

MINISTRY OF EDUCATION
AND TRAINING

MINISTRY OF HEALTH

NATIONAL INSTITUTE OF HYGIENE & EPIDEMIOLOGY

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**SITUATION OF JAPANESE ENCEPHALITIS,
SOME CHARACTERISTICS OF ITS VECTORS
AND CAUSATIVE AGENT IN THE CENTRAL
HIGHLANDS DURING 2005 – 2018**

Major: Public Health

Code: 62720301

SUMMARY OF PhD THESIS ON MEDICINE

HANOI – 2020

**THESIS PERFORMED AND COMPLETED AT THE
NATIONAL INSTITUTE FOR HYGIENE &
EPIDEMIOLOGY**

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This doctoral thesis will be defended at the Dissertation
Committee of Institutional level at:

National Institute of Hygiene & Epidemiology

on 2020

This doctoral thesis can be found at:

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INTRODUCTION

Japanese encephalitis (JE), has been and is a problem of health concern, due to its consequences and burden of disease, as well as difficulties in preventing and controlling this epidemic in the community. In the world, JE is recorded the annual average number of about 67,900 cases, the general rate is 1.8 / 100,000 people and possess always the risk of outbreak.

In Vietnam, since 1959, the acute encephalitis syndrome has been discovered in children, then it happened in large areas for many years. In the Central Highlands, during 2000-2001, 21 cases of JE were identified. In the period 2002-2005, there were 283 cases of JE, of which 50 cases died. Up to now, there was not any comprehensive study covered the disease situation, including disease vector and molecular structure of Japanese encephalitis virus in the Central Highlands region. Therefore, this thesis was conducted with the following objectives:

1. To describe the current situation of Japanese encephalitis at 4 provinces of the Central Highlands during period of 2005–2018.
2. To determine the species composition, distribution and prevalence of Japanese encephalitis virus among the mosquitoes of *Culex* genus in the Central Highlands region during 2005 - 2018.
3. To describe some molecular characteristics of Japanese encephalitis virus isolated from the Central Highlands during 2005-2018.

New contributions in science and practical value of the thesis

The thesis is a systematic and complete research on the current situation of JE, disease transmission vectors and molecular characteristics JE virus in Central Highlands during the period of 2005-2018.

The thesis described the current status of an acute encephalitis syndrome (AES), Japanese encephalitis (JE); determined the prevalence of Japanese encephalitis virus infection among some mosquitoes of *Culex* genus. The thesis also described the molecular characteristics of JE virus isolated from mosquitoes, identified the differences in nucleotide sequence within genome E of JE-GI virus at the Central Highlands in comparison with those of other areas in the country, in the region, and identified 8 changing positions of amino acids, contributing in the gene bank of E gene sequence region of 4 JE virus strains with the codes of HM228922, HM228923, AB728500 and AB728499, respectively.

The structure of thesis

Thesis consisted of 123 pages, excluding references and appendices, 21 tables and 26 figures.

Of which, 2 pages of the Introduction; 33 pages of Literature overview; 19 pages of Subjects and Research methods; 38 pages of Research Results; 28 pages of Discussion; 2 pages of Conclusion and 1 page of Recommendation.

Chapter 1. LITERATURE OVERVIEW

1.1. Epidemiology of Japanese encephalitis

1.1.1. History of disease occurrence in the world and in Vietnam

Research history of JE disease has been recorded by Japanese scientists since 1871 with the symptoms of encephalitis in horses and humans. It is estimated that there are about three billion people in the world living in the endemic areas of the JE virus and at risk of infection by *Culex* mosquitoes, especially *Cx.tritaeniorhynchus* and *Cx.vishnui* due to their surviving condition related to rice water cultivation and pig farming in Asia.

In Vietnam, for the first time, JE was recorded in 1953 with the preliminary report of two French scientists, Puyuelo H. and Pre'vot M., on about 98 cases of JE recorded among the French expeditionary army in North Vietnam. At the same time, the role of JE virus transmission among *Cx.tritaeniorhynchus* mosquito was also confirmed by virus isolation from this mosquito species.

At Central Highlands, according to documents of the National Institute of Hygiene and Epidemiology (NIHE), statistics on the situation of "acute encephalitis syndrome" in Vietnam from 1979-1990 showed that the incidence rate per 100,000 population ranges from 1.62 (in 1990) to 5.96 (in 1984) and the mortality rate varied from 0.18 (1990) to 0.76 (1983). In the years 2000-2001, 21 cases of JE were identified. In the period of 2002-2005, over 283 cases of encephalitis were detected, of which 50 died, 46 JE cases were detected from 74 samples taken from patient with AES in Gia Lai province.

