MINISTRY OF EDUCATION AND TRAINING

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EPIDEMIOLOGICAL, CLINICAL, PATHOGENIC AND IMMUNOLOGICAL CHARACTERS OF MEASLES IN THE NORTH VIET NAM, 2013 - 2014

Specialization: epidemiology

Code: 62 72 01 17

SUMMARY OF PHD THESIS

The Thesis was finished at

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HA NOI – 2018

LIST OF ABBREVIATION

Abbreviation	English
ARN	Acid ribonucleic
BYT	Ministry of Health
ELISA	Enzyme Linked Immunosorbent Assay
GAVI	Global Alliance of Vaccine & Immunization
GMC	Geometric mean concentration
IFN	Interferon
IgA, IgG, IgM	Immunoglobulin
IL	Interleukin
MR	Measles - Rubella
MMR	Measles – Mumps -Rubella
MMRV	Measles – Mumps -Rubella– Varicella
PCR	Polymerase Chain Reaction
SYT	Provincial Health Department
SSPE	Subacute sclerosing Panencephalitis
TCMR	National Program of Expanded Immunization
TTYTDP	Provincial Preventive Medicine Center
UN	United Nations
UNICEF	United Nations Children’s Fund
VSDT	Hygene and Epidemiology
WPR	Western Pacific Region
WHO	World HealthOrganization

RATIONALE

Measles is an acute respiratory infection, caused by measles virus, circulating all over the world and is a common disease in young children. The disease may progress to self recover but in some cases it leads to complication that causes death. The disease is preventable by highly effective vaccines that help reducing morbidity and mortaligy dramatically. However there still have several epidemics in the world. 2013 and early 2014 saw an incresed number of measles cases. In WPRO, measles cases increased in some countries including Viet Nam. In Viet Nam, measles vaccine for children under 1 year – old has been introduced in EPI since 1985. Nevertherless outbreaks occurred every 3 – 4 years. From late 2013 to early 2014, epidemic occurred in almost all provinces nationwide, especially in the North. Most of cases were children under 10 years, particularly children under 9 months. Death mainly concentrated in this region. In order to have scientific evidences on measles epidemic in 2013 – 2014, contributing to measles control activities, aiming to measles elimination in coming years, we conducted the research **"Epidemiological, clinical, pathogenic and immunological characters of measles in the North Viet Nam, 2013 - 2014".**

Research objectives:

1. Describe epidemiological and clinical characters of measles in the North, 2013 - 2014.

2. Identify molecular epidemilogical characters of measles virus in the North, 2013 - 2014.

3. Evaluate measles immune situation of children until 9 years old and women 16 to 39 years old in Ha Noi in 2013, before the epidemic.

NEW CONTRIBUTION OF THE THESIS

The study has some new contributrions as it gave fairly sufficient description on epidemiological and clinical characters of cases and deaths involving measles in the North during 2013-2014, revealling that children without vaccination had high rate of being infected and deceased from measles;measles virus in the epidemic was not novel, mostly belonged to genotype H1 which had been circulating in Viet

Nam;children under 1 and young women (16-19 years old) had the low rate of protective IgG antibody level.

From the research, we recommend guaranteering sufficient, punctual and high vaccination coverage, especially in Northern mountainous areas to reduce as much as possible numbers of cases and deaths; monitoring and reporting the rate of vaccination regularly at commune level to have in time plan for supplement activities or immunization campaigns; when outbreak occurred, it is needed to estimate the spreading risk of outbreak to implement vaccinating people living in risk areas and surrounding places to minimize transmission; it is necessary to categorize levels of treatment, limit hospital admission of mild cases; the hospitals needs to stream-line examination, localize patients to reduce the risk of cross infection; considering vaccinating children 6 - 8 months old in the epidemic and giving booster doses of measles containing vaccines (MR, MMR...) to women before pregnancy to enhance passive immune in order to protect young children who are under ages of measles vaccination.

STRUCTURE OF THE THESIS

The Thesis includes 127pages, 4 chaptes: Rationale: 2pages, Chapter 1Background - 35pages, Chapter 2Research Methodology- 16pages, Chapter 3 Results - 36pages, Chapter 4 Discussion - 35pages, Conclusion- 2 pages, Recommendation- 1 page. The Thesis has 11tables, 11figures, 31charts, 107national and international reference documents.

CHAPTER I. BACKGROUND

1.1. General information of measles

1.1.1. Cause of the disease: Measles is caused by measles virus. Measles virus belongs to Paramyxoviridae family, sub family Paramyxovirinae, branch Morbillivirus, it transmits all the year. Measles virus causes disease on human only. Patients are the unique transmision source. No asymtomatic patients. No animal reservoir and vector.Measles virus releases with upper respiratory mucus. The virus is weak in the outer environment but is highly transmitted, highly geneticaly stable, both in lab and field.

1.1.2. Epidemiology of measles:

Measles is one of most transmitted infectious disease in human. The disease occurs all over the world, all year round.

Morbidity in men and women is equal. Young children usually receive antibody from their mothers. The passive antibody decreases after 6 months. After 9 months the antibody from mother is not enough to protect the child. People who have not been infected or vaccinated are succeptible. Immune obtained by measles infection or vaccination is permanent and enhanced by exposure with patients.

Before measles vaccination program, outbreak occured every year and epidemic occured every 2 – 3 years. It is estimated that every year there are 30 millions cases and more than 2 deaths world wide. More than 95% people infected before age of 15 years.

After the EPI, the number of cases increases every 3 – 4 years, compatible to an outbreak, then decreasing, big epidemic occurs every 7 -8 years. The intervals between outbreaks are time to accumulate succeptible people. In developing countries, low vaccination coverage, high birth rate and high resident condense, the transmission is high in young children and pre-school children. High morbidity swifts to older children including adolescents and youths when vaccination rate increases, because of immune gap in those ages.

1.1.3. Surveillance of measles:

Definition: Suspected measles case is the case with fever, rash and at least one of those symptoms as cough, running nose, conjunctivitis, lymph nodes (neck, occipital, ear), arthralgia.

Categorization of cases

- Lab confirmed case: the case is positive with measles virus IgM antibody by ELISA or specific gen of measles virus is detected by PCR or measles virus is isolated.

