Pasteur du Laos (Nr 9).

Serological testing

Samples were tested with commercial ELISA kits (Euroimmun) to determine IgG antibody levels against diphtheria and tetanus according to the manufacturer's instructions.

Anti-diphtheria titers were grouped into 'No protection' (<0.01 IU/mL), 'Uncertain protection' (0.01–0.099 IU/mL), 'Immunisation protection present' (≥ 0.1 IU/mL) and 'Long-term immunisation protection' (>1.0 IU/mL) [17].

Anti-tetanus titers were grouped into 'Insufficient immunity, booster vaccination recommended' (<0.1 IU/mL), 'Immunity given, booster vaccination will provide long-term immunity' (0.1–0.5 IU/mL), 'Sufficient immunity, booster vaccination in 2–5 years' (>0.5–1.1 IU/mL), 'Sufficient immunity, booster vaccination in 5–10 years' (>1.1–5.0 IU/mL) and 'Sufficient immunity, booster vaccination in approximately 10 years' (>5.0 IU/mL) [18].

If the samples exceeded the detection limits for the ELISA kits, they were retested in dilution if deemed useful considering resources and time constraints.

Data analysis

Data analyses were conducted using R software [19] (detailed description of the packages in Supplementary Data). For bivariate and multivariable analysis, values >0.5 IU/mL were considered to be 'sufficient immunity' against tetanus and ≥ 0.1 IU/mL 'immunisation protection' against diphtheria. Chi-square test and Fisher's exact test were applied as appropriate to identify variables associated with being protected against diphtheria and tetanus. Independent variables associated the outcome with a $p < 0.2$ in bivariate analyses were included in the multivariable modelling. Logistic regression models were conducted to estimate the odds ratio (OR), 95% confidence interval (CI) and p -values. The models were probed for a possible interaction between the variables sex and age and post-hoc analyses were performed. The correlation between anti-diphtheria or anti-tetanus titre and the age of the participants was assessed by calculating the spearman coefficient (ρ). A p -value < 0.05 was considered statistically significant. All code used in the analyses is publicly available on [GitHub](#).

RESULTS

Population characteristics

Most of the participants were of Tai-Kadai ethnicity (Vientiane Capital: 98%; Bolikhamxay province: 93%). The median age of the participants was 15 years and 49% were male (Vientiane Capital: 47%, Bolikhamxay province: 51%). A detailed description of the study cohort has been previously published [16]. When asked about their vaccination history, 3 students could provide their home-based vaccination cards including the childhood immunisations and 13 students had records of additional vaccinations. Among those, 10 students (1.3% of all participants) had a record of a recent tetanus vaccination. All these students (aged 14–18 years) were female and from Bolikhamxay province.