1.1.2. The causative of disease

The causative agent is JE virus, a mosquito-borne Arbo virus of the genus *Flavivirus*, family *Flaviviridae*. The virus can

survive at -70°C for a few years, or for much longer at lyophilized stage stored at -70°C. The natural reservoirs of JE virus are mainly birds and pigs. Wild birds carry the virus but do not develop any disease, therefore is a source of virus infection for mosquitoes living in the wild. The pathogenesis of JE depends on the factors such as host susceptibility, the availability of a vector carrying JE virus and the virus reservoir surrounding human living. The virus is transmitted by mosquitoes into the bloodstream, where it can multiply and travels throughout the body. Using its neurotropic properties, the virus invades nerve cells, reproduces and grows rapidly there, causing encephalitis reactions leading to neurological symptoms appearing. JE disease has no specific treatment. Use JE vaccine is the most effective measure to prevent the disease.

1.2. Characteristics of Culex mosquito and its role in Japanese encephalitis virus (JE) transmission

Culex mosquito is both a reservoir of virus and a vector of virus transmission to humans. In Vietnam, some Culex species, including *Cx. tritaeniorhynchus*, are identified as the main vector of JE transmission. Based on the database and confirmed by the actual observations, the normal cycle of JE virus in nature is the "bird-mosquito" cycle. Over the summer season, this cycle develops a virus amplification cycle of "mosquito-pig", which in turn can lead to a special "mosquito-human" cycle. The "mosquito-human" cycle can be called the last cycle, because JE virus is not transmitted from person to person through mosquito vector.

1.3 Molecular characteristics/molecular biological epidemiology of JE

In the field of medical microbiology, molecular characteristics/molecular biological epidemiology is the most modern method today to determine the molecular characteristics of the pathogen, the origin of the pathogen, their propagation over time and space at the molecular level. It has become an essential and accurate tool in epidemiological surveillance because it can: identify the origin, emergence and spread of the pathogen; track changes and evolution of pathogenic strains at the molecular level over time and over geographic zones; identifying relevant disease trends for pathogens that carry high or low virulence genes.

Chapter 2. SUBJECT AND STUDY METHODS

2.1. Study subjects, place and time

2.1.1. Study subjects

Subject for Objective 1: Suspected cases of viral/JE infections with their records (Appendix 1) at 4 provinces of the Central Highlands, during period of 2005-2018.

Subject for Objective 2: Some Culex mosquito strains collected at 4 provinces in the Central Highlands during 2005-2018 and JE virus strains isolated from collected Culex mosquitoes of the same period.

Subject for Objective 3: Nucleotide and amino acid sequences of genome E region of JE virus strains isolated from Culex mosquito collected at 4 provinces of the Central Highlands.

2.1.2. Study place

Research was carried out at 4 provinces of Central Highlands: Kon Tum, Gia Lai, Dak Lak và Dak Nong.

2.1.3. Study time frame

Research was conducted from 2017 to 2018.

2.2. Study methods

2.2.1. Study design

Cross-sectional descriptive study.

2.2.2. Sample size and method for selection the sample

Sample size and sample selection method for Objective 1:

713 suspected patients of AES due to virus were collected and surveyed by the questionnaires during 2005-2018 at 4 provinces of the Central Highlands, of which 168 cases were identified by laboratory results.

Sample size and sample selection method for Objective 2:

138 samples of *Culex* mosquitoes were collected including 5,368 mosquito individuals in the period 2005-2007 and 236 samples with 8,351 mosquitoes collected in the period 2012-2014 for describe and testing JE infection; 372 mosquito individuals were collected in the field during 2 years (2017-2018), of these, 166 mosquito samples were used for virus isolation.

Sample size and selection method for Objective 3:

04 virus strains were isolated from *Culex* mosquitoes collected in 2007 during the *Culex* mosquito sample collection period (2005-2007); 05 strains of virus isolated from *Culex* mosquitoes collected in 2018 (2017-2018).

2.2. Research content

2.2.1. Information collection techniques and tools

Data collection techniques and tools, the implementation and results evaluation for Objectives 1

Methods of data collection: From the system of reporting, statistics, monitoring the epidemic situation, obtained patient information (age, gender, occupation, ethnicity, test results, etc). Used method of observation, recording the available data according to the questionnaire.

Techniques and tools to collect information, conduct and evaluate results for objectives 2

Methods of data collection: All data concerning the composition, distribution, and role of virus transmission of Culex mosquitoes collected in the period 2005-2016 were investigated. Mosquitoes were caught from the field for investigating and identifying for statistic analysis, describing the composition and distribution of these Culex mosquitoes.

Research technique: Mosquito collection technique; Culex mosquito identification technique; techniques for preserving and transporting mosquito samples after identified from the field

Techniques and tools to collect information, conduct and evaluate results for objectives 3

Implementation technique: Purification of RT-PCR product for sequencing by Sanger method; building phylogenetic trees.

2.3. Ethics in research

Research design was approved by the Biomedical Ethics Council of the National Institute of Hygiene and Epidemiology.