- Epidemiologically confirmed case: the suspected case without samples collection but has epidemiological connection with a case confirmed by lab testing or by epidemiology (contacted or might contact with confirmed case in the same environment, in which the interval time between 2 cases is 7 – 21 days);

1.1.4. Prevention:

- For people who have not contacted the case: using vaccines for long term prevention. EPI is giving free measles vaccines for children 9 months old and 18 months old.

- For people who have contacted the case: giving measles vaccines within 72 hours may help preventing.

1.1.5. Measles situation in the world and in Viet Nam:

- In the world: thanks to measles vaccines, from year 2000 to year 2011 the number of death caused by measles decreased by 71%, however we still encountered several outbreaks. As of 2011, there were 158.000 deaths and more than 20 million children have not been vaccinated. In 2012, there still had epidemics in 15 countries in Europe, Africa, South Asia and South East Asia.

- In Viet Nam: Measles is circulating in the whole country, all around year but usually becoming outbreaks in winter – spring, when cold and west weather gives favourable condition for more complications in patients, especially respiratory complication resulting in death. Since measles vaccine has been introduced in EPI for free vaccination in 9 months children since 1985, measles disease has been well controlled, number of cases in 2012 decreased by tens compared to that in 1984. Since 2011 measles vaccine has been given freely for 18 months old children as booster doses.

1.2. Distributrion of measles virus genotypes

In general, 3 patterns of measles virus genotype distribution have been described, depending on situation and containment of measles. In countries that still have endemic transmission, the majority of cases are caused by one or several endemic genotypes that are distributed geographically. In countries that have eliminated measles, the small numbers of cases are caused by a number of different genotypes that reflect various sources of imported virus and suggest that the lack of sustained transmission of an endemic genotype or genotypes. The third pattern occurs in countries or regions that have had very good measles control but are experiencing an increase in the number of susceptible individuals because of failure to maintain high vaccination coverage rates – the outbreaks are associated with a single genotype of virus with nearly identical sequences.

According to WHO virologic surveillance, during 2007 – 2009, genotypes B3, D4, D8, D9, H1 were found in European region. In South

East Asia the majority genotypes included D4, D5, D8. In Western Pacific region, genotype H1 was still endemic in China. H1a was circulating in Viet Nam and caused an outbreak in 2009. Genotypes H1, D9, G3 và D5 were found in other countries.

In Viet Nam, from 2006 to 2012 there were 61 throat swabs found positive with measles virus, mostly genotype H1.

1.3. Epidemiology:

Measles virus has only one serotype, but it has 8 clades designated A, B, C, D, E, F, G, H. Within these clades, there are 23 recognised genotypes.

Measles immune can be obtained after getting disease naturally or by vaccination.

1.3.1. Immunological responses to natural infection:

The early nonspecific immune responses occur during the prodromal phase of the illness. Specific immune response includes measles virus-specific antibody and cellular immune response. The first measles virus-specific antibody produced after infection are of the IgM subtype, followed by IgG.

The duration of protective immune following wild-type measles virus infection is generally thought to be life-long. Immune response to measles increases with measles infection but decrease to other antigens, lasting for several weeks to months after the acute illness.

1.3.2. Immunological responses to immunization:

Measles vaccines induce humoral and cellular immune response. After 1 dose injection at 8 – 9 months old, 89.6% vaccinees develop immune response, this rates reach 92.2% and 99% in children 9 – 10 months old and 11 – 12 months old respectively.

The studies show 97% children that have no immune response after first vaccination develop immune response at second injection. The second dose is designed to induce immune response in children who have not been protected at the first injection and to immunize unvaccinated children.

Children born to vaccinated mothers have passively-acquired maternal antibodies but with lower level compared to children born to naturally infected mothers, so they are still susceptible.

CHAPTERII. RESEARCH METHODOLOGY

2.1. Objective 1: Description of epidemiological and clinical characters of measles in the North, 2013 – 2014

2.1.1. Research target

Reports of cases and deaths related to measles (Investigation Form for suspected measles/rubella) according to guidance of surveillance and control of measles/rubella issued by the Ministry of Healch, investigation reports according to Measles Medical Record Form issued by National Institute of Hygiene and Epidemiology. Definition for measles cases and deaths were:

2.1.1.1. Definition of measles cases:

a)Surveillance cases (clinical cases): the case who has symptoms: fever, maculopapular rash (not haemorrhagic or vesicle) and at least one of those symptoms as cough, coryza, conjunctivitis (red eyes), lymph nodes(neck, occipital, ear) or arthralgia.

b) Confirmed cases: lab confirmed and/or epidemiologically confirmed cases.

2.1.1.2. Definition of measles involving deaths:

Deaths due to any specific cause and are confirmed by a central hospital that measles was the cause.

2.1.2. Place of research

- 28provinces, cities in the North (to Ha Tinh province).
- National Institute of Hygiene and Epidemiology.
- Pediatric Hospital, Tropical Diseases Hospital, Bach Mai Hospital.

2.1.3. Time of research:From 1/2013 to 12/2014.

2.1.4. Research design:retrospective description.

2.1.5.Sample size and choosing sample:choosing all measles-related cases and deaths reported.

2.1.6. Variables:ages;gender; address; clinical symptoms; contact history; admission times; places of treatment; vaccination history; test results; cause of death.

2.1.7. Data collection: retrospective investigation of cases and deaths relating to measles based on reports from provinces, institutes, hospitals.

2.1.8. Analysing data:

- Data were processed using biostatistic method with Epi. Info 2000 and STATA software. Statistic indicators were numbers of cases, deaths, percentage (%) of cases, deaths, crude rates of cases per 100.000 population, case fatality rate, statistic tests were used to compare the different between groups.

- Using Arc GIS softwear for measles epidemiology map.

2.1.9. Ethic issues

The research was retrospective, using surveillance data reported to the Ministry of Health as regulated so there was no concern about morality in bioresearch. Individual information was coded and kept close.

2.2. Objective 2: Identify molecular epidemiological characters of measle virus in the North, 2013 – 2014

2.2.1. Research target

Respiratory samples and sera collected from scarlet febril patients and measles cases in North provinces.

2.2.2. Place of research: 28 provinces, cities in the North, Central Pediatric Hospital, Bach Mai Hospital.

2.2.3. Time of research

Collecting samples: From 01/2013 to 12/2014.

