MINISTRY OF EDUCATION AND TRAINING

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SEVERAL VIRAL ETIOLOGIES CAUSE ACUTE ENCEPHALITIS SYNDROM IN BACGIANG PROVINCE, 2004-2017

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2. Duong Thi Hien, Do Phuong Loan, Nguyen Thanh Luan, Bui Minh Trang, Phan Thi Nga, (2018), "Molecular characterization of Japanese encephalitis virus in Bacgiang province, 2004-2017", *Vietnam Journal of Preventive Medicine*, episode 28(7): 105-114.

3. Duong Thi Hien, Do Phuong Loan, Pham Hong Quynh Anh, Bui Minh Trang and Phan Thi Nga, (2019), "Epidemiology and molecular characterization of enterovirus causing acute encephalitis syndrome in bac giang province, 2004-2017", *Vietnam Journal of Preventive Medicine*, episode 29(3): 9-17.

ACRONYM

ABBREVIATIONS	ENGLISH	VIETNAMESE
Arbo	Arthropodborne	Mang bởi côn trùng tiết túc
ADN	Deoxyribonucleic acid	Axit Deoxyribonuclêic
ARN	Ribonucleic acid	Axit ribonucleic
BAV	Banna virus	Vi rút Banna
cDNA	Complement DNA	ADN bổ sung
DNT	Cerebrospinal fluid	Dịch não tủy
ELISA	Enzyme Linked Immunorbent assay	Thử nghiệm miễn dịch gắn enzyme
HCVNC	Acute Encephalitis Syndrome	Hội chứng viêm não cấp
HSV	Herpes simplex virus	Vi rút Hec-pec
MAC-ELISA	IgM Antibody Capture ELISA	Kỹ thuật ELISA tóm bắt IgM
NDiV	Nidovirus	Vi rút Nam Định
p	Probability	Xác suất
RT-PCR	Reverse transcriptaze polymerase chain reaction	Phản ứng chuỗi phiên mã ngược
VNNB	Japanese Encephalitis Virus	Viêm não Nhật Bản
VRĐR	Enterovirus	Vi rút đường ruột
WHO-TCYTTG	World Health Organization	Tổ chức Y tế Thế giới

INTRODUCTION

Acute encephalitis syndrome (AES) due to viruses is a common health problem worldwide because of high mortality rate (10-20%) or may lead to the serious neurological sequelae, which is a burden for the family and society. In the world, the proportion of people who acquired AES is from 3.5 to 7.4 per 100,000 and this disease can be found at any ages but it has higher incidence in younger population, especially children. In Vietnam, Japanese encephalitis virus (JEV) is the main cause of AES in most rural plains or mountainous areas, which is also the leading causative agent in AES cases among Vietnamese children. Apart from that mentioned reason, intestinal virus ECHO 30, Banna virus (BAV), Nam Dinh virus (NDiV), herpes virus are also the other causes of AES in Vietnam.

Bac Giang is a northern mountainous province. In 1999, an outbreak of encephalitis was reported with 203 cases, in which more than 30% the number of deaths, and JEV was the major cause of these deaths with about 33.3%, other cases were unknown. Since 2000, vaccines against JEV for children in Bac Giang province have been increasingly used, but surveillance of AES cases showing the number of cases were still a significal publich health problem. To boost the surveillance, diagnosis, treatment and prevention of AES, a research named "*Several viral etiologies cause acute encephalitis syndrom in Bacgiang province, 2004-2017*" was conducted with two aims:

1. To identify several Arbo virus pathogens, Enterovirus and Herpes virus causing acute encephalitis syndrome in Bac Giang, 2004-2017.

2. To describe several molecular characteristics of Japanese encephalitis virus, Enterovirus causing acute encephalitis syndrome in Bac Giang, 2004-2017.

PRACTICAL MEANING AND NEW CONTRIBUTION OF THE THESIS

- **Novelty**: This is the first study on the epidemiological, virological and molecular biology characteristics of several viral causes of acute encephalitis syndrome in Bac Giang.

- **Practical applications**: The study provides scientific data for Vietnam and other countries in the world about the incidence, epidemiological and molecular characteristics of several viruses causing acute encephalitis syndrome. Research results have practical applications in surveillance, diagnosis and prevention of acute viral encephalitis syndrome, which are significant in teaching as well as research.

STRUCTURE OF THE THESIS

The thesis consists of 137 pages (excluding references and appendices), including 4 chapters, 21 tables, 30 pictures, 1 photo. Recomment 2 pages; Chapter 1: Overview (39 pages); Chapter 2: Subjects, materials and research methods (19 pages); Chapter 3: Research results (38 pages); Chapter 4: Discussion (34 pages); Conclusion (2 pages); 1 page petition; List of works published (1 page). References: 140 references; 12 appendices.