Chapter 3. RESULTS

3.1. Current situation of Japanese encephalitis at 4 provinces in the Central Highlands, during period of 2005-2018

Table 3.1. Rate of morbidity and mortality due to acute encephalitis syndrome and Japanese encephalitis at 4 Central Highlands provinces during 2005- 2018

Year	Case AES	Death	AES case/ 100.000 people	Case JE	Death	JE case/ 100.000 people	JE death/ 100.000 people
2005	40	4	1.11	10	4	0.28	0.11
2006	46	3	1.24	11	3	0.30	0.08
2007	16	0	0.32	2	0	0.04	0.00
2008	20	2	0.53	7	2	0.18	0.05
2009	28	2	0.72	11	2	0.28	0.05
2010	21	2	0,52	4	2	0.10	0.05
2011	40	0	0.99	13	0	0.32	0.00
2012	11	0	0.27	2	0	0.05	0.00
2013	46	0	1.10	8	0	0.19	0.00
2014	63	3	1.43	7	3	0.16	0.07
2015	107	9	2.48	33	9	0.76	0.21
2016	90	0	2.02	20	0	0.45	0.00
2017	113	7	2.45	28	7	0.61	0.15
2018	72	2	1.56	12	2	0.26	0.04
Total	713	34	1.21	168	34	0.29	0.06

713 cases of acute encephalitis syndrome (AES) were detected with the rate of 1.21/100,000 population. Of which, 168 cases of Japanese encephalitis (JE) were identified with the positive rate of 23.56%. The general incidence of JE was 0.29/100,000

population. The number of deaths due to JE was 34 cases, made the overall death rate of 0.06/100,000 population.

Table 3.2. Rate of death/incidence due to JE at 4 Highland provinces, during 2005 – 2018

Province Year	Gia Lai		Kon Tum		Dak Lak		Dak Nong	
	C/ 10 ⁵ people	D/C%	C/ 10 ⁵ people	D/C %	C/ 10 ⁵ people	D/C%	C/ 10 ⁵ people	D/C%
2005	0.89	40.00	0.00	0.00	0.00	0.00	0.00	0.00
2006	0.67	37.50	0.79	0.00	0.00	0.00	0.00	0.00
2007	0.17	0,0	000	0.00	0.00	0.00	0.00	0.00
2008	0.58	28.57	0.00	0.00	0.00	0.00	0.00	0.00
2009	0.88	18.18	0.00	0.00	0.00	0.00	0.00	0.00
2010	0.30	50.00	0.00	0.00	0.00	0.00	0.00	0.00
2011	0.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2012	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2013	0.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2014	0.46	4,86	0.00	0.00	0.00	0.00	0.00	0.00
2015	1.00	35.71	0.21	0.00	0.75	2.43	0.70	25.00
2016	0.96	0.00	0.99	0.00	0.05	0.00	0.00	0.00
2017	1.41	14.29	0.56	10.00	0.20	25.00	0.00	0.00
2018	0.67	10.00	0.37	50.00	0.00	0.00	0.00	0.00
Total	0.70	19.10	0.23	28.57	0.07	21.05	0.05	25.00

Gia Lai was a province with the highest incidence of JE (0.7/100,000 people), and the lowest rate was found in Dak Nong (0.05/100,000 people). The death/morbidity rate in Kon

Tum was the highest (28.57%) and the lowest was in Gia Lai (19.1%).

Table 3.3. AES and JE case distribution by district/town/city in the Central Highlands, 2005-2018

Province	District	Case of	No of district found case	Rate (%)
Gia Lai	17	AES	16	94.12
		JE	15	88.24
Kon Tum	10	AES	9	90.00
		JE	7	70.00
Dak Lak	15	AES	15	100.00
		JE	8	53.33
Dak Nong	8	AES	5	62.50
		JE	3	37.50
Total	50	AES	45	90.00
		JE	33	66.00

AES was recorded in 45/50 (90%) districts/cities/towns of 4 provinces. JE was identified in 33/50 (66%) districts/city/town, in which Gia Lai has the highest number of districts detected with JE (15/17 districts, 88.24%).

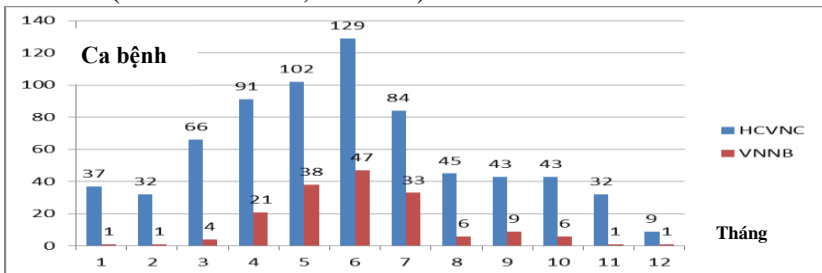


Figure 3.2. AES and JE case distribution by month at 04 Central Highland provinces, 2005- 2018

Cases appeared all year round, gradually increased in the last months of dry season and early rainy season (summer months), peaking in June with 129 cases of AES and 47 cases of JE.