2.2.4. Research design: Cross sectional description.

2.2.5. Sample size and choosing sample:

Choosing all samples from patients who were relevant to be research target.

2.2.6.Variables:place and time of sample collection, virus strain, virus genotype, measles virus sequence, phylogenetic trees of N and H gens of measles virus.

2.2.7. Data collection

- Respiratory samples were collected within 5 days after rash appears, in severe patients, samples could be collected within rash duration. Sera were taken within 28 days after rash occurs.

- Samples were stored at required temperature before testing.

2.2.8. Testing technique

Diagnostic tests were done in Measles Virus Lab of National Institute of Hygiene and Epidemiology according to WHO procedures.

- Virus isolation: throat swabs were used to isolate virus by Vero/SLAM cells and incubated for 7 days.The cells were observed daily to detect cytopathic effect (CPE).

-Identify virus multiplying by indirect fluorescent technique.

-Identify measles virus genotype: positive isolations, throat swabs were collected for RNAs extraction using QIAamp Viral RNA Mini Kit (QIAGEN Sciences, Germantown, MD, USA).

-The genomic RNAs then were used as template for RT-PCR using the QIAGEN OneStep RT-PCR Kit and primer set provided by Centers for Disease Control and Prevention, USA (MeV216: 5'-TGG AGC TAT GCC ATG GGA GT-3 'MeV214: 5'-TAA CAA TGA TGG AGG GTA GG-3').

-To increase sensitivity in detecting genetic material of measles in sera, the 2nd round of PCR were applied using GoTaq® Green Master Mix (Promega, WI, USA) and inner primer set provided by National Institute of Infectious Diseases (NIID), Japan (pMvGTf2: 5 ' - AGTA TTA GGG CA GAG ATG GT-3 '; pMvGTr2: 5'-GAG GGT AGG CFF ATG TTG TT-3').

- After purified with ExoSAP-IT (Affymetrix Inc., Santa Clara, CA, USA), the PCR products were subjected to sequencing reaction by using the BigDye Terminator Cycle Sequencing ReadyReaction Kit, version 3.1 (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences were determined by using an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems).

- Measles genotypes were identified by comparing nucleotide sequences of samples (N-450) – COOH with that of prototype strains designated by WHO.

2.2.9. Analysing data

Sequence alignment was performed using Clustal W included in the MEGA (v6.02) software package. The phylogenetic tree was constructed using the Maximum Likelihood method with boostrap probability after 1000 replicate trials. Phylogenetic tree was constructed using Kimura – 2 parameter. Similarity/difference of nucleotides was calculated by the same method.

2.2.10. Ethic issues

Research protocol was approved by Biomedical Research Ethic Committee of National Institute of Hygiene and Epidemiology.

2.3. Objective 3: Evaluate measles immune situation of children until 9 years old and women 16 to 39 years old in Ha Noi in 2013, before the epidemic

2.3.1. Research target

Sera samples from non-infectious patients until 9 years old and patients 16 – 39 years old in Saint Paul and Thanh Nhan Hospitals.

2.3.2. Place of research: Saint Paul Hospital, Thanh Nhan Hospital.

2.3.3. Time of research: Samples were collected from 1 – 12/2013.

2.3.4. Research design*:*Retrospective cross sectional description.

2.3.5. Sample size and choosing sample*:*

All sera met criteria from 2 study sites were stored at national biosample bank of National Institute of Hygiene and Epidemiology.

2.3.6. Variables:ages, gender, measles-specific IgG antibody, measles IgG geometric mean.

2.3.7. Data collection

-Kept the residual sera after completing diagnostic tests (minimum 0.2ml) from microbiology laboratories at study sites (except samples from infectious patients).

- Samples were stored at -25 to -15^{o}C before testing.

2.3.8. Testing technique

ELISA method was applied to detect measles IgG antibody following procedure of Measles Virus Lab at NIHE.

Virion/Serion Measles IgG ELISA Test Kit (German) (≥ 99% sensitivity and 93% spesificity) were used. IgG concentration was measured by photometer at wavelength of 405nm and expressed by mIU/ml.

IgG ≥ 275mIU/ml was considered protective. From ≥ 200mIU/ml to< 275mIU/ml was intermediate and <200mIU/ml was unprotective.

2.3.9. Analysing data*:*

Data were gathered by Excel, using R software for calculating rates, antibody geometric mean, statistic tests for comparision.

2.3.10. Ethic issues*:*

The use of these sera samples was approved by the Director of National Institute of Hygiene and Epidemiology. Research protocol was approved by Biomedical Research Ethic Committee of National Institute of Hygiene and Epidemiology.

CHAPTERIII. RESULTS

3.1. Epidemiological and clinical characters of measles in the North, 2013 – 2014

The 2013 – 2014 measles outbreak in the North of Viet Nam recognised 9,584 comfirmed cases, of which 4,628 cases were lab-confirmed (48.3%), 4,956 cases were epidemiologically confirmed (51.7%).

3.1.1. Distribution of measles cases by timing

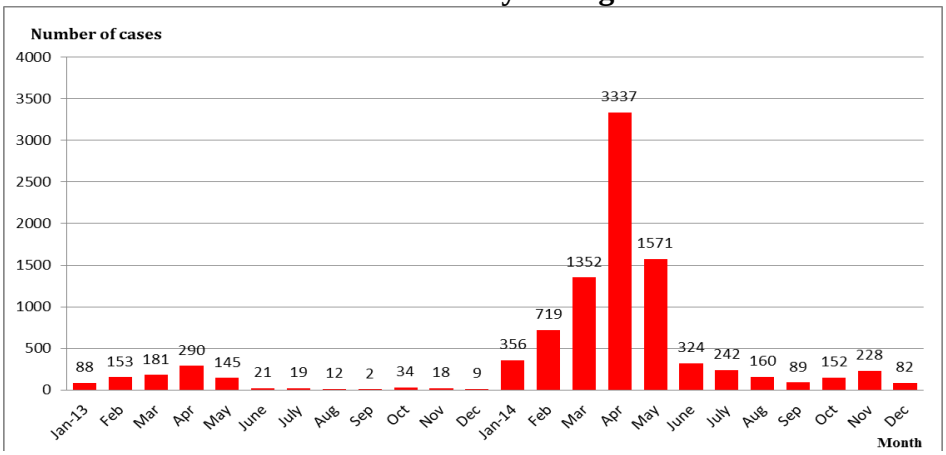

Chart3.1: Distribution of measles by months

Measles cases were scattering in northern mountainous provinces from early 2013. Outbreak was recognised since April 2013 then the numbers of cases went down until Dec. 2013 and came up back

in several northern provinces from early 2014, reaching peak in April 2014 and graduately decreased from June 2014. December 2014 saw the least cases.