Chapter I. OVERVIEW

1.1. Characteristics of acute encephalitis syndrome

1.1.1. Acute encephalitis syndrome in the world

Acute encephalitis syndrome often causes severe clinical conditions, along with a long hospital stay, the use of many diagnostic techniques and costly treatments, but the disease may cause a lot of serious sequelae, even lead to death.The majority of cases with acute encephalitis syndrome are not identified the direct cause from the brain organization but must be determined the causative agent via serological, immunological and molecular biology tests from the cerebrospinal fluid samples, blood samples or specimens taken from

outside the central nervous system areas. In an analysis of Jmor et al. 2008, an analysis of 87 studies on acute encephalitis syndrome worldwide shows that in Western countries in recent years , the figure for people developing AES was 7.4/100,000. in which children accounted for 10.5 to 13.8 /100,000 in compared with adults is about 2.2 /100,000.

1.1.2. Acute encephalitis syndrome in Vietnam

In Vietnam, AES has been studied since 20th century, in which Japanese encephalitis has been reported since 1952 by the announcement of two French authors, Puyuelo H and Prévot M. In 1953 these two French authors had a report about 98 cases of Japanese encephalitis during the French expeditionary army in Northern Vietnam. The suspected surveillance of AES by viruses is the basis for the diagnosis/monitoring patients with Japanese encephalitis, and at different periods of time, it can be seen that the incidence of Japanese encephalitis has changed due to impacts of preventive vaccines. In particular, the annual incidence of Japanese encephalitis ranging from 4.16 to 4.78 per 100,000 people (1994-1996); from 2.57 to 4.16 per 100,000 people (1996-2000) and between 2.75 and 2.82 per 100,000 people (2001-2004).

1.1.3. Acute encephalitis syndrome in Bac Giang

Bac Giang is a mountainous province in the Northern of Vietnam with the lowland or delta land, the midland and the mountainous region. In agriculture, the city has mainly developed pig raising and wet rice cultivation. Viral AES has always been a dominant health problem of Bac Giang province among the group of infectious diseases recorded for many years. In Bac Giang, the incidence of AES for the period 1995-1999 was 12.5 per 100,000 people, in 2000-2004 it was 7.44 per 100,000 people. Studies related to the detection of AES agents in Bac Giang were mentioned after the acute encephalitis epidemic occured in 1999 with 203 cases, 32.5% mortality/infection, viral etiology Japanese encephalitis was identified with about 33.3%, the remaining were unknown. To control the number of cases and deaths caused by viral

AES in Bac Giang, Japanese encephalitis vaccine has been widely used to prevent the disease for children aged 1-5 in the province but the number of viral AES suspect cases caused by viruses did not reduce. In fact, the outbreaks of AES still occur in a 2-3-year cycle (1999, 2001, 2004). Moreover, other agents contributed to the disease have not been systematically studied, so it is necessary to have a comprehensive study that helps to identify the pathogens, which is the scientific basis to orient the effective prevention of disease.

1.2. Classification of viral etiology causing acute encephalitis syndrome

(1) Group of viruses transmitted by respiratory gastrointestinal tract such as several Entero-Picorna enteric viruses: Nipah virus, Coxsackie virus type B, type 1, 2, 3, 4, 5, 6; ECHO viruses types 2, 5, 6, 7, 9, 14, 16 and 30; Entero virus types 70, 71. (2) Group of viruses transmitted by arthropods, such as mosquitoes, ticks ...: Japanese encephalitis virus (JEV), West Nile virus, Venezuelan horse encephalitis virus, Eastern horse encephalitis virus, Western horse encephalitis virus, Banna virus (BAV). (3) Group of viruses that can cause encephalitis-opportunistic meningitis: Epstein Barr virus, Herpes simplex virus (HSV) type 1 and type 2, Varicella Zoster virus, Cytomegalo virus.

1.3. Characteristics of several viruses that cause acute encephalitis syndrome

1.3.1. Japanese encephalitis virus

JEV virus belongs to family *Flaviviridae*, genus Flaivivirus, the virus genome is single-stranded RNA of approximately 11kb in length. JEV virus is the leading cause of AES in Asia, with an estimated 67,900 cases annually, mortality rates between 10%-30% and rates of neurological or mental sequelae can be as high as 30% -50%.

1.3.2. Banna virus

Banna virus belongs to genus Seadornavirus, *Reoviridae* family, genome of Banna virus is double-stranded RNA with 12 segments. Banna virus was

isolated from cerebrospinal fluid of AES patients and from blood of unknown fever patients in Yunnan province, China. And then the virus were isolated in different areas from patients, mosquitoes in China, Indonesia and Vietnam.

1.3.3. Nam Dinh virus

NDiV is a member of the *Mesoniviridea* family, the virus is spherical, the virus genome is single-stranded RNA with 20,192kb in length. NDiV is a new Arbo virus transmitted by mosquitoes, isolated from *Culex quinquefasciatus* and *Aedes albopictus* mosquitoes. Database on incidence, distribution characteristics by age, geography, gender, time in Vietnam and around the world are still poor.

1.3.4. Enterovirus

Enterovirus is a virus belonging to Enterovirus genus of family *Picornaviridae*. Enterovirus A-D (EVs) is an important cause of morbidity and mortality worldwide. The incidence of AES caused by intestinal viruses varies by geographic region, climate, and season. The disease occurs more frequently in tropical and temperate regions, in summer and autumn, but is high year round in tropical and subtropical countries. The incidence of AES caused by intestinal viruses accounts for about 21%-22% of cases identified in different geographical regions.

