Measles neutralization antibodies and some related factors in women of Reproductive-age in Hanoi, Vietnam

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ABSTRACT

Aims: Our study aimed to determine neutralization antibodies against measles virus and some related factors in women of childbearing-age from 18 to 30 years old, in Hanoi, Vietnam.

Methodology: In 2018, a cross-sectional study was conducted on 1,235 childbearing-age women from 18-30 years old in Bavi district, Hanoi, Vietnam. The study participants were interviewed by using a structured questionnaire which included demographic information, and history of measles. The measles neutralization antibodies (MNA) of these participants were quantified by a plaque reduction neutralization test (PRNT). The data were analyzed using the Epidata 3.1 and Stata 14.2 software.

Results: The results showed that only 58.5% had MNA at sufficient protective levels (≥120mIU/ml), up to 12.5% were negative for MNA, and 358 women (29%) had MNA at insufficient protective levels. In addition, we found that the participants' working as public employees, having two children, and being ≥ 25 years of age were all positively associated with the sufficient protective antibody levels (P<0.05). A weak positive correlation between age and MNA titers was determined (r= 0.26).

Conclusion: Approximately half (41.5%) of the women of childbearing age in this study did not have sufficient protective antibodies against measles virus. The study results suggest that it is necessary to boost measles vaccine to prevent the sporadic measles infection in women of childbearing age in Hanoi and to quantify the dynamics of maternal measles antibody levels in infants born from these women, so as to provide evidences to control measles incidence in children up to 9 months of age in the future.

Keywords: measles, neutralization antibody, reproductive- age woman, Vietnam

1. INTRODUCTION

Measles is one of the most common infectious diseases in young children. According to the World Health Organization (WHO), prior to the availability of measles vaccine, about 90% of children had been infected with measles before reaching the age of 15 [1]. About 2.6 million deaths and 15,000-60,000 infants had been blinded by measles per year worldwide [2]. Currently, deaths due to measles have decreased to approximately 134,200 globally. Measles vaccination resulted in a 75% reduction in measles deaths from 2000 to 2013 worldwide and active measles immunization was considered the best means of preventing the disease [1]. Although many efforts were made globally for the control and elimination of measles in at least five WHO Regions which were targeted by 2020, within 2017-2018, measles outbreaks occurred in Americas, Eastern Mediterranean, Europe, Africa, and Southeast Asia. In some countries and regions, measles re-emerged after being eliminated, and the goal of eliminating measles in 5/6 regions of the world by 2020 has not been achieved [3].

In Vietnam, from 1979 to 1984, the measles incidence was from 69.4 to 150.5 per 100,000 population with a death rate due to measles ranged from 0.23 to 0.6. Thanks to the effectiveness of the expanded program on immunization (EPI) over the years, the measles incidence rate in Vietnam has decreased from 150.5/100,000 population in 1984 to 8.5/100,000 population in 2002. However, measles still remained the ninth leading cause of death in children in the period of 1996 - 2000 [4]. In 2014, measles epidemic occurred in most of the provinces/cities in the country, mainly in the group of 1 - 4 years old (32.2%), followed by a group of children under 1-year-old (22.8%). Particularly, the incidence rate of the group of children under 9 months of age was 13.2%, and that of the group from 9 to 11 months accounted for 9.6%. There were 148 deaths from measles after 12 consecutive years without measles deaths, including the deaths of children before the age of vaccination [5].

In Hanoi, although there have been many efforts made for measles control and prevention, in 2008-2009, there was a measles outbreak with a total of 946 confirmed measles cases, the patients were mainly children aged 15 years and older (71.1%) [6]. Four years later, from December 2013 to the end of 2014, a big measles outbreak occurred with 5,970 confirmed cases and 14 deaths. Most cases (57.1%) were children under 5 years old, and 24.6% was children under 9 months [7]. Measles cases in children under 9 months of age, who have not yet reached the age of vaccination were thought to be due to part of low maternal antibodies. The reason for the low measles antibody titer from the mothers may be that the immunity acquired through vaccination is weaker than the immunity from natural infection, or that the vaccine has reduced the number of measles cases, thus reducing the chance of booster effect on measles virus. Therefore, in 2019, Nguyen and colleagues conducted a study on 401 women giving birth at General Hospital of Ba Vi District, Hanoi. The results showed that 77.06% of women were positive for measles-specific IgG antibodies, and that 72.00% of newborns from mothers aged under 25 and 93.53% those from mothers over 30 years old were measles seropositive. The proportion of measles IgG antibodies together with geometric mean titer (GMT) decreased from 82.97% (506.96 mIU/ml) at the age of three months to 23.19% (45.22 mIU/ml) at the age of nine months [8]. Following up the previous study, we measured measles neutralization antibodies in women of childbearing age and related factors to prepare for an intervention study on evaluation of the effectiveness of measles vaccination to reduce measles incidence in women of childbearing age and their babies up to 9 months in Hanoi, Vietnam.