3.1.2. Distribution of measles cases by provinces and regions

Provinces that had higher numbers of cases were Ha Noi (4,226 cases, accounting for 44.1% cases in the North), Ha Giang (996 cases, equal to 10.4%), Lao Cai (653 cases, 6.8%), Yen Bai (360 cases, 3.8%), Son La (356 cases, 3.7%). Some provinces recognised few cases were Bac Can (1 case), Cao Bang (5 cases), Bac Giang (34 cases).

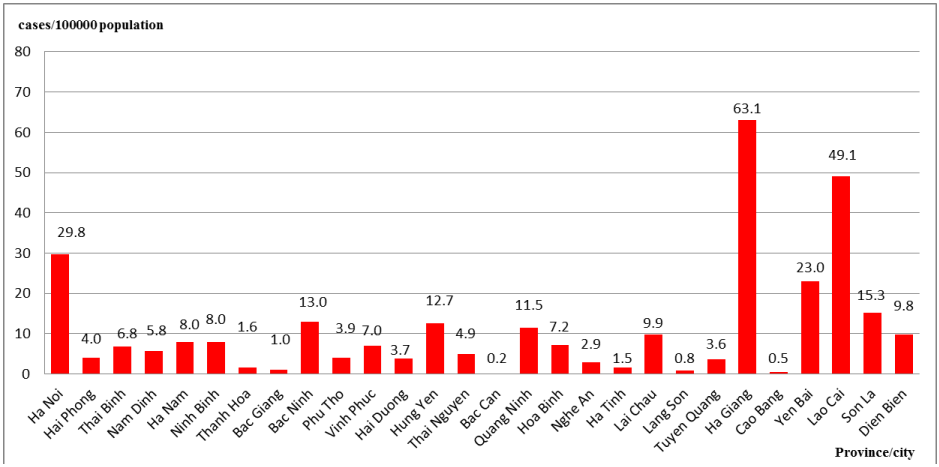

Chart3.3: Distribution of measles cases/100.000 popupation by provinces

In Chart 3.3, Ha Giang had the highest rate of cases/100.000 population (63.1), following by Lao Cai (49.1), Ha Noi(29.8) and Yen Bai (23).

By regions, Hong river plain had the rate of 15.3/100.000 population, that rate were 13,2 in the North East, 11.3 in the North West, 2.1 in the Central North. The difference of rates between regions was statistically significant ($p<0.001$).

In Hong river plain region, cases were spreading in the cities/towns. In mountainous provinces, cases mainly concentrated in hamlets of H'Mong ethnic group.

3.1.3. Distribution of cases by time

The outbreak started from Lai Chau with the first case recognised on April 29, 2013. However, in 8 months long it was localized only in 4 provinces bordering with Lai Chau: Ha Giang (360 cases), Lao Cai (294 cases), Yen Bai (74 cases), Lai Chau (66 cases). Till December 2013, the first case of Hong river plain region was recognised and after only 1 month (in February 2014), the outbreak spread to all provinces of North West, Hong river plain and some provinces of North East (23/28 provinces).

3.1.4. Distribution of cases by ages and gender

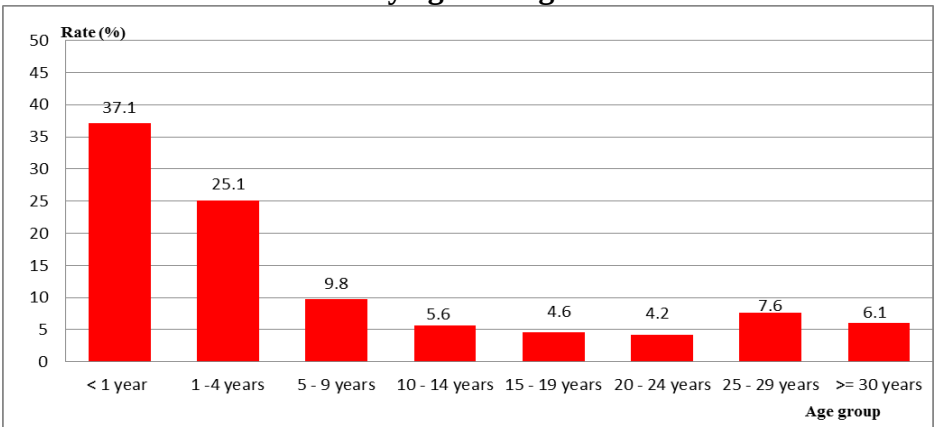

Chart 3.5: Percentage of cases by age group

Chart 3.5 showed children under 1 year old accounted for highest rate (3,558 cases, equal to 37.1%), followed by group of 1 – 4 years old (2,404 cases, equal to 25.1%) and other age groups. The rate of cases/100,000 population in children < 1 year old was highest (441.2), followes by group 1 – 4 years old (95.3).

Among cases < 1 year old, children < 6 months old was 26.3%, cases aged 6 – 8 months old was 43.2% and cases from 9 – 11 months old was 30.5%. Cases under 9 months old accounted for 25,8% of total cases and 69.5% of under 1 year old cases. The difference between male and female rates was not significant with p = 0.5.

3.1.5. Vaccination and exposure histories of measles cases

Table3.2: Vaccination histories of measles cases

Vaccination	Numbers (person)	Rate (%)
Measles vaccination (n = 9,584))		
Yes	2,283	23.8

Vaccination	Numbers (person)	Rate (%)
No	5,135	53.6
Unknown	2,166	22.6
Numbers of doses (among those reported yes) (n = 2,283)		
1dose	1,279	56.0
2 doses	435	19.0
Unknown	569	25.0
Rates of measles vaccination by ages		
1 dose/patients ≥ 9 months old*	1,279/7111	18.0
2 doses/ patients ≥ 2 years old*	435/5.247	8.3

** Patients who knew their vaccination histories*

Among measles cases, only 23.8% were vaccinated, of those 19% received 2 doses, 56% received only 1 dose. Among patients from 9 months old, rate of having 1 dose of measles vaccine was only 18%. In patients from 2 years old, there were only 8.3% had 2 doses.