1.3.5. Herpes virus

The virus of the *Herpesviridae* family is spherical in shape, 120-200nm in diameter, the virus genome is double strand DNA. Currently, there are 8 types of HSV that cause disease in humans. Of which, type 1 is the most likely to cause encephalitis, accounting for 90%. Encephalitis due to HSV-1 is seriously pathological and has a high mortality rate.

Chapter II.

SUBJECTS AND METHODS OF THE STUDY

2.1. Research subjects

-Patients are clinically diagnosed with AES suspect cases due to a virus according to the criteria suggested by the Ministry of Health and the World Health Organization.

- JEV, EVs isolated from CSF patients AES.

2.2. Site and time of the research

- Implementation time: 2015-2018

- Sampling and investigating about AES patients' information collected at Luc Ngan Regional General Hospital, Provincial General Hospital and Obstetrics and Gynecology Hospital of Bac Giang Province.

- Virological research was conducted at the Center for Disease Control in Bac Giang and National Institute of Hygiene and Epidemiology.

2.3. Research Methods

- Methods of cross-sectional epidemiological research, retrospective and prospective studies combined with analytical laboratory research.

- Cases with clinical diagnosis of suspected AES caused by virus

+ From 2004 to 2014: Retrospective questionnaires, taking all patients with enough samples of double serum samples and CSF.

+ From 2015 to 2017: In the study, all patients were sampled dual serum and CSF for testing.

- Laboratory diagnosis identifying virus JEV, BAV, NDiV, EVs, and HSV causing AES.

- Description of molecular characteristics of JEV and EVs: Sequencing all positive samples from isolates, all positive samples were isolated from or/ and NS1-ELISA (JEV), building phylogenetic tree.

- Laboratory materials and techniques:

+ MAC-ELISA technique to diagnose JEV from serum or CSF: Use JEV diagnostic bio-kit to test IgM detection enzyme (MAC-ELISA), provided by the National Institute of Hygiene and Epidemiology.

+ ELISA IgM technique indirectly detects IgM against Banna virus, Nam Dinh virus from CSF: Using purified Banna virus antigens, purified Nam Dinh virus antigens to attach plates by Institute of Tropical Medicine, School Nagasaki University provided, in collaboration with Sigma.

+ Realtime RT-PCR technique to detect genetic material of EVs from CSF: Using Sacace Enterovirus Real-TM, REF V16-50FRT biological kit.

+ Realtime PCR technique to detect genetic material of HSV from CSF: Using QIAgen Artus HSV-1/2 QS-RGQ kit.

+ Virus isolation on cell lines C6/36 (JEV) and Vero/Rda cells (EV). JEV was identified by RT-PCR technique amplifying gene region E, collecting PCR products for product purification techniques and sequencing by Sequencing machine. EV was type by NGS sequencing the entire VP1 genome (866bp) on Illumina Miseq machine with "paired-end" method.

+The data of the research results were processed by bioinformatics software such as GraphPad software, DNA Star biological software (Lasegene), MEGA 6.0

2.6. Data processing

Data were processed, entered into computers and managed by Epidata software 3.1, Stata 14 software.

Chapter III. RESEARCH RESULTS

3.1. Identify several Arbo virus, Enterovirus, Herpes virus causing acute encephalitis syndrome in Bac Giang, 2004-2017

3.1.1. Diagnosis of virus origin causing AES

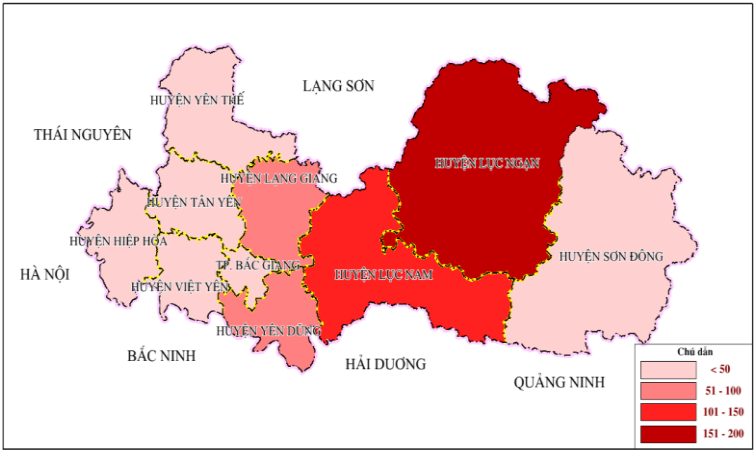

Figure 3.1. Distribution of cases with AES in Bac Giang, 2004-2017

In the 2004-2017 period, AES cases were recored in 10/10 districts and cities of Bac Giang province with a total of 594 cases.