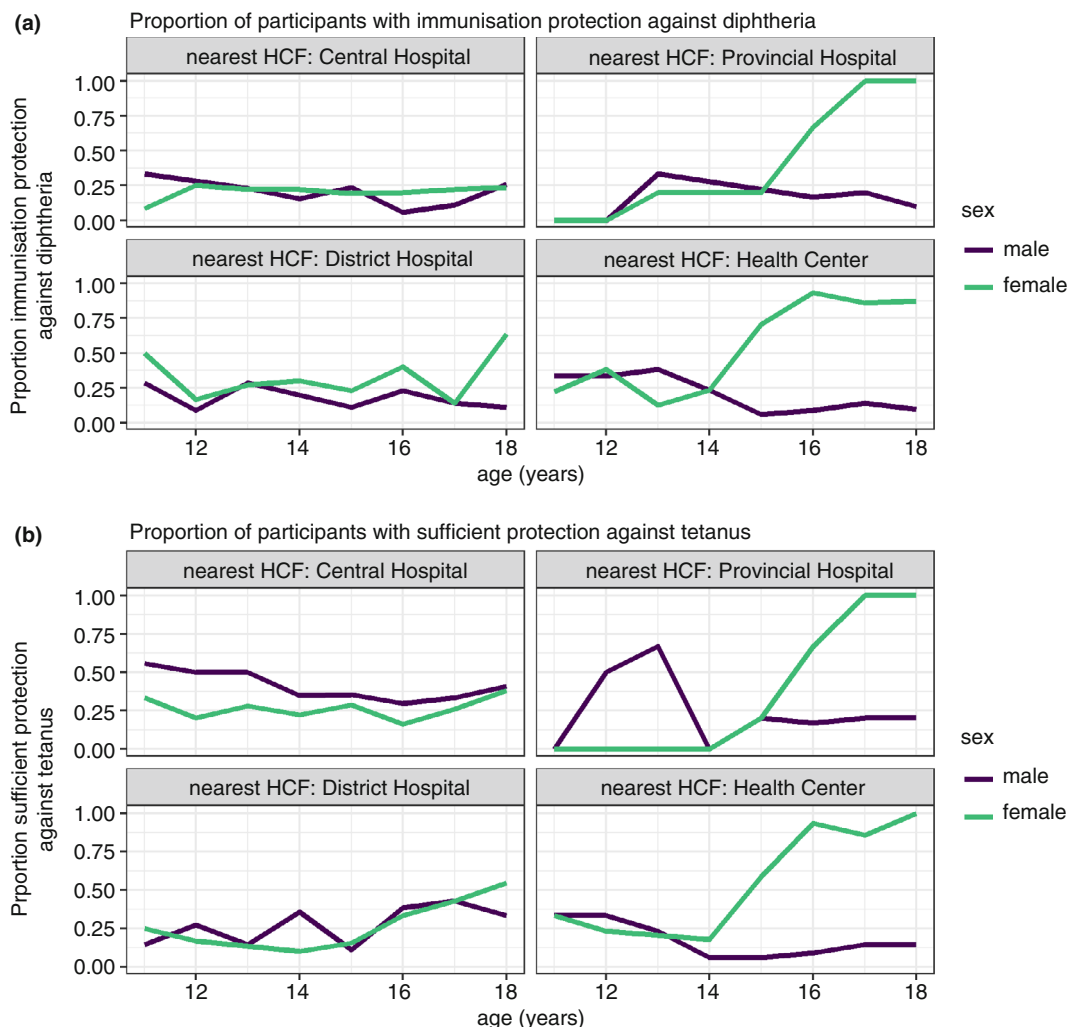


FIGURE 1 Proportion of participants with immunisation protection (≥ 0.1 IU/mL anti-diphtheria titre) against diphtheria (a) and sufficient immunity (> 0.5 IU/mL anti-tetanus titre) against tetanus (b) according to age and stratified by sex and nearest health care facility. HCF, health care facility.

Seroprotection against diphtheria

Overall, 25.8% of participants had at least anti-diphtheria IgG antibody titres ≥ 0.1 IU/mL, indicating immunisation protection (Table S1). Very few participants (5.1%) were not protected against diphtheria and 3.6% showed long-term protection. The majority of participants (69.1%) had antibody levels corresponding to ‘uncertain protection’ and fewer participants had antibody levels corresponding to ‘immunisation protection present’ (22.2%) (Table S1; Figure S1). We observed differences in the proportion of participants protected against diphtheria according to age, sex and type of nearest health care facility: The proportion of female participants with antibody levels corresponding to ‘protection present’ or ‘long-term protection’ was highest in the age groups 15 to 18 years and those living close to a Provincial hospital or health center (Figure 1, Figure S1). The same trend was observed by looking at the titre data: Age and anti-diphtheria titre were positively correlated among female participants ($\rho = 0.3$; $p < 0.0001$), but not among

male participants ($\rho = -0.02$; $p = 0.673$) (Figure S2). Four of all tested samples had an anti-diphtheria titre above the detection limit of the kit and were not retested.