Table 3.4. AES and JE rate distribution by age group at 04 Central Highland provinces, 2005- 2018

Age (y.o)	AES			JE		
	Case	(%)		Case	(%)	
<1	70	9.82	59.61	4	2.38	69.64
1-4	131	18.37		40	23.80	
5-9	134	18.80		51	30.36	
10-14	90	12.62		22	13.10	
≥15-70	288	40.39		51	30.36	
Total	713	100.00		168	100.00	

AES found in the group of <15 years old was accounted for 59.61%, and 40.39% for the age group of ≥15-70. JE detected in the group of <15 years old rated a highest incidence (69.64%) compared with that of group ≥15 years old (30.36%).

Table 3.9. AES and JE rate distribution by sex at 04 Central Highland provinces, 2005- 2018

Province	Case	Male		Female		Total	
		No	(%)	No	(%)	No	(%)
Gia Lai	AES	372	62.63	222	37.37	594	100.00
	JE	79	60.31	52	39.69	131	100.00
Kon Tum	AES	29	61.70	18	38.30	47	100.00
	JE	10	71.43	4	28.57	14	100.00
Dak Lak	AES	33	55.93	26	44.07	59	100.00
	JE	9	47.37	10	52.63	19	100.00

Province	Case	Male		Female		Total	
		No	(%)	No	(%)	No	(%)
Dak Nong	AES	3	23.08	10	76.92	13	100.00
	JE	2	50.00	2	50.00	4	100.00
Total		437	61.29	276	38.71	713	100.00

437 cases of AES were man, accounting for 61.29%, women were 276 cases (38.71%). Of the 168 cases of JE, males were 59.52%, higher than females (40.48%).

Table 3.10. AES and JE rate distribution by ethnic group at 04 Central Highland provinces, 2005- 2018

Province	Case of	Kinh		Gia Rai		Others (Xo Dang ⁽¹⁾ , E De ⁽²⁾ , M'Nong ^{(3)...})		Total	
		No	%	No	%	No	%	No	%
Gia Lai	HCVNC	144	24.24	445	74.92	5	0.84	594	100.00
	VNNB	12	9.6	119	90.84	0	0.00	131	100.00
Kon Tum	HCVNC	5	10.64	0	0.00	42	89.36	47	100.00
	VNNB	0	0.00	0	0.00	14 ⁽¹⁾	100.00	14	100.00
Dak Lak	HCVNC	30	50.85	0	0.00	29	49.15	59	100.00
	VNNB	7	36.84	0	0.00	12 ⁽²⁾	63.16	19	100.00
Dak Nong	HCVNC	8	61.54	0	0.00	5	38.46	13	100.00
	VNNB	3	75.00	0	0.00	1 ⁽³⁾	25.00	4	100.00
Total	HCVNC	187	26.23	445	62.41	81	11.36	713	100.00
	VNNB	22	13.10	119	70.83	27	16.07	168	100.00

119 cases of JE (70.83%) were detected among Gia Rai ethnic, the remaining 27 cases (16.07%) were from other ethnic groups (Xo Dang, Ede, Ba Na, etc.). The Kinh were 22 cases, accounting for 13.10%.

3.2. Species composition, distribution and prevalence of JE infection among mosquitoes of *Culex* genus at the Central Highlands, 2005-2018

Table 3.11. Composition and distribution of some *Culex* mosquitoes in the Central Highlands, 2005-2018

No	Name of strain	Gia Lai	Kon Tum	Dak Lak	Dak Nong	Total (%)
1	<i>Cx. tritaeniorhynchus</i>	2,043	1,039	2,075	911	6,068 (43.06%)
2	<i>Cx. vishnui</i>	1,649	2,199	823	426	5,097 (36.17%)
3	<i>Cx. fuscocephala</i>	212	451	63	201	927 (6.58%)
4	<i>Cx. gelidus</i>	132	208	337	119	796 (5.65%)
5	<i>Cx. malayi</i>	118	112	156	67	453 (3.22%)
6	<i>Cx. quinquefasciatus</i>	180	143	77	41	441 (3.13%)
7	<i>Cx. khazani</i>	33	88	32	32	185 (1.31%)
8	<i>Cx. whitmorei</i>	66	0	0	0	66 (0.47%)
9	<i>Cx. pseudovishnui</i>	58	0	0	0	58 (0.41%)
Total mosquito individuals		4,491 31.87%	4,240 30.09%	3,563 25.29%	1,797 12.75%	14,091 100%

09 species of mosquitoes were determined, of which 43.06% were *Cx. tritaeniorhynchus*. 07 mosquito species were found at all 4 provinces, 02 species of *Cx. pseudovishnui* and *Cx. whitmorei* were available only in Gia Lai province.