Table 3.1. Determination of Arbo virus agent causing AES by ELISA in Bac Giang, 2004-2017

Type of sample		JEV	BAV	NDiV
CSF (n=594)	Total number of samples	594	594	594
	Positive	97	112	28
	Positive rate (%)	**16,33**	**18,86**	**4,71**
Serum I (n=594)	Total number of samples	594	-	-
	Positive	97	-	-
	Positive rate (%)	**16,33**	-	-
Serum II (n=594)	Total number of samples	594	-	-
	Positive	120	-	-
	Positive rate (%)	**20,20**	-	-

Out of 594 viral AES cases, the positive rate for JEV is 20.20%, Banna virus is 18.86%, and for Nam Dinh virus is 4.71%.

Table 3.2. Determination of several virus agents causing AES by molecular biology techniques in Bac Giang, 2004-2017

Virus agent	Realtime PCR (CSF)		Realtime RT-PCR (CSF)		Positive rate / total (%)
	Number of samples tested	Positive	Number of samples tested	Positive	
HSV	594	0	-	-	0/594 (0,00)
EV	-	-	594	78	78/594 (13,13)

In 594 cases of viral AES, of which 78 cases identified positive for intestinal virus, the positive rate was 13.13%. In this study, no cases was detected with specific primers of herpes viruses.

3.1.2. Characteristics of AES epidemiology due to viruses

3.1.2.1. Several characteristics of AES epidemiology due to Enterovirus

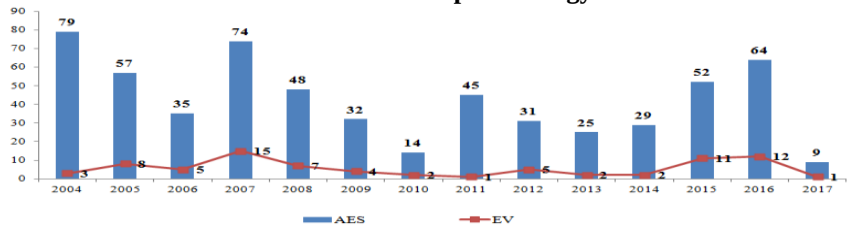

Figure 3. 4. Distribution of AES caused by Enterovirus by year, 2004-2017

The prevalence of viral AES due to EV was recorded in all years, the years with higher cases reported in 2007 (15 cases), 2016 (12 cases).

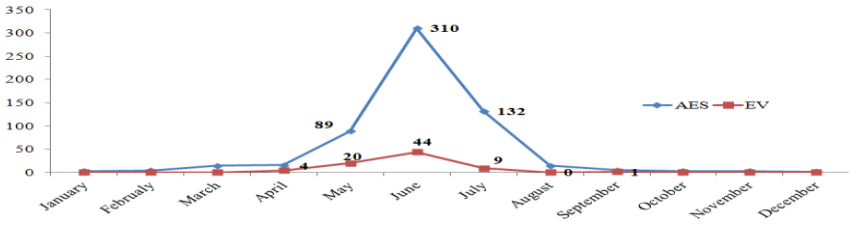

Figure 3. 5. Distribution of AES cases due to Enterovirus in months, 2004-2017

The number of EV cases were mostly recorded from April to July of the year, suggesting the seasonal occurrence of the disease during summer months 93.59% (73/78) with the peak in June.

Table 3.5. Distribution of AES cases caused by Enterovirus in age groups, 2004-2017

Age group		AES			EV		
		n	%	**Cases /100.000**	**n**	%	**Cases /100.000**
Two groups	<15	464	78,12	**8,60**	61	78,21	**1,13**
	≥ 15	130	21,88	0,77	17	21,79	0,10
Total		**594**	**100**	**2,67**	**78**	**100**	**0,35**

Most of AES cases were in age group under 15 years old (8.6/100,000) is 11 times higher than the age group over 15 years old upward (0.77/100,000).

3.1.2.2. Several characteristics of the epidemiological due to JEV

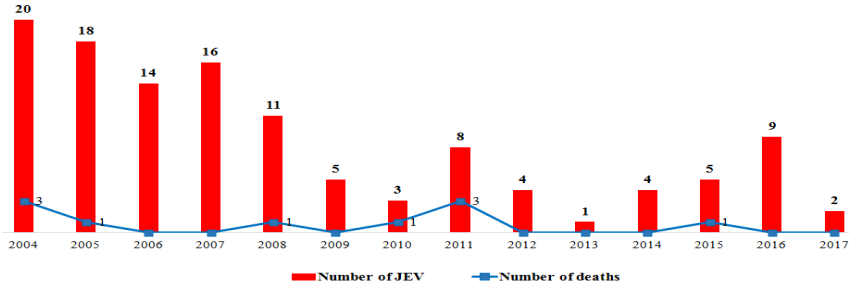

Figure 3.8. Distribution of JEV cases by years, 2004-2017

JEV distributed in all years in the 2004-2017 period, of which high concentration ranging from 2004 to 2008 and between 2011 and 2016.