Exposure histories of measles cases with scarlet febril cases were unclear. Only 7.4% of measles cases contacted scarlet febril cases, 15.8% of measles cases reported there were suspected cases near their houses of at working sites,

3.1.6. Clinical symptoms

In 9.584 measles cases, typical symptoms of measles were observed with high rates: fever and rash 99.9%, cough 94.2%, running nose 77.8%, conjunctivitis 59.2%... (Chart 3.11).

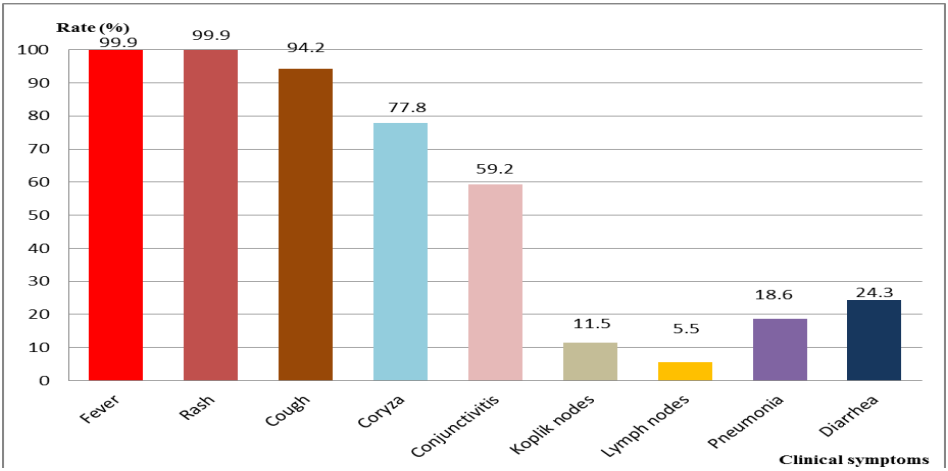

Chart 3.11: Distribution of clinical symptoms in measles cases

3.1.7. Measles - involving deaths

In that oubreak in the North, there were 145measle-involving deaths, including 70 deaths (48.3%) in Ha Noi.

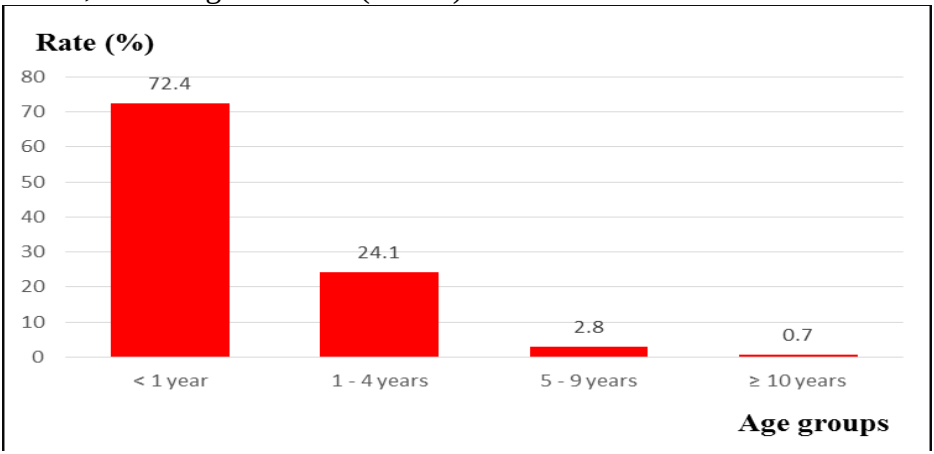

Chart 3.15: Distribution of measles - involving deaths by age groups

In 145 deaths, there were 140 under 5 years old, accounting for 96.5%, of those the highest rate belonged to children under 1 year old (105 deaths). Children under 6 months old were 17.2%, 6 – 8 months old were 40.9%, 9 – 11 months old were 41.9% of deaths under 1 year old.

In the outbreak, the case fatality rate (CFR) in children < 1 year old were highest (3.0%) and that was lower in older ages (1.5% in group

1 – 4 years old). The difference between two rates was statistically significant with p <0.005.In children <1 year old, the CFR in children < 6 months old was 1.9%, lower than that of children from 6 – 11 months (3.3%). That difference was significant (p < 0.05).

In 145 deaths, there were 104 cases had scarlet fever before admission (71.8%) and 34 cases (23.4%) developed fever and rash after admission.Of which, 19 cases (55.9%) got rash 5 days after admission, equal to 13.1% of deaths. Among 104 cases acquired rash before admission, therw were 50 cases (47.6%) admitted within only 1 day after getting rash.

Most of deaths stayed long in the hospitals (72 cases - 49.6% staying from 7 – 20 days; 30 cases - 20,7% staying for more than 20 days). Causes confirmed were mainly measles with complications of pneumonia/bronchitis/respiratory failure (106 cases, 73.1%).

Among measles-involving deaths, there were only 6 cases vaccinated (4.1%). Unvaccinated were 92 cases (63.4%), of which 42 cases (45.7%) aged from 9 months old. In 84 children from 9 months – who should have been vaccinated, there was only 4.8% received 1 dose of vaccine.

3.2. Molecular epidemiological characters of measles virus in the North, 2013 - 2014

448 throat swabs and 5,667 sera samples were collected. 473 samples were found positive using RT-PCR and nRT-PCR.