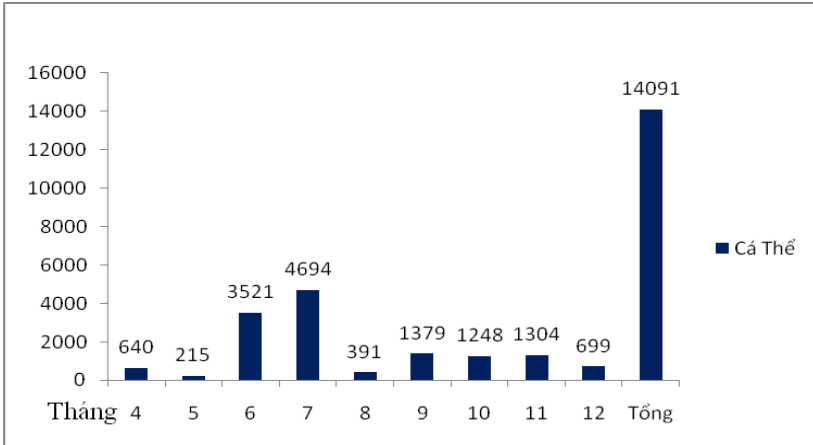


Figure 3.3. The distribution of Culex mosquitoes collected over time (months) at 4 Central Highlands provinces, 2005-2018

Mosquitoes collected from April to December, of which those found in July was the largest number with 4,694 individuals, accounting for 33.31%.

Table 3.15. Isolation of JE virus by C6/36 cells from some Culex mosquitoes collected in Central Highlands, during 2005-2018

Mosquito catching time	Number of isolates	Number of samples caused cell destruction	Number of samples confirmed by RT-PCR
2005-2007	138	17	04
2012-2014	236	26	0
2017-2018	166	51	05

Total	540	94	09
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94 samples were found causing destruction on C6/36 mosquito cells, 9 samples were tested positive, including 04 samples from *Culex* mosquito collected in 2007 and 05 samples collected in 2018.

Table 3.16. Results of JE virus isolation by cells C6/36 from *Culex* mosquitoes collected in the Central Highlands, 2005-2018

No	Name of strain	Isolated samples	Number of samples caused cell destruction	Positive samples
1	<i>Cx. fuscocephala</i>	45	7	1
2	<i>Cx. gelidus</i>	74	9	0
3	<i>Cx. khazani</i>	7	0	0
4	<i>Cx. malayi</i>	31	0	0
5	<i>Cx. pseudovishnui</i>	2	0	0
6	<i>Cx. quinquefasciatus</i>	62	6	0
7	<i>Cx. tritaeniorhynchus</i>	176	49	4
8	<i>Cx. vishnui</i>	140	23	4
9	<i>Cx. whitmorei</i>	3	0	0
Total mosquito strains		540	94	09

09 samples were tested positive with JE virus, all were identified as *Cx. fuscocephalus* (1 sample), *Cx. tritaeniorhynchus* (4 samples) and *Cx. vishnui* (4 samples).

Table 3.17. Minimum infection rate of JE virus among *Culex* mosquito species collected in Central Highlands, 2005-2018

Name of strain	No of isolated samples/No of identified mosquitoes	Number of positive samples	Minimal JE virus infection rate (%)
<i>Cx. tritaeniorhynchus</i>	176 (6,068)	02	0.33
<i>Cx. vishnui</i>	140 (5,097)	02	0.39

Rate of JE virus infection among *Cx. tritaeniorhynchus* was 0.33% and among *Cx. vishnui* was 0.39%.

3.3. Description of some molecular characteristics of Japanese encephalitis virus isolated from Central Highland provinces, 2005-2018

Table 3.18. Information of virus strains isolated from Culex mosquitoes collected in the Central Highlands, 2005-2018

Strain code	Mosquito species	Collection time	Province	JE typing by RT-PCR
07VN310	<i>Cx. tritaeniorhynchus</i>	6/2007	Kon Tum	Positive
07VN311	<i>Cx. tritaeniorhynchus</i>	6/2007	Gia Lai	Positive
07VN479	<i>Cx. vishnui</i>	7/2007	Kon Tum	Positive
07VN486	<i>Cx. vishnui</i>	7/2007	Kon Tum	Positive

Strain code	Mosquito species	Collection time	Province	JE typing by RT-PCR
18VN76	<i>Cx. fuscocephalus</i>	6/2018	Kon Tum	Positive
18VN77	<i>Cx. vishnui</i>	6/2018	Kon Tum	Positive
18VN129	<i>Cx. tritaeniorhynchus</i>	6/2018	Gia Lai	Positive
18VN130	<i>Cx. tritaeniorhynchus</i>	6/2018	Gia Lai	Dương tính
18VN139	<i>Cx. vishnui</i>	6/2018	Gia Lai	Dương tính

4 virus strains collected in 2007 were identified as JE virus; 5 strains collected in 2018 showed to have high similarity (99%) with Manglie virus strain (MH807827.1).