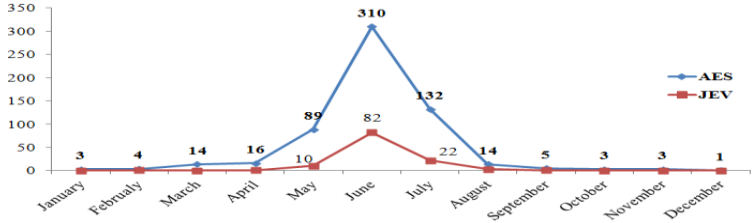

Figure 3.9. Distribution of JEV cases in months, 2004-2017

The number of cases infected with JEV appeared from February to September in the year, of which the number of cases concentrated in the months of 5-7 accounted for 95%. In June alone, the highest number of cases accounted for 68.33% (82/120).

Table 3.8. Distribution of JEV according to the average age, 2004-2017

Periods	Average age of Japanese encephalitis			
	JEV	Average age	Median	95%CI
2004-2008	79	7,27	6	6 – 9
2009-2013	21	9,76	9	7 – 13
2014-2017	20	10,2	12	7 – 13

Periods	Average age of Japanese encephalitis			
	JEV	Average age	Median	95%CI
Total	**120**	**8,20**	**7,00**	**7 – 9**

The average age patients suffer from JEV based on 120 cases is 8.2 years, the average age is in the range of 7-9 with 95% confidence. In the period of 2004-2008, the average age of the disease was 7.27 lower than the period of 2014-2017, 10.2 years, the difference was statistically significant (p <0.05)

Table 3.9. Distribution of Japanese encephalitis cases by age group, 2004-2017

Age group		Infection		Death		Infection/Death Rate (%)
		n	%	n	%	
Two groups	<15	105	**87,50**	9	90,00	8,57
	≥ 15	15	12,50	1	10,00	6,67
Total		**120**	**100**	**10**	**100**	**8,33**

JEV meets in all age groups, but high concentration is <15 years old. The prevalence in the age group <15 is for 87.50%. Group ≥15 accounts for only 12.50%. The difference is statistically significant with p <0.05.

3.1.2.3. Several characteristics of the epidemiological epidemic are caused by Banna virus

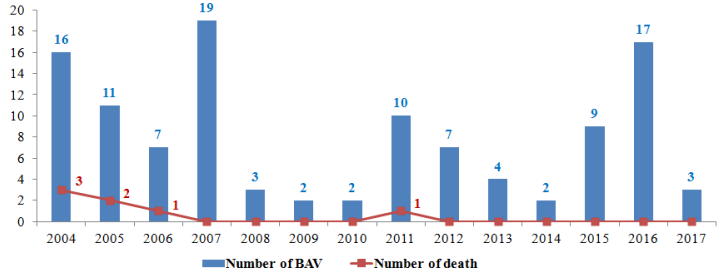

Figure 3.16. Distribution of AES by Banna virus by year, 2004-2017

Encephalitis caused by BAV were recorded in all years, out of 112 cases, 2004-2017, most of cases were recorded in 2004, 2007, 2011 and 2016.

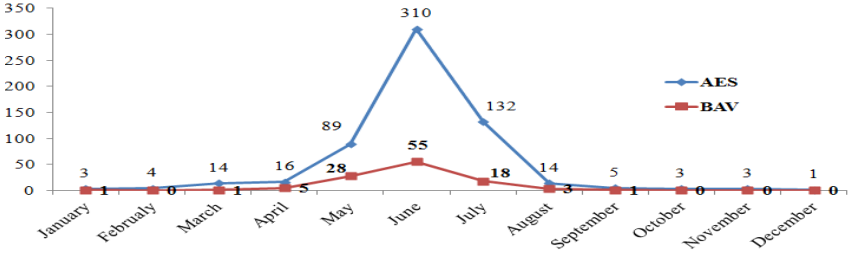

Figure 3.17. Distribution of AES by Banna virus by months, 2004-2017

AES caused by Banna virus appears from January to September of the year. The number of cases increased gradually from April to August.The total number of cases in summer months is 90.18% with the peak 49.11% in June.

Table 3.13. Distribution of Banna cases by age groups, 2004-2017

Age group		Infection		Death		Infection / Death Rate (%)
		n	%	n	%	
Two groups	<15	81	**72,32**	7	100	8,64
	≥ 15	31	27,68	0	0,00	0,00
Total		**112**	**100**	**7**	**100**	**6,25**

Banna cases were recorded in all age groups, but most of <15 years old.

3.1.2.4. Several characteristics of the epidemiological epidemic are caused by Nam Dinh virus

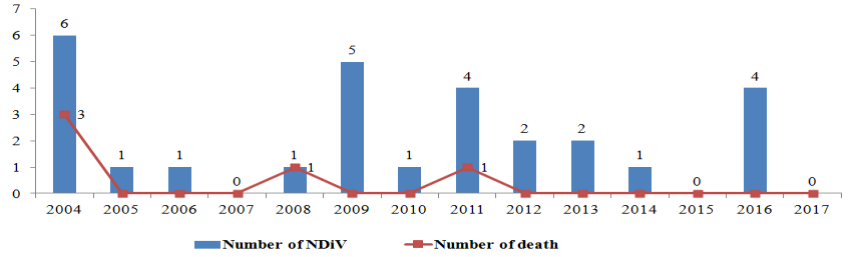

Figure 3.23. Distribution of AES by Nam Dinh virus in years, 2004-2017

AES determined by Nam Dinh virus accounted for 4.71%.