2. MATERIAL AND METHODS

2.1. Study design

A cross-sectional study was conducted from April 2018 to May 2018 in Ba Vi district where the vaccination rate has been high since the implementation of EPI in Vietnam in 1981 and where there had not been any measles epidemic reported for many years [9].

Ba Vi is one of the 30 districts and towns of Hanoi city. It is a district in the semi-mountainous region, located to the northwest of Hanoi capital. Ba Vi has a total of 424km2, with a population of more than 265 thousand people, including 3 ethnic groups: Kinh, Muong, and Dao. Ba Vi has 31 communes, inclusive of 7 mountainous communes. Ba Vi district is bordered to 3 mountainous provinces in the north of Vietnam, Hoa Binh province to the south, Phu Tho province to the west and Vinh Phuc province to the north. The location of the study is shown in Figure 1.

Women with the following criteria were selected to the study: living in Ba Vi district, age 18-30, agree to participate in the study, consent to provide information on sociodemographic and clinical information, are not immunocompromised people, have no acute illness at the time of participation to the study, and agree to donor their blood for measles antibodies testing.

A total of 1,235 women aged 18-30 were included in the study. The subjects who aged from 18 to 30 years old were selected as a convenience with a written agreement to participate in the study. Study participants were invited to their commune health stations. At first, the participants were interviewed on a structured questionnaire including information about demographics, history of measles, acute/chronic illness, marriage status, number of children... Then, 3ml of venous blood of these participants were taken in order to quantify the neutralization antibodies against the measles virus by a plaque reduction neutralization test (PRNT).