Figure 3.10. Phylogenetic plants built from nucleotide sequence E genome of JE virus isolated from Culex mosquitoes collected in the Central Highlands.

Four strains coded as 07VN310, 07VN311, 07VN479 and 07VN486 isolated from Culex mosquitoes collected in Gia Lai and Kon Tum provinces were identified as JE genotype I (GI) virus and were registered, issued code in gene bank as HM228922, HM2289923, AB728500 and AB728499, respectively.

Table 3.19. The difference in nucleotide levels between JE-GI viruses isolated in Central Highlands, in Vietnam and in the region

Genotype	Comparison criteria	p – distance	Value range
Genotype I	Among the JE-GI viruses isolated from provinces of Central Highlands	1.4%	0.1%–1.8%
	Among the JE-GI viruses isolated from Central Highlands anh in Vietnam	2.7%	1.1%–4.9%
	Among the JE-GI viruses isolated from Central Highlands anh in the region	4.8%	1.6%–9.1%

Nucleotide differences between 4 strains of JE viruses isolated from Culex mosquitoes collected at 4 provinces of Central Highlands was 1.4%; this rate was 2.7% in comparison to GI strains isolated from other areas of Vietnam and 4.8% when compared with other GI strains isolated in the region.

Table 3.20. Amino acid substitution of JE GI virus detected in the Central Highlands compared with standard GI strains

No	Amino acid location	Amino acid substitution		Substitution type
		Standard strain	Strains isolated from Central Highlands	
1	316	Tyr	Asp	Non-coservative
2	328	Ser	Met	Non-concervative
3	410	Arg	Cys	Non-concervative

No	Amino acid location	Amino acid substitution		Substitution type
		Standard strain	Strains isolated from Central Highlands	
4	414	Leu	Ser	Non-concervative
5	421	Glu	Gly	Non-concervative
6	424	Thr	Ile	Non-concervative
7	427	Lys	Ser	Non-concervative
8	603	Arg	Ser	Non-concervative

There were 8 amino acid substitution positions found in E genome region, all were of non-conservative substitution pattern.

Table 3.21. Haplotyping of JE virus isolated in Central Highlands

Strain code	Amino acid location on E gene								Haplotype
	10	34	36	65	123	209	227	408	
07VN310	D	M	N	V	A	L	S	S	NKSS
07VN311	D	M	N	V	A	L	S	S	NKSS
07VN479	D	M	N	V	S	L	S	S	SKSS
07VN486	N	S	N	V	S	L	S	S	SKSS

4 amino acid positions, 123, 209, 227 and 408, respectively. were predicted to be under pressure of selection for NKSS (2 strains coded as 07VN310 and 07VN311) and SKSS (2 strains coded as 07VN479 and 07VN486).

Chapter 4. DISCUSSION

4.1. Situation of Japanese encephalitis at 4 provinces of the Central Highlands, during 2005-2018

In this study, the incidence of AES caused by the virus at 4 provinces in the Central Highlands during the period of 2005-2018 was 1.21/100,000, lower than that obtained in 1984 (5.96/100,000). Out of 713 cases of AES caused by virus, 168 cases of JE were identified with the positive rate of 23.56% and the average rate of JE virus infection per 100,000 population was 0.29 (0.04-0.76). The results of this study were higher than that obtained by Duong Thi Hien et al. in Bac Giang in the period 2006-2015 with the average incidence of JE per 100,000 population of 0.25 (0.06-0.65). Our results were much lower than those obtained by Phan Thi Nga et al. in their study conducted in the period of 1979-1990 in the North of Vietnam with the incidence of JE per 100,000 population ranged from 1.62 (in 1990) to 5.96 (in 1984); and the rate of JE tested positive among children <15 years old was fluctuating between 50-70%. The rate of death due to JE in the period 2005-2018 was 0.06/100,000, lower than that of 1990 (0.18/100,000) and of 1983 (0.76/100,000 inhabitants).

In our study, 713 cases of AES and 168 cases of JE were recorded year round, of which the case number found in the first months of the year was low, then increased gradually in the summer months and reached a peak in June, then decreased gradually to the last months of the year (Figure 3.2). The number of JE cases among humans was found related to the growth cycle of *Culex* mosquito vector in hot, rainy

months, when mosquitoes breed and develop in wet rice cultivation areas and depends on farming activities of local people.

Figure 3.3 showed that during the period of 2005-2018, in the Central Highlands region, *Culex* mosquitoes were most collected in June (3,521 individuals) and peaked in July (4,694 individuals).

Up to 90% (45/50) districts reported to have AES cases, notably, JE appeared in 66% of districts (33/50) at all 04 Central Highlands provinces (Table 3.3), of which Gia Lai had the highest number of districts infected with JE in the region with 88.24% (15/17) of districts.