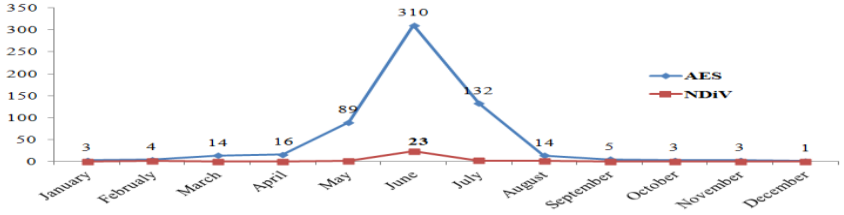

Figure 3.24. Distribution of Nam Dinh cases by months, 2004-2017

About 28 out of 594 positive cases, most of cases were recorded from April to August in the year, the highest was in June, accounting for 82.14%.

3.1.2.5. Several characteristics of viral encephalitis confirming etiology

Table 3.15. Several epidemiological characteristics of identified viral encephalitis, 2004-2017

Characteristics of viral encephalitis		Number of cases (n=302)	Rate (%)	Rate per 100.000
Seasonal distribution	Spring	10	3,31	0,05
	Summer	283	93,71	1,27
	Autumn	9	2,98	0,04
	Winter	0	0,00	0,00
Regional distribution	City	21	6,95	1,15
	Midlands	130	43,05	1,06
	Mountain	151	50,00	1,86
Gender distribution	Male	181	59,93	1,65
	Female	121	40,07	1,08
Distribution by age groups	Ages <1	14	4,64	3,78
	Ages 1-4	84	27,81	5,97
	Ages 5-9	82	27,15	4,84
	Ages 10-14	57	18,87	2,97
	Ages < 15	237	78,48	4,39
	Ages ≥ 15	65	21,52	0,39

The prevalence of encephalitis virus in the mountainous areas is 1.6 times higher than in the midland and city areas, the incidence in men is higher than female, the group under 15 years of age is 11.26 times higher than the group over 15 years old upward.

3.2. Several molecular characteristics of JEV, EVs causing AES in Bac Giang, 2004-2017

3.2.1. Several molecular biological characteristics of JEV

Figure 3.30. Genetically generated tree species of gen E (1917bp) of JEV GI and GIII in Bac Giang, 2004-2017

Based on phylogenetic tree creating, there was demonstrated 01 strain of JEV virus coded 04VN76 isolated from JEV patients in 2004 is GIII, in the same group with the strains of JEV GIII isolated in Vietnam before 2004. JEV virus samples were detected from cerebrospinal fluid (03 samples), of which 02 samples detected in 2007 coded 07CSF215, 07CSF235 and 01 samples detected in 2017 coded 17CSF02 belonging to GI subtype GI-b.

Table 3.18. Differences in nucleotide levels between JEV GI and GIII viruses in Bac Giang with other JEVstrains in Vietnam and in the region

Genotype	Comparative criteria	p–distance	Value range
Genotype I	Among JEV GI in Bac Giang	2,6%	0,6%–3,7%
	Between JEV GI in Bac Giang and GI strains in Vietnam	3,5%	0,3%–6,8%
	Among JEV GI in Bac Giang and other strains in the region	6,1%	1,7%–9,9%
Genotype III	Between GIII strains in Bac Giang and GIII strains in Vietnam	2,5%	0,4%-5,6%

The difference in terms of nucleotide between JEV GI virus in Bac Giang and other GI strains in Vietnam is 3.5%. The comparison of nucleotide level between JEV GI viruses in Bac Giang and other GI-b strains in the region such as Thailand, Japan, Cambodia, South Korea and China is 6.1%. The nucleotide difference between GIII Bac Giang strains and other GIII strains of Vietnam fluctuated within 2.5%.

Table 3.19. Characteristics of substituted amino acids of JEV were detected in Bac Giang compared to generic genotype I strains (Consensus* strain)

No.	Amino acid position	Amino acids substituted		Substitution type
		Standard strain	JEV in Bac Giang	
1	316	Tyr	Asp	No conservation
2	328	Ser	Met	No conservation
3	410	Arg	Cys	No conservation
4	414	Leu	Ser	No conservation
5	421	Glu	Gly	No conservation
6	424	Thr	Ile	No conservation
7	427	Lys	Ser	No conservation
8	603	Arg	Ser	No conservation

It can be seen from the table, the results of Egene of JEV GI virus in Bac Giang have identified 8 amino acid change sites with non-conserved replacement types. Analysis of haloptype at 4 amino acid positions of 123, 209, 227 and 408 of JEV GI viruses detected in Bac Giang all had haloptype as NKSS (Asparagine-Lysine-Serine - Serine).