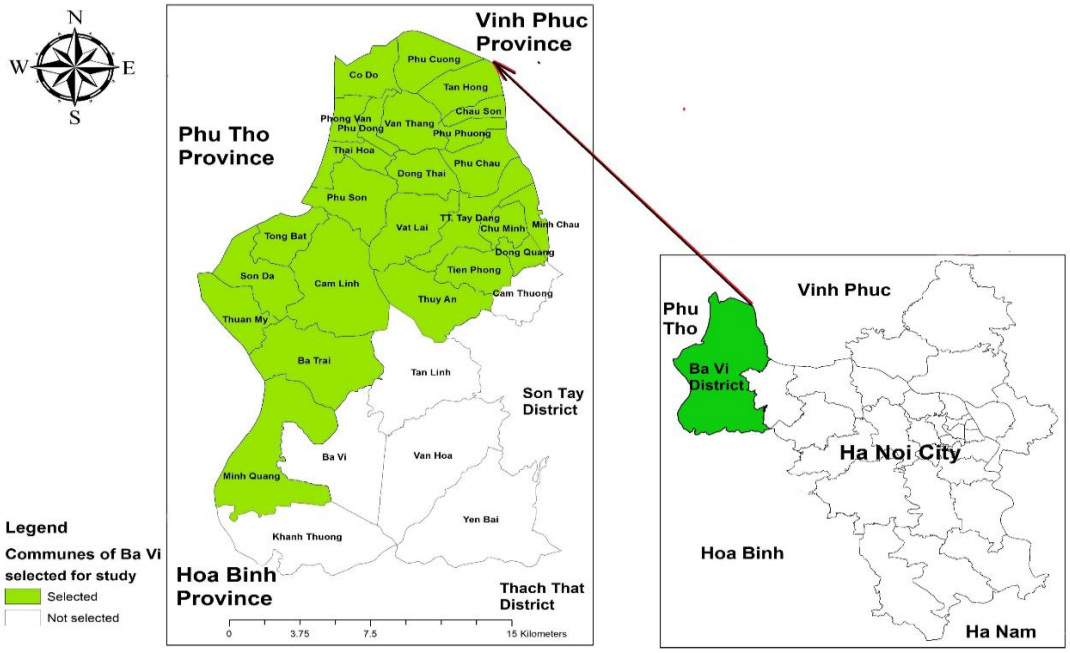

Figure 1. Location of the study area

A. Map of Ba Vi district; B. Map of Hanoi city

2.2. Laboratory techniques

In brief, PRNT was implemented as below [10,11]: Sample serum and International Standard Measles serum (3rd International Standard, NIBSH, 97/648) were heat inactivated at 56^{o}C for 30 minutes. Then 6 fourfold dilutions starting from 1/4 to 1/4096 of each heat inactivated serum were prepared in 2% MEM. An equal volume of a challenge virus (AIK-C strain) at the concentration of 100 PFU/100 µl was added to the volume of serum dilution. Then the mixtures were incubated at 32°C for 60 min. After incubation, the virus-serum mixtures were infected onto monolayer Vero/SLAM cells, which were previously cultured on a 24-well cell culture plate. The plates were further incubated in a CO_2 incubator at 32^{o}C with 5% CO_2 for 60 minutes. After exposing the cells to the virus-serum suspension for 60 minutes, the cells were overlaid with 1 ml of 1% methylcellulose solution (Cat.M0512, Sigma). The plates were incubated in a CO_2 incubator at 37^{o}C with 5% CO_2 for 5 days. The cells then were fixed with 0.5 ml of 10% formaldehyde solution for 1 hour. The fixative was removed and the cells were stained with 0.5 ml of 5% Crystal Violet staining solution (Cat. no. 548-62-9, Merck) at room temperature for 30–45 minutes. The plates were rinsed under running water and the number of plaques in each well was counted. Fifty percent endpoint titers (Neutralizing Dose, ND50) were calculated using the Karber formula:

log10 ND50 = m- Δ ($\sum$p – 0.5), where:

m: is the log10 of the highest dilution

Δ: is the constant interval between dilution expressed as log10

$\sum$p: is the sum of all the proportions of number of plaques/average number of plaques for virus control.

ND50 results were transformed into mIU/ml: a unitage constant was calculated by dividing the concentration of antibody in the international standard serum by the ND50 of the international standard serum (3,000 mIU/ml/ND50). ND50 results of test sera were then multiplied by unitage constant to obtain results in mIU/ml.

A serum sample was considered as a sufficient protective level when neutralizing antibody concentration is greater than or equal to 120mIU/ml [10,11].

2.3. Data collection and analysis

The data were entered and analyzed using the Epidata 3.1 and Stata 14.2 software. Algorithms including percentage calculation, geometric mean, OR, and univariate logistic regression analysis were used in the process.

3. RESULTS

The research subjects were mainly from the age group of ≥25 years old, accounting for 72.9%. The mean age of the study group was 26.2 ± 2.9. Regarding the subjects' professions: public employees were 314/1,235 (25.4%); workers (15.1%); farmers (28.4%); freelance laborers (19.1%) and housewives (12.0%). Among the participants, 81.2% had a high school/university degree, 17.0% graduated from secondary school, and 1.8% had primary school education. Altogether, 619/1,235 participants (50.1%) had 1 child, 40.1% had 2 children, and 3.6% had 3 or more children. Most of the subjects (72.9%) reported that they had not had measles in their lives (Table 1).

Table 1. Demographics of research subjects (n=1.235)

Characteristics	Number (n)	Percentage (%)
Age (years)		
≤20	56	4.5
21 – 24	279	22.6
≥25	900	72.9
Mean ± SD	26.2 ± 2.9	
Profession		
Farmers	351	28.4
Public employees	314	25.4
Freelance laborers	236	19.1
Workers	186	15.1
Housewives	148	12.0
Education background		
Primary school	22	1.8
Secondary school	210	17.0
High school	454	36.8
College	351	28.4
University/graduate	198	16.0
Number of children		
No children yet	76	6.2
One child	619	50.1
Two children	495	40.1
≥ 3 children	45	3.6
History of measles infection		
Infected	195	15.8
Not yet infected	900	72.9
Unknown	140	11.3

Of the total 1,235 women enrolled in the study, only 722 (58.5%) women had sufficient protective neutralization antibody titers (≥120 mIU/ml). Particularly, up to 154 (12.5%) participants were negative for measles neutralization antibodies, and 359 (29.0%) women had measles neutralization antibodies (MNA) at insufficient protective levels (Figure 2).