JE was recorded in all ages from children under 1 year old to the adults of 70 years old. However, the prevalence rate concentrated highest in the group under 15 years old, accounting for 69.64%; in the group of ≥ 15 years old, this rate was 30.36% (Table 3.4). This result is relatively consistent with the results reported by Phan Thi Nga et al (2000-2001), showing that JE occurred mainly among children under 15 years old (83.18%).

Overall, men suffered from JE with the rate of 59.52%, higher than that of women (40.48%), of which at Gia Lai province, 60.31% (79/131) cases of JE were men, higher than this rate in women (39.69%). The results were not much different from some other studies, such as according to the survey conducted by Le Duc Hinh et al. at Bach Mai Hospital, among 116 medical records during 1978-1980, 68 were men and 48 were women, etc.

The JE prevalence among Gia Rai people was the highest (70.83%, 119/168), followed by this rate of other ethnic groups like Xo Dang, Ede, M’Nong, etc. accounting for 16.07% (27/168), Kinh people accounted for 13.10% (22/168). This result is higher than results obtained previously by Dang Tuan Dat et al. in Gia Lai, in the period of 2003-2007, showed the highest rate of JE among Gia Rai clan (59.7%).

4.2. Species composition, distribution and prevalence of Japanese encephalitis virus of Culex mosquitoes in the Central Highlands region during 2005-2018

9 species of Culex mosquitoes were collected, including 14,091 mosquito individuals used for JE virus isolation, of which 7 species of mosquito were found at 4 provinces. 2 species identified as *Cx.pseudovishnui* (58 individuals) and *Cx.whitmorei* (66 individuals) were collected only in Gia Lai province. The most dominant mosquito species was *Cx.tritaeniorhynchus*. They were found at all survey points, with a rate of 43.06%, followed by *Cx.vishnui* (36.17%) and *Cx.fuscocephala* (6.58%), while the other 06 species collected at the lower rate, ranging from 0.41% –5.65%.

The number of mosquitoes was the most collected in July and June, corresponding to 4,694 individuals (33.31%), and 3,521 individuals (24.99%), respectively (Figure 3.3). This was also the time to record the highest number of AES and JE cases in the year, in particular, 129 cases of AES and 47 JE cases were detected in June (Figure 3.2).

By this study, 14,091 mosquito individuals belonging to 9 species were collected and a total of 540 samples of Culex

mosquitoes were isolated including the those collected in 2005-2007 (138 samples), in 2012-2014 (236 samples) and in 2017-2018 (166 samples). 94 samples showed to causing pathological phenomenon for cells, only 9 samples were identified as possibly JE virus, including 4 samples isolated from *Culex* mosquitoes obtained in 2007 and 5 samples isolated from mentioned above mosquitoes obtained in 2018. Of which, only 4 virus strains isolated in 2007 were identified as JE virus.

By gene sequencing technique to analyse E genome region, 2 JE virus strains were isolated and identified from mosquito *Cx.tritaeniorhynchus* and 2 other JE strains isolated from *Cx.vishnui*. The minimum JE infection rate among *Cx.tritaeniorhynchus* and *Cx.vishnui* population for the period of 2005-2018 in the Central Highlands region was 0.33% and 0.39%, respectively (Table 3.17). Compared with other study in Vietnam during period of 2006-2008 showing the minimum prevalence of JE virus in the population of *Cx.tritaeniorhynchus* and *Cx.vishnui* was 0.71 and 1.30, respectively, our result were lower.

4.3. Some molecular characteristics of Japanese encephalitis virus isolated from mosquitoes in Central Highlands

9 virus strains isolated from mosquitoes were JE positive by RT-PCR, but by the sequencing, only 4 strains isolated from mosquitoes collected in 2007 were confirmed as JE viruses, including strains coded 07VN310, 07VN311, 07VN479 and 07VN486. These JE virus strains were identified as GI type, showing to have the nucleotide difference compared

with other GIs strains isolated from 4 Central Highlands provinces, in Vietnam and in the region with the rate of 1.4% - 2.7% - 4.8%, respectively. The E genome sequence of these 4 JE virus strains has been coded in the gene bank as HM228922, HM228923, AB728500 and AB728499, respectively. 05 strains isolated from *Culex* mosquitoes in Central Highlands in 2018 were found to have the similarity of the whole genome up to 99% in comparison to that of Manglie virus strain coded MH807827.1 by the gene bank, which was isolated in China in 2011.

Amino acid analysis of GI viruses in the Central Highlands has identified 8 amino acid change locations, but they were all non-conservative pattern. In terms of haplotype characteristics of JE virus isolated from mosquitoes caught in Central Highlands provinces, two types of haplotype, NKSS and SKSS, were found, which are the two most common haplotype types of JE virus among mosquitoes and pigs in Vietnam. In which, the typical haplotype for GI genotype is NKSS with a special feature of the NKSS haplotype type that contains asparagine (N) at amino acid position 123, which coded the ability to multiply as well as the ability of pathogenicity of JE virus strains.