Table3.7: Distribution of measles virus strains in the North

No.	Province/city	H1		D8		Total
		2013	2014	2013	2014	
1	Bac Giang	-	9	-	-	9
2	Bac Ninh	-	2	-	-	2
3	Dien Bien	-	6	-	-	6
4	Ha Giang	41	46	-	-	87
5	Ha Nam	-	1	-	-	1
6	Ha Noi	4	44	-	-	48
7	Ha Tinh	-	2	-	-	2
8	Hai Duong	-	3	-	2	5
9	Hai Phong	-	1	-	-	1
10	Hoa Binh	-	7	-	-	7
11	Hung Yen	-	6	-	-	6
12	Lai Chau	11	1	-	-	12

No.	Province/city	H1		D8		Total
13	Lang Son	-	1	-	-	1
14	Lao Cai	11	2	-	2	15
15	Nam Dinh	-	1	-	-	1
16	Ninh Binh	-	4	-	1	5
17	Nghe An	-	44	-	2	46
18	Phu Tho	-	1	1	-	2
19	Son La	-	8	-	-	8
20	Thai Binh	-	1	-	-	1
21	Thai Nguyen	-	2	-	-	2
22	Thanh Hoa	-	2	-	1	3
23	Yen Bai	13	68	-	-	81
24	Vinh Phuc	-	3	-	-	3
	Total	80	265	1	8	354
		345		9		

Totally 354 measles virus strains were sequenced to identify genotypes. Two circulating genotypes were H1 and D8, in which H1 genotype (97.5%) were more predominant than genotype D8(2.5%).

In early phase of the outbreak in 2013, most of strains were H1 genotype (80 strains), belonging to 5 provinces, cities in the mountainous (Ha Giang, Lai Chau, Lao Cai, Yen Bai) and Ha Noi. Till 2014, genotype H1 spread to at least24/28 provinces, cities in the North with 265 strains. These were previous circulating strains in Viet Nam.

In the phylogenetic tree, H1 strains belonged to two different sub-lineages with the p-distance between two sub-lineages was 1.9%. The sub-lineage 1 contained strains mainly in Ha Giang (75 strains), Ha Noi (15 strains) and one strain in Bac Ninh. The p-distance within this sub-lineage was 0.1%, ranging from 0 – 0.2% and the closest strains with Vietnamese strains in this sub-lineage were some sporadic strains in China, Australia, Hong Kong and Russia. The p-distance between Vietnamese strains and others was 1.9%.

The rest strains in H1 genotype belonged to sub-lineage 2 with the p-distance was 0.2%. The closest strains with Vietnamese strains in this sub-lineage were endemic strains or predominant strains in China, with the p-distance was 0.5%.

The D8 strains detected in this research belonged to variant Frankfurt-Main with the bootstrap value of 99% and the p-distance

between these strains was 0.1%. The p-distance between D8 strains in the North, the South and Highland was 0.1%.

3.3. Measles immune situation of children until 9 years old and women 16 – 39 years old in Ha Noi, 2013

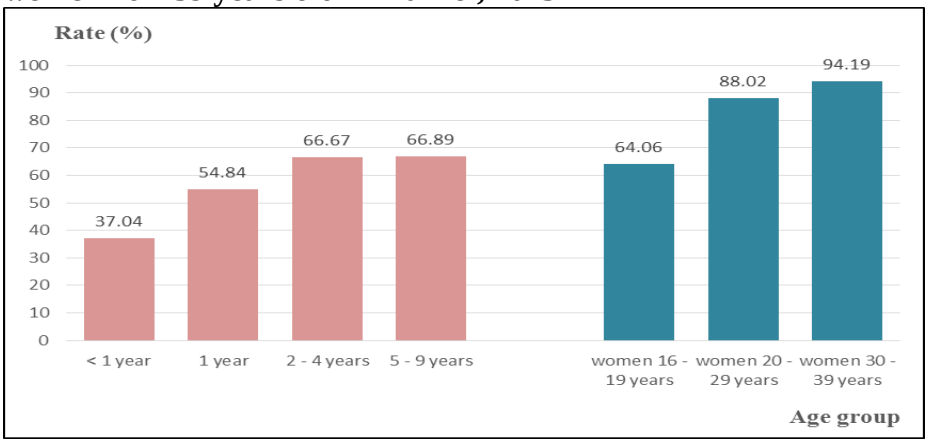

Chart 3.24: Percentage of protective IgG antibody by age groups

In 2013, 976 sera samples were collected in Thanh Nhan and Saint Paul hospitals from patients mainly in Ha Noi and in some neighboring provinces.

In Chart 3.24, among 54 patients < 1 year old (most of them were not vaccinated), only 37% had protective IgG antibody. This percentage increased in older ages, especially in women from 30 years old, which reached 94.2%.

In children ≤ 9 years old, in both two groups who had protective IgG and unprotective IgG, the differences between males and females were not significant with p = 0,6.In 128 women from 16 – 19 years old, 64.1% had protective IgG, this rate in women 20 – 29 years old was 88% and in group 30 – 39 years old was high as 94.2%. The differences between rates of protective and unprotective IgG in groups aged from 16 years old were statistically significant with p < 0,001.

Average IgG antibody levels in all groups were higher than protection threshold, lowest level was in children < 1 year old (331,3 mIU/ml). This concntrate was higher in older groups. The differences between average IgG levels in all age groups were significant with p<0,001.

CHAPTERIV. DISCUSSION

4.1. Epidemiological and clinical characters of measles in the North, 2013 – 2014

4.1.1. Distribution of cases by geographical areas and time

In year 2013 – 2014 measles cases were recognised in all provinces in the North, morbidity was high in Hong rive plain and North Eastareas. Ha Noi owned the most cases and deaths in the region.

In mountainous provinces, the outbreakstarted from hamlets of H'Mong ethnic group and then spreading to the whole provinces. This reflected immune gaps in this group, because the people were living in remote and isolated areas, besides, they had nomadic habits, giving birth at homes and in several locations, people refuse vaccination. For these reasons, though resident density was lower than that at plain areas, the unequal vaccination rates resulted in measles immune gaps that facilitated the outbreak and its trasmission to the whole hamlets andcommunes. While in plain areas, towns/cities and industrial zones where there were alot of cases recognised, cases were not concentrated in one commune/ward but scattering. That meant high quality of vaccination and high coverage remained for years helped creating good immune levels in these areas.

In mountainous areas, transmission was slow, that might be due to population density was low, geographic was unequal, with several isolated locations. Thus remaining timely high rates of epidemic-responded vaccination in high risk groups in surrounding areas is necessary and may help containing outbreaks in other areas, diminishing new cases. Besides, it is needed to isolate, localise the outbreaks, limit the contacts with measles cases to reduce transmission.