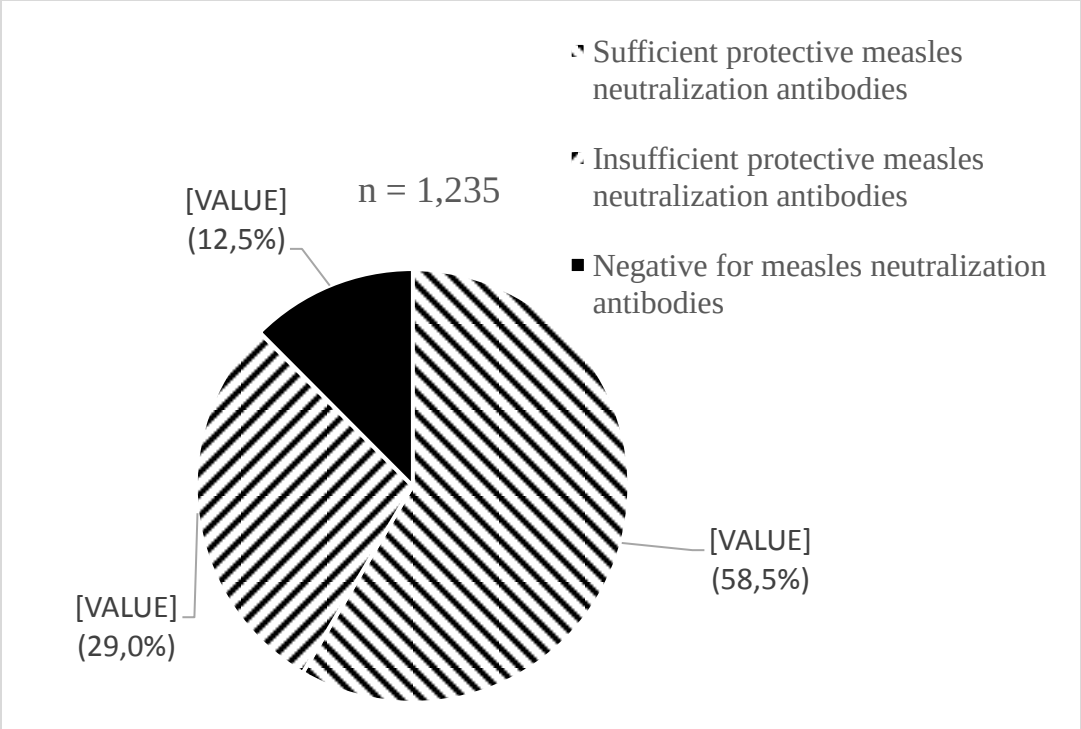

Figure 2. Proportion of women aged 18-30 years with protective measles neutralization antibodies (n=1,235)

The geometric mean titer (GMT) analysis of the measles neutralization antibody (Table 2) showed that groups of participants who had GMT at a protective level were those who were over 25 years of age (149.2 mIU/ml), those with 2 children (145.6 mIU/ml) and 3 children (125.1 mIU/ml), and those who worked as freelance laborers (129.3 mIU/ml) and public employees (137.0 mIU/ml). Those who occupied as farmers and workers, those with no or one child, and those who were under 25 years old had GMT at an insufficient protective level (<120mIU/ml).

Factors associated with having sufficient protective MNA among groups of participants were analyzed. The results showed that there were significant differences between age groups of >25 years old and under 20 years old (OR=1.94; *P*=0.01), between groups of having 2 children and having no child (OR=1.86, *P*=0.01), and between group of public employees and farmers (OR=1.43, *P*=0.02). People who reported ever having measles had sufficient protective MNA 1.94 times higher than those without measles (OR=1.94; *P*=0.00) (Table 2).