3.2.2. Several molecular biological characteristics of intestinal virus

Table 3.20. Annual distribution of serum types of intestinal virus causing AES, 2004-2017 *

Year	Serum typ				
	E4	E6	E30	E33	Total
2006		1	1		**2**
2009	1				**1**
2010			1		**1**
2012		4	1	2	**7**
2013			2		**2**
2014			1		**1**
2015			1		1
Total	**1 (6,7%)**	**5 (33,3%)**	**7 (46,7%)**	**2 (13,3%)**	**15 (100%)**

Among 78 confirmed positive cases with EV primers, only 15 EV strains were isolated from RDa cells (19.2%) and identified serotype based on the entire region VP1 gene.

Table 3.21. Differences in the nucleotide level between EV in Bac Giang and with other strains in the same serotype

Genotype	Strains	Similarity at nucleotide level (%)	Oscillation range (% - %)
E30	Group 1 - Bac Giang	94,1	92,8- 97,9
	Subgroups 1 - other countries	91,6	89,0-95,8
	Subgroups 2 - Bac Giang	94,7	89,8-99,4
	Subgroups 2 - other countries	95,0	88,8-99,2
	Strains in Bac Giang	86,1	83,2- 99,4
E6	Strains in Bac Giang	97,6	94,0-100,0
	Bac Giang - another country	89,9	85,5-97,0
E33	Strains in Bac Giang	100	-
	Bac Giang - another country	85,3	83,3-98,7
E4	Bac Giang - another country	87,2	79,7-98,3

From phylogenetic trees, the E30 strains detected in Bac Giang in this study were divided into two subgroups with the similarity between the two groups being 86.1%. The E6 strains detected in this study had 97.6% nucleotide similarity.

Both strains of E33 of Bac Giang which were discovered in 2012 had absolute similarity in VP1 gene region (100%). When compared on Genbank, they have the highest similarity with strains in China and Thailand with 85.3% similarity.

In database of the gene Bank, E4 serotype strains were found mainly from China, Thailand, Australia, USA, France and Japan. In this study, the first time E4 strain was detected with nucleotide level similarity with E4 strains in other countries as 87.2%.

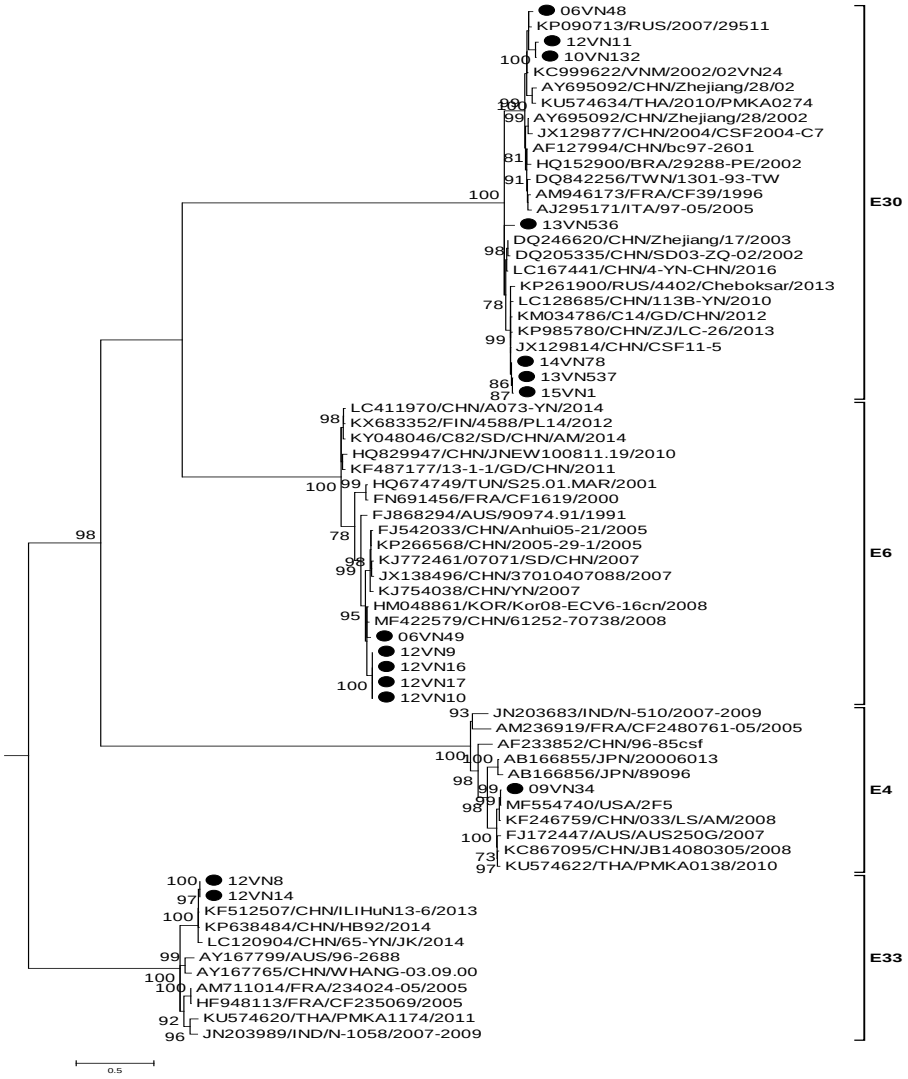

Figure 3.32. Phylogenetic tree of VP1 genome (866bp) Enterovirus in Bac Giang, 2004 - 2017.