In bivariate analysis, female participants, participants older than 16 years, participants recruited in Bolikhamxay province, especially in Pakkading district, and participants whose closest health facility was a health centre were more likely to be protected (Table S2). The variables ‘province’, ‘district’ and ‘nearest health care facility’ were highly correlated: most students recruited in Vientiane Capital lived close to a Central hospital while most students recruited in Pakkading, a rural area, lived closest to a health centre (Figure S3). The best fitting model included the variables ‘sex’, ‘age’ and ‘nearest health care facility’; and an interaction between sex and age (Table S3). After post-hoc analyses of the final model, female participants older than 16 years were significantly more likely to be protected against diphtheria compared to males ≤ 13 years of age (OR = 2.89; 95% CI: 1.92–4.34; $p < 0.0001$) (Table 1). Participants living closest to a health centre were also more likely to be protected than

TABLE 1 Model estimates after post-hoc analyses of the binomial generalised linear model for immunisation protection against diphtheria and for sufficient immunity against tetanus.

Model	Variables	Estimate	SE	Odds	OR	95% CI	<i>p</i> Value		
Immunisation protection against diphtheria	Type of nearest HCF - Central hospital	-1.58	0.15	0.21	Ref				
	Type of nearest HCF - Provincial hospital	-1.35	0.30	0.26	1.25	0.65-2.42	0.906		
	Type of nearest HCF - District hospital	-1.24	0.20	0.29	1.40	0.87-2.28	0.516		
	Type of nearest HCF - Health centre	-0.52	0.14	0.60	2.89	1.92-4.34	<0.0001		
	Interaction (Age: Sex)								
	Age group ≤13 years: Male	-1.24	0.24	0.29	Ref				
	Age group 14-16 years: Male	-2.01	0.25	0.13	0.47	0.24-0.89	0.196		
	Age group >16 years: Male	-1.79	0.27	0.17	0.58	0.29-1.15	0.630		
	Age group ≤13 years: Female	-1.40	0.23	0.25	0.86	0.46-1.61	1.000		
	Age group 14-16 years: Female	-0.63	0.18	0.53	1.85	1.06-3.21	0.255		
	Age group >16 years: Female	0.05	0.21	1.05	3.64	1.99-6.64	<0.001		
	Sufficient immunity against tetanus	Interaction (Age: Sex)							
		Age group ≤13 years: Male	-0.56	0.20	0.57	Ref			
Age group 14-16 years: Male		-1.23	0.19	0.29	0.51	0.30-0.88	0.143		
Age group >16 years: Male		-0.95	0.21	0.39	0.68	0.38-1.20	0.763		
Age group ≤13 years: Female		-1.60	0.24	0.20	0.35	0.19-0.65	0.011		
Age group 14-16 years: Female		-0.77	0.17	0.46	0.81	0.49-1.34	0.962		
Age group >16 years: Female		0.29	0.20	1.34	2.35	1.45-4.07	0.029		

Abbreviations: 95% CI, 95% confidence interval; HCF, health care facility; OR, odds ratio; SE, standard error.

participants living closer to a Central hospital (OR = 3.64; 95% CI: 1.99-6.64; $p < 0.0001$).

Seroprotection against tetanus

In total, 30.9% of participants had an anti-tetanus IgG titre >0.5 IU/mL corresponding to 'sufficient immunity' (Table S4). Serological profiles were relatively similar at both study sites: the proportion of participants with sufficient immunity was 31.7% in Vientiane Capital and 30.2% in Bolikhamxay province (Table S4). Similar to the anti-diphtheria data, female participants aged 15 and older showed highest proportion of 'sufficient immunity' (Figure S4). The same trend was also observed looking at the titre data: Age and anti-tetanus titre were positively correlated in female participants ($\rho = 0.23$; $p < 0.0001$) and negatively in males ($\rho = -0.14$; $p < 0.008$) (Figure S5). Of all samples, 49 sera had an anti-tetanus titre exceeding the detection limit of the ELISA kit and were tested again in dilution (1:400). After retesting, anti-tetanus IgG titers of 12 samples remained above the detection limit and were not retested.

When the proportion of sufficiently protected participants was plotted according to age and stratified by sex and type of nearest health care facility, the observed pattern was similar to the anti-diphtheria data: a higher proportion of older female students living closest to the provincial hospital or a health centre were protected as compared to younger males (Figure 1).