Table 2. Measles neutralization antibody titer among women of reproductive age by sociodemographic and clinical factors

Characteristics		GMT (mIU/ml)	Protective neutralization antibody levels			
			Not enough protection N(%)	Enough protection N(%)	*P*	OR (95%CI)
Age (years)						
	≤20	66.5	29 (5.6)	27 (3.7)	-	1
	21 – 24	65.1	163 (31.8)	116 (16.1)	0.36	0.76 (0.4 – 1.4)
	≥25	149.2	321 (62.6)	579 (80.2)	**0.01**	**1.94 (1.1 – 3.3)**
Number of children						
	No children yet	61.2	40 (7.8)	36 (5.0)	-	1
	One child	110.0	271 (52.8)	348 (48.2)	0.15	1.43 (0.9 – 2.3)
	Two children	145.6	185 (36.1)	310 (42.9)	**0.01**	**1.86 (1.1 – 3.0)**
	≥ 3 children	125.1	17 (3.3)	28 (3.9)	0.12	1.83 (0.9 – 3.9)
Profession						
	Public employees	137.0	116 (22.6)	198 (27.4)	**0.02**	**1.43 (1.05 – 1.95)**
	Workers	107.0	80 (15.6)	106 (14.7)	0.57	1.11 (0.78 – 1.59)
	Farmers	111.2	160 (31.2)	191 (26.5)	-	**1**
	Freelance laborers	129.3	92 (17.9)	144 (19.9)	0.11	1.31 (0.94 – 1.83)
	Housewives	106.1	65 (12.7)	83 (11.5)	0.73	1.07 (0.73 – 1.57)
History of measles infection						
	Infected	196.4	57 (11.1)	138 (19.1)	0.000	**1.94 (1.39 - 2.72)**
	Not yet infected	104.4	401 (78.2)	499 (69.1)	-	1

Unknown	139.9	55 (10.7)	85 (11.8)	0.243	1.24 (0.86-1.78)
Ethnic groups					
Kinh	116.6	484 (94.4)	672 (93.1)	0.37	0.8
Others	166.2	29 (5.6)	50 (6.9)		

There was a weak positive correlation (r=0.26) between the titers of MNA and the age of the study subjects (Figure 3). The equation showed that for every one-year increase in age, the MNA titer would increase by 38.2 mIU/ml, *P*=0.0001 (Table 3).

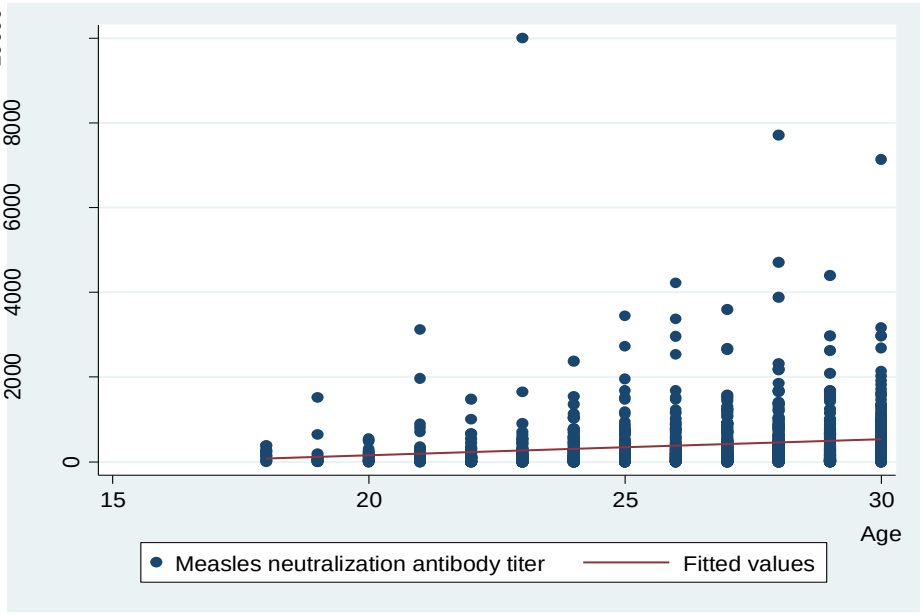

Figure 3. Correlation between measles neutralization antibodies and the age of woman 18-30 years old, in Hanoi, Vietnam (r = 0.26)