CONCLUSION

1. Current situation of Japanese encephalitis at 4 Central Highlands provinces, during 2005–2018

Out of 713 suspected cases of viral acute encephalitis infections found at 4 provinces of the Central Highlands during 2005-2018, 168 JE cases were detected by MAC-ELISA

technique, with the rate of 23.56%. The incidence rate of JE was 0.29/100,000 inhabitants, recorded in 33/50 (66%) districts/towns/cities, especially high in Gia Lai with 15/17 districts/towns affected (88.24%).

JE is scattered throughout the year, gradually increased in the last months of dry season and early rainy season (summer months), peaking in June. JE found with the higher rate among population of <15 years old than among those aged ≥ 15 years olds (69.64% versus 30.36%). Men suffered from the disease more than women (59.52% versus 40.48%). The Gia Rai ethnic group has higher incidence (70.83%) compared to other ethnic groups such as Xo Dang, Ede, M 'Nong, etc. (16.07%) and Kinh people (13.10%).

2. Species composition, distribution and prevalence of Japanese encephalitis virus among Culex mosquitoes in Central Highlands, 2005–2018

Nine species of mosquitoes of *Culex* genus have been identified, distributed throughout the investigation sites, in which *Cx. tritaeniorhynchus* species accounted for the highest rate (43.06%), followed by *Cx. vishnui* (36.17%); the remaining 07 species have this rates ranged from 0.41% –6.56%.

9 virus strains were isolated from *Culex* mosquitoes collected in 2007 and in 2018 and were tested positive for E gene region primers by RT-PCR technique. Of these, 4 virus strains isolated from *Culex* mosquito in 2007 were identified as JE virus, in particular, 2 JE strains were isolated from *Cx. tritaeniorhynchus* and 2 JE strains isolated from *Cx. vishnui*; other 5 virus strains that isolated from *Culex* mosquitoes in

2018 were the new strains discovered for the first time in Vietnam and showed to have 99% of the genome sequence similar to Manglie virus (MH807827.1). The detected minimum JE infection rate among *Cx.tritaeniorhynchus* population was 0.33% and among *Cx.vishnui* was 0.39%.

3. Some molecular features of Japanese encephalitis virus isolated from Central Highlands during 2005-2018

4 strains of JE virus isolated from *Culex* mosquitoes collected in 2007 were identified as JE genotype I. 05 strains isolated from *Culex* mosquitoes collected in 2018 are not JE but new viruses, discovered for the first time in the Central Highlands.

The difference was observed in nucleotide sequences of genome E region of 4 JE GI virus strains isolated in 4 provinces of Central Highlands and in comparison to that of JE GI strains isolated from other provinces of Vietnam and those isolated in Asia Pacific region with the rates of 1.4%, 2.7% and 4.8%, respectively. These virus strains showed to have 8 changing amino acid positions, but all were non-conservative replacement with two types of haplotype, NKSS and SKSS, of which NKSS haplotype is rare type.

RECOMMENDATION

- 1) It's necessary to continue the monitoring epidemiological surveillance of AESs, JE and to monitor the annual JE vector, as a basis for epidemiological assessment in order to propose positive and timely measures to prevent the disease more effectively.

- 2) Continuing to study and monitor the epidemiology of JE virus in humans and disease transmission vectors; research and evaluation of the effectiveness of vaccine prophylaxis; conduct research on the factors of social conditions, customs and habits, etc. among some ethnic minorities with JE situation in the Central Highlands.

**LIST OF PUBLISHED SCIENTIFIC ARTICLES
RELATED TO THIS THESIS**

1. **Pham Khanh Tung**, Ngo Thi Tu Thuy, Truong Xuan Toan, Vo Gia Bac, Dang Tuan Dat, Phan Thi Nga (2019), “Some epidemiological characteristics of Japanese encephalitis in the Central Highlands, 2005–2018”, *Journal of Vietnam Preventive Medicine*, No 12(29): pp. 94-101.
2. **Pham Khanh Tung**, Bui Minh Trang, Nguyen Viet Hoang, Dang Tuan Dat, Nguyen Van Sinh, Truong Xuan Toan, Phan Thi Tuyet Nga, Nguyen Hong Dan, Do Phuong Loan, Bui Manh Tuan, Nguyen Thi Hong Yen, Phan Thi Nga (2019), “Determination of Culex mosquito species composition and Japanese encephalitis virus infection rate of some Culex species in the Central Highlands, 2005–2018”, *Journal of Vietnam Preventive Medicine*, No 12 (29): pp. 118-127.
3. **Pham Khanh Tung**, Pham Hong Quynh Anh, Do Phuong Loan, Dang Tuan Dat, Bui Minh Trang, Nguyen Vinh Dong, Phan Thi Nga (2020), “Some molecular characteristics of Japanese encephalitis virus isolated from mosquitoes in Central Highlands ”, *Journal of Vietnam Preventive Medicine*, No.1(30): pp. 9-18.