4.1.2. Distribution of cases by age groups

The outbreak in 2013 – 2014 in the North mainly focused in children < 5 years old (accounting for 62.2%). There was no difference between rates of males and females, similar to the situation in 2008 – 2012 in the North.Among patients <5 years old, the highest rate was children < 1 year old (63.6%), especially infants <9 months (41.5%), like results of study on epidemiological characters of measles in Viet Nam 2013 – 2014 done by Dang Thanh Huyen et al which showed

children < 1 year old took highest morbidity, followed by children 1 – 4 years old. Under 9 months old children were not vaccinated, their immune depends on passive antibody from their mothers. If the mother had not been infected or vaccinated, they could not give child antibodies. In general, most children from 6 – 9 months have no passive antibodies from mothers. They become high risk group of measles infections. That explained the rate of 6 – 8 months old children was as high as 43.2%. Patients from 9 – 11 months old accounted for the rate of 30.5% of cases under 1 year old. They might have only 1 dose of measles vaccine or have not been vaccinated due to late schedule. This result was same as that of a study on epidemiological and clinical characters in the whole country in 2013 – 2014 that showed patients from 6 – 8 months old rated 39.9%, patients from 9 – 11 months old had same rate in patients under 1 year old, 99.9% cases in this age group (those who could have received 1 measles vaccine dose) were not vaccinated.

4.1.3. Vaccination and exposure histories

There were more than 70% of cases were unvaccinated or did not remember the history. In 23.8% vaccinated patients, less than 20% got 2 doses, 56% of these patients got only 1 dose. In patients aged from 9 months, rate of children who received 1 vaccine dose was very low (18%), in > 2 years old patients –those who should have 2 vaccine doses, the rate of having 2 doses was less than 10%. Same as the outbreak in the North, measles situation in 20 provinces in the South at the same time showed that there were 64.3% of cases not vaccinated. It proved the fact that unvaccination or unfully vaccination put the children at high risk of being infected. Study on measles outbreak in Ha Noi 2014 taken by Nguyen Ngoc Quynh and others showed the rate of over 74% cases were unvaccinated of not fully vaccinated. The unvaccinated people had the risk of getting measles at 3.5 times higher than fully vaccinated people, unfully vaccinated ones had the risk of getting measles at 2.1 times higher than fully vaccinated ones.

4.1.4. Clinical symptoms

In measles outbreak of 2013 – 2014, all cases developed typical symptoms like fever, rash which rated nearly 100%. Nevertheless,

exposure histories were unclear. There were no unexpected clinical symptoms detected.

4.1.5. Deaths

Deaths were mainly patients < 5 years old (96.5%), mostly patients < 1 year old (72.4%). Among under 1 year old deaths, infants ≥ 6 months old were 82.2%, that rate is higher than infants < 6 months old (17.2%) – those infants still had passive antibodies from their mothers.

The case fatality rate of patients under 1 year old were highest (3.0%). In those children, the CFR of patients 6 – 11 months old was higher than patients under 6 months old (3.3% and 1.9%), especially that of patients from 9 – 11 months old (4.1%). These children were not protected by passive antibodies from mothers and might not be vaccinated due to late schedule.

Vaccination rate was very low in these deaths: in 84 children from 9 months old – those who were obligated for vaccination, the rate of having 1 vaccine dose were only 4.8%. Vaccination was an effectivemeasure because it could not only prevent children from getting disease but also shorten the duration.

In 104 children admitted after developing rash, nearly 50% were admitted just after 1 day. These children may get other diseases together with measles before hospital admission. Some cases acquired measles after entering hospitals. When staying in the hospitals, children would have got other diseases because their immune reduced when they engaged measles, that made them easy to be co-infected, especially in hospital environment. In addition, measles could make other diseases worse including congenital heart diseases, respiratory diseases, hereditary diseases.

More than 70% of deaths stayed long time in the hospitals, this was one reason that made children be at risk of getting other diseases in the hospitals. Though recent data cannot confirm this theory, but the study done by Le Khanh Nguyen Hang et al on hospitalized patients showed that samples collected with 5 days after admission had lower rate of co-infection (13.8%) compared to that of samples collected afterward (72.2% samples collected from day 6th to day 10th were co-infected).

4.2. Molecular epidemiological characters of measles virus

According to WHO reports, the wild type of H1 genotype strains were first detected in the outbreak in 1993 – 1994 in China. Until now, H1 genotype has been still endemic genotype in China and in other countries in the Western Pacific Region. In Viet Nam, the first report about the circulation of H1 genotype was in 2003 in Nha Trang. In 2012, Viet Nam started conducting molecular epidemiological surveillance of measles virus in WHO program. The results showed that the predominant strains in the North of Viet Nam were H1 genotype.

In the outbreak in 2013 – 2014, the endemic strains in the North of Viet Nam were still H1 genotype, but they divided into two different sub-lineages with the p-distance between two sub-lineages was 1.9%. This result showed that measles strains circulating in this outbreak had two different ancestors. In another word, they came from two different origins: the endemic strain had the same ancestor with Chinese strains and the rest strain might be imported or had the same ancestor with strains in other neighboring countries as Lao and Cambodia. However, there were no genotype information of measles virus in those countries although there also had big epidemics in Lao and Cambodia at the same period of time.

Measles virus D8 is endemic in India but the variant Frankfurt-Main was first detected in 2011 in Germany. The strains of this variant disseminated widely in Germany, spreading to Sweden. The Viet Nam D8 strains belonged to "D8 Frankfurt-Main" variant and they closely genetically related to European strains than strains circulating in the region. It showed that D8 genotype strains in Viet Nam had the same ancestor with strains in Europe and they were different from other D8 genotype strains in neighboring countries in the region.

4.3. Measles immune situation of children until 9 years old and women 16 – 19 years old in Ha Noi, 2013

Despite that measles vaccine has been introduced in EPI Viet Nam since 1985 to vaccinate 1 dose for children from 9 months old, and from 2011 the schedule changed to give one more booster dose for children from 18 months old, but the assessment in children until 9 years old and women 16 – 39 years old in Ha Noi 2013 showed a low protective IgG rate in children under 1 year old (37%). That rate increased in older groups, the highest rate was seen in women 30 – 39

years old. The low protective IgG rate could be explained by the fact that lots of children <1 year old were younger than vaccination time, and another reason was many parents wanted to use commercial vaccination (as in Ha Noi). At research time, combined measles vaccines were indicated for children at 1 year old, meaning children under 1 year were waiting for commercial vaccination. Besides, one main reason that made the children vaccinated later than scheduled in Ha Noi was the children were ill. Study of Nguyen Thanh Hue on vaccination situation for 8 types of vaccines in Ha Noi suburb in 2016 showed that children who were hospitalized atleast once in the year has lower vaccination rate than that of children who were not admitted to hospitals (OR = 0.65).