Table 3. Correlation between measles neutralization antibody titer and age of the study subjects

Characteristics	Coefficient a	*P*	95%CI
Measles neutralization antibody titer	38.2	0.0001	25.4 – 50.9
Equation	Neutralization antibody titer = 38.2*age – 614.2		

4. DISCUSSION

This research was selectively conducted in Ba Vi where there had been high vaccination rates for children reported in the EPI in many years with measles vaccination rates for children from 9 months of age always ranged from 95% to 99% [9]. In addition, Ba Vi was one of the districts of Hanoi that has not had a measles outbreak since 1995 according to the regulations of the Ministry of Health of Vietnam on measles epidemic surveillance and prevention [12]. In this regulation, the Ministry of Health clearly stated that a measles outbreak occurred when there were ≥ 3 epidemiology linked or laboratory-confirmed cases of measles in a district within 1 month, when the rash onsets of two cases must be within 7 and 21 days apart, and when at least 2 cases were confirmed by laboratory diagnosis [12]. Therefore, the results of this study would also be a premise for the implementation of further intervention studies to reduce the incidence of measles in childbearing – age women and their children under 9 months of age in Hanoi in the future.

A total of 1,235 women aged 18-30 participated in the study, of which only 58.5% participants had sufficient protective MNA, 41.5% of participants did not have MNA or had MNA at insufficient protective levels (Figure 2). These results may explain why the prevalence of measles in the over 15 years old group in recent measles outbreaks in Hanoi were quite high. Specifically, during the measles outbreaks in 2008-2009 and 2013-2014, the prevalence of measles in the age group over 15 years old were 71.1% and 30.9%, respectively [6,7]. In addition, analysis of geometric mean titer (GMT) of MNA of the subject groups showed that participants who occupied as farmers and workers, those of older age group and more children may have had natural infections which gave them higher GMT and longer immunity. The younger age group were more likely to have experienced only vaccine-induced immunity which is likely to be less than natural immunity for measles. These research results suggested that periodic booster shots of measles vaccine should be given to women of childbearing age, paying special attention to the group of women under 25 years old, childless or having one child, working as farmers and workers to prevent sporadic measles infections in this age group.

A number of discrete studies on the MNA of childbearing-age and pregnant women in some localities in Vietnam have also been carried out. The results showed that there were different proportions with sufficient protective MNA as found in the previous studies. For example, in 2016, the Dang et al.'s reported that only 71.7% pregnant women in Dong Anh district, Hanoi city had IgG antibodies against measles virus. Among them, up to 90.5% of women over 30 years old had measles antibodies [13]. Another study by Nguyen et al. in 2013 indicated that the percentage of women aged 16-30 years old in some northern provinces of Vietnam having MNA was 70.1%, of which the group of women over 30 years old having MNA was higher and reached 94.2% [14]. In a study by Nguyen et al. in 2016, also in Ba Vi, Hanoi, out of 401 women giving birth at the Ba Vi district hospital, 77.06% was seropositive for MNA. Among them, the group of women aged 30 years and older having MNA was 88.06%, higher than those of mothers aged ≤ 25 (66%) [8]. The difference in the prevalence of neutralization antibodies against measles virus in this study from previous studies in Vietnam may be due to the differences in study locations, measles vaccine coverage rates, research subjects, study time, the different endemic regions, and laboratory testing methods. However, all these research results showed that there was a rather large proportion of women of childbearing age, pregnant women or women at birth were negative for MNA or had MNA at insufficient protection levels. Therefore, there is a need for conducting a nationwide study on the persistence of MNA in childbearing-age women to have an effective strategy to control measles in this age group in the nation.