In bivariate analysis (Table S2), age and sex were associated with being sufficiently immune and both variables were included in the logistic regression model. There was a significant interaction between the two variables, indicating that the protection against tetanus by age depends on the sex of the participants and vice versa (Table S5). In post-hoc analyses, when all possible participant groups were compared to males ≤13 years, female participants ≤13 years were 0.35 times less likely to have sufficient immunity against tetanus (95% CI: 0.19-0.65; $p = 0.01$) (Table 1). Female participants >16 years were 2.4 times more likely to have sufficient immunity (95% CI: 1.45-4.07; $p < 0.029$).

DISCUSSION

In this study, we found low sufficient or immunisation protection against tetanus and diphtheria among Lao adolescents from Vientiane Capital and Bolikhamxay province. The proportion of participants with sufficient levels of antibodies against diphtheria was only 26% (vs. around 50% in a study from Thailand) [20]. A low diphtheria seroprotection is concerning and may indicate susceptibility in younger ages where the disease is more severe [15]. Indeed, although the number of reported cases in Lao PDR has decreased since introduction of the vaccine in infants, WHO reported 73 cases in 2019 [13] and a study of outbreaks in 2012/2013 found a median age of 10.5 years [14]. In the current study, the seroprotection increased significantly with

age in female participants. This increase with age for anti-diphtheria, as well as for anti-tetanus antibodies, was strongest in those women whose closest health facility was a Provincial hospital or a health centre, maybe reflecting better vaccination coverage in those locations or possibly exposure to the pathogen.

The overall protection against tetanus was 30.9%, lower in male adolescents (28%) than in females (34%). Although females have the risk of MNT, adolescent and adult males have other routes of exposure such as from contaminated wounds, and men make up a large proportion of cases in low-income countries [21]. Indeed, even in those few protected males in the current study, lower antibody levels were seen with increased age underlining the high susceptibility of adult males.

Although females had higher overall protection against tetanus, the younger adolescent girls had a slightly lower chance to be protected than boys. However, protection in girls increased significantly with age and those >16 years were more likely protected than young girls or boys of the same age. This might be due to the policy to vaccinate females aged 15–49 with tetanus containing vaccines and thereby reducing the risk of MNT. The introduction of Td combined vaccine within the last 10 years also explains the similar increase in diphtheria antibody titres in older female adolescents. MNT was eliminated from the Lao PDR according to a validation study in 2013 [22] due to a concerted effort by the Lao Ministry of Health to vaccinate women of reproductive age and to improve birth practices and umbilical cord care. Here, although we see that the policy resulted in an increased seroprotection of adolescent females, a substantial proportion (66.4%) were still not sufficiently protected (titres <0.5 IU/mL). This compares to less than 5% with these antibody levels in a study in neighbouring Thailand, where boosters—for males and females—are implemented at the age of 18 months and 4–6 years and are recommended at 11–12 years of age and every 10 years thereafter [20]. In 2017 in Vientiane Capital and Bolikhamxay province, only 49.4% and 55.9% of women of child-bearing age were estimated to be protected against tetanus, indicating a need to strengthen the existing vaccination policy [6]. Furthermore, in the Lao PDR, there are still many young pregnancies, especially in rural areas, where females below 15 years of age would benefit from a booster vaccination [23].

A limitation of the current study was that we did not have access to vaccination history of the vast majority of the participants. Therefore, the reason for low tetanus and diphtheria seroprotection could be either low vaccination coverage or waning of vaccine-induced antibodies or both. Also, caution is advised when extrapolating data to the whole country, since the study took place in only two provinces.

Taken together with our previous observations of missed opportunities to vaccinate during early ANC visits [12], these data suggest a strengthening of tetanus and diphtheria vaccination coverage and booster doses in females before or during adolescence should be considered to maintain elimination of MNT and reduce susceptibility to diphtheria infection. The

Lao Ministry of Health should also review the burden of tetanus and diphtheria in adolescent and adult males and consider Td vaccination boosters in females and/or males before adolescence. A cost-benefit analysis may also provide valuable insights into which vaccination strategy and which vaccine combination may be most suitable for the Lao PDR.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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