This result is compatible to result of study on measles immune remains in children 2 – 9 months old in Tu Ky, Hai Duong in 2015 done by Dang Thanh Huyen and colleagues which showed 86.9% of those children had no protective measles IgG. 2 months old children had highest rate of having protective IgG (35.1%), followed by children 3 – 5 years old (21.3%) and the lowest rate was in children 6 – 9 months old (0.5%).

In ≤ 9 years old children, children from 5 years old had a higher rate of having protective IgG. There was no difference between males and females in terms of IgG concentrations. That was relevant to characters of the 2013 – 2014 outbreak which mainly focused on children under 5 years old and cases distributed equally to boys and girls.

In women from 16 years old, women from 30 years old gained the highest rate of having protective IgG (94.2%), those people were born before EPI time and most of them were infected with natural measles diseases, next group was women from 20 - < 30 years old (88%). Women 16 – 19 years old (born from 1993 – 1997) had lower rate of protective IgG than groups over 20 and over 30 years old, though they received 1st measles vaccine doses in routine immunization and booster doses during 2002 vaccination campaign in the North. The reason might be low coverage or low quality of vaccination.That result is similar to the result of study done by Dang Thanh Huyen on Dong

Anh hospital, Ha Noi in 2016 that showed pregnant women aged from 30 years old had highest rate of positive anti-measles IgG, followed by women 25 – 29 years old and 18 – 19 years old.

After years implementing EPI, measles morbidity decreased dramatically. However, the result is reproductive age women (who obtained immune by vaccination) cannot pass enough protective antibodies to their children. Thus, to improve protective IgG concentrations in young children, it is necessary to consider giving measles vaccines to women before pregnancy. According to a study result on anti-measles IgG in mothers and their children in 2016, babies born from vaccinated mothers had GMC of anti-measles IgG higher significantly than that of babies born from unvaccinated mothers.

CONCLUSION

1. Epidemiological and clinical characters of measles in the North, 2013 – 2014

- Measles epidemic in the North Viet Nam 2013 – 2014 occurred in almost all provinces and cities, concentrating in outbreaks in mountainous area and scattering in plain areas. The outbreak started from Lai Chau in April 2013, spreading to neighboring mountainous provinces and to plain provinces from February 2014. Morbidity was high in Hong river plain and North East areas.Most of cases were children under 1 year old (37,1%) and 1 – 4 years old (25,1%). Morbidity was higher in children who were not or not fully vaccinated (children under 9 months-old accounted for 69,5% of cases under 1 year old, more than 70% cases were not vaccinated or their vaccination histories were unknown).

- Typical symptoms of measles such as fever, rash, cough … occurred in high rates (over 94%). There were no unexpected symptoms. Exposure histories were not clear (92,7% reported no exposure with scarlet fever cases).

- Most of deaths were children under 5 years – old (96,5%), of which 42% were children under 9 months – old, before vaccination schedule for measles. Deaths occurred mainly in central hospitals, main

causes might be cross infected measles in patients who was suffering from other existing diseases. Immunization coverage in vaccination – aged children was very low (4,8%).

2. Molecular epidemiological characters of measles virus in the North, 2013 - 2014

Measles virus strain in the epidemic 2103 – 2014 was not novel.

- Genotype H1 prevailed (97,5%) and was in the same ancestor with previous Viet Nam and China H1 strains

- Genotype D8 occurred at very low rate (only 2,5%) and closely related to European variants. There were 99,9% similarity between D8 strains in the North, South and in Central Highland.

3. Measles immune situation of children until 9 years old and women 16 – 39 years old in Ha Noi, 2013

- Children under 1 year – old in Ha Noi in 2013 had low rate of protective IgG titre (37%). The older age groups had higher rate of protective IgG.

- Women 16 - 19 yearsold (born after EPI) had rate of protective IgG level lower than women 30 - 39 years-old (born before EPI) (64,1% and 94,2% respectively).

RECOMMENDATIONS

1. Guarantee sufficient, punctual and high vaccination coverage, especially in Northern mountainous areas to reduce as much as possible numbers of cases and deaths; monitoring and reporting the rate of vaccination regularly at commune level to have in time plan for supplement activities or immunization campaigns

2. When outbreak occurred, it is needed to estimate the spreading risk of outbreak to implement vaccinating people living in risk areas and surrounding places to minimize transmission; it is necessary to categorize levels of treatment, limit hospital admission of mild cases; the hospitals needs to stream-line examination, localize patients to reduce the risk of cross infection

3. Consider vaccinating children 6 - 8 months old in the epidemic and giving booster doses of measles containing vaccines (MR,

MMR...) to women before pregnancy to enhance passive immune in order to protect young children who are under ages of measles vaccination.

LIST OF RELATED MANUSCRIPTS

1. Nguyen Minh Hang, Pham Quang Thai, Do Thi Thu, Nguyen Van Binh, Nguyen Tran Hien (2016), "Some epidemiological and clinical characters of measles in 2013 – 2014 in the North of Viet Nam", *Vietnam Journal of Preventive Medicine*, vol. XXVI, 15 (188), 2016, pages 21-31.

2. Nguyen Minh Hang, Le Thi Quynh Mai, Do Thi Thu, Pham Quang Thai, Nguyen Tran Hien (2017), "Measles immune situation of some children and women groups in some provinces in the North Viet Nam, 2013", *Vietnam Journal of Preventive Medicine*, vol. XXVII, 7, 2016, pages 26-33.

3. Do Phuong Loan, Trieu Thi Thanh Van, Nguyen Thi Mai Duyen, Nguyen Minh Hang, Komase Katsuhiro, Nguyen Tran Hien (2017), "The first appearance of measles genotype D8 in Northern Vietnam during 2013-2014 outbreak", *Vietnam Journal of Preventive Medicine*, 27 (12), pages 29-37.