Analysis of the results of MNA at sufficient protective levels by the age group showed that the group of over 25 years old had 1.94 times higher sufficient protective antibodies than the group of subjects under 20 years old did (*P*=0.01)(Table 2). The study results also indicated that the MNA titers gradually increased and for every additional one year of age, the MNA titers increased to 38.2mIU (*P*=0.0001) (Table 3). In addition, there was a weak positive correlation between ages and MNA titers (r=0.26) (Figure 3). The reason could have been that the older the age, the greater the chance of having been exposed to the measles virus, so the MNA titers would be higher. Another reason would be that from 2000-2010, Hanoi continuously organized additional measles vaccination campaigns for children 6 years of age, and 10-12 years old. Therefore, the age group over 25 years old had a higher chance of receiving more booster doses of measles vaccine, thus having higher antibodies. The study also indicated that the occupational group of public employees had 1.43 times higher sufficient protective MNA than those of the farmer group did (*P*=0.02) (Table 2). Such difference may have occurred because the group of public employees had more knowledge and consideration to the disease than the farmer group so they can get MMR vaccine, which is not part of EPI, but recommended for women of childbearing-age. Therefore, their MNA titers were higher than those of the farmer group.

Of the 1,235 participants, 154 (12.5%) were negative for MNA (Figure 2), while it was reported that the vaccination rate for children under 1 year old in EPI, and for children aged 5- 6, and 10 - 12 years old in the booster measles vaccination campaigns in Ba Vi district regularly achieved to >99% [9]. Thus, there is a need to review and evaluate the accuracy of the EPI reporting system in Ba Vi district.

In this study, of the 1,235 women aged 18-30 years old who enrolled in the study, up to 41.5% of women did not have enough protective MNA (Figure 2). The studies worldwide showed that there were significant differences in the status of neutralization antibodies against measles virus in pregnant/childbearing-age women in different regions and countries around the world. A study by Brugha et al in the UK (1996) showed that up to 23% of women vaccinated with measles vaccine at their childhood did not have sufficient protective antibodies to measles (MNA <200 mIU/ml) [15]. A study in Belgium (2010) showed similar results to the study in the UK that 26% of the vaccinated women had insufficient protective antibodies [16]. In 2013, another study by Martins et al. in India indicated that 4% of pregnant women did not have enough measles protective antibodies [17]. Qian et al. (2010) reported that 88.68% of women in Shanghai, China had positive MNA; the rate of positive measles antibodies decreased gradually with age [18]. In 2013, a study in Catalonia (Spain) mentioned that 89% of pregnant women had enough protective antibodies against measles virus [19]. Recent research in Guangzhou, China by Lu et al. in 2016 published that the positive rate of MNA in the mother group was 87.3% [20]. In most countries worldwide, children who are under 9 months old are not eligible to receive their first shot of the measles vaccine in EPI. Meanwhile, measles infection in unvaccinated infants have been reported in several countries [21]. On the other hand, WHO does not recommend measles vaccination of 6-month of age, due to the immaturity of the immune system as well as the presence of maternal antibody, but recommends vaccination of 6-months of age during measles outbreak [22]. The surveillance system needs to be strengthened so that outbreaks can be detected at early stage and emergency vaccination responses can be made. That a proportion of the children who are under 9 months old have measles may be due to the fact that the mothers have not sufficient protective measles antibodies. This raises further the need to give one shot of measles vaccine booster dose for women before pregnancy, and to measure the dynamics of maternal MNA levels in infants born from these women in order to provide evidences to control measles incidence in women of childbearing-age and their children up to 9 months of age in the future.

5. CONCLUSION

Approximately half (41.5%) of the women of childbearing age in this study did not have sufficient protective measles virus antibodies. The study results suggest that it is necessary to provide a measles booster vaccination to prevent the sporadic measles infection in women of childbearing age in Hanoi and quantify the dynamics of maternal measles antibody levels in infants born from these women, so as to provide evidences to control measles incidence in women of childbearing age and their children up to 9 months of age in the future.

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COMPETING INTERESTS

None to declare

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration of all authors. Author THD designed the study, managed the field work, performed data collection and analysis, and wrote the first draft of the manuscript. Author LDP performed the laboratory testing. Authors AKTN, KK and SI managed the analyses of the study and wrote the first draft of the manuscript. Authors HDH and HNT designed the study. All authors read and approved the final manuscript.

CONSENT

All authors declare that written informed consents were obtained from all study participants for publication of this work. A copy of the written consents is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

The study was approved by the Medical Ethics Committee of the National Institute of Hygiene and Epidemiology, Vietnam, code IRB-VN011057/IORG 0008555.

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