(The strains marked with black circles (●) are the strains discovered in Bac Giang in the period 2004 - 2017 in this study).

Chapter IV. DISCUSS

4.1. Identify of Arbo virus, Entertovirus, Herpes virus causing acute encephalitis syndrome in Bac Giang, 2004-2017

4.1.1 Diagnosis viruses causing AES

In a total of 594 cases of AES in Bac Giang during the 2004-2017 period, 50.84% of cases of AES were identified due to one of the four kinds of viral agents, namely EV, JEV, BAV and NDiV. In this study, no cases of AES infection were detected due to the Herpes virus. The number of confirmed cases of at least due to one agent out of the total number of AES cases was 302/594 (50.84%). The figure of detecting the etiology of the causative agent virus in our research results is similar to that of other authors in the world and Southern of Vietnam with proportion detecting the agents around 30 %-60%.

4.1.2. Several characteristics of AES due to viruses

4.1.2.1. Several characteristics of AES due to Entertovirus

The study in Bac Giang on prevalence of AES due to EV accounted for 13.13% (78/594). The proportion determining AES cases caused by intestinal virus varies by geographic region, the results of research to determine etiology of gastrointestinal tract virus in the Central period 2015-2017 (11.2%), South Vietnam period 1996–2008 (9.3%), in Son La in 2015 (2.3%). Differences in proportions by geographic region have also been confirmed in other researchs from countries around the world, AES caused by EV in the United States during 2011-2014 (51.6%), in the UK during 2004-2013 (29% -52%), in China from 2013-2016 (22.1%; Jinan) from 2014-2017 (46.79%; ZheJiang).

4.1.2.2. Several characteristics of AES due to Japanese encephalitis virus

In our study, proportion determining etiology of JEV virus causing AES was 20.20%. In compare the incidence rate of JEV in Bac Giang in the period of 2004-2017 with the previous period, it was decrease.The average age of JEV cases in the period 2004-2008 (7.27) is lower than, 2009-2013 (9.76) and

2014-2017 (10.2). Therefore, it is a shift in the age group of JEV in Bac Giang in the direction the age of JE cases is higher than previous years. Most of JE cases have not been vaccinated (64.17%), or incomplete shots (35.83%). Thus, it is necessary to raise public awaress via multi media on the implementation of vaccination against JEV and follow the booster injection according to the manufacturer's instructions to enhance the immuse system against JEV inode to ensure the effectiveness of JEV prevention.

4.1.2.3. Several characteristics of AES caused by Banna virus

AES caused by Banna virus is the second cause behind JEV causing AES in Bac Giang. Among positive cases, the group with high incidence is over 15 years old. This result is consistent with the author Hoang Minh Duc's study on the distribution by age group in AES due to Banna virus. However, when analyzing the incidence rate per 100,000 people by age group, it was found that high incidence was found in the group under 10 years old the lowest incidence in the group of over 15 years old. This result is argreed with the general characteristics of AES caused by Arbo virus, mainly in children under 15 years old.

4.1.2.4. Several characteristics of AES due to Nam Dinh virus

The positive rate of NDiV is 4.71%, with the scattered cases in 2004-2006, 2008-2014 and 2016. The detection rate is lower when compared with the research results in Northern Vietnam in the period of 1998-2004, the proportion positive of AES caused by Nam Dinh virus is 12% among AES cases. Nam Dinh virus is a newly discovered agent, further studies should be conducted on virology, pathogenetic mechanisms and vector of disease transmission in order to protect public health from the disease.

4.2. Molecular biological characteristics of JEV, EV causing AES in Bac Giang, 2004-2017

4.2.1. Several molecular biological characteristics of JEV

The results of genetic analysis was used to identify the isolated virus strain 2004 coded 04VN76 as the virus JEV genotype III, and 03 JEV samples in the

year 2007 and 2017 JEV viruses were identified to be genotype I. In this study, JEV genotype I viruses were directly detected from 3 clinical samples of JE patients is a remarkable achievement. Analysis of several amino acid changes of JEV GI viruses in Bac Giang, 8 amino acid change sites were identified but they are all non-conservation substitutes. The results of haplotype analysis of JEV virus in Bac Giang all are showed NKSS. This is a specific haplotype for the GI genotype, with the special feature of this haplotype, which contains asparagine (N) at amino acid position 123.

The position of amino acid 123 determines the ability to multiply as well as the possibility of being infected by the virus. Position 123 is located on domain II, plays an important role in forming the second structure of the E protein, and the amino acid changes on domain II also affect the virulence of the virus due to the space structure of the virus. According to published data, up to now, only four JEV strains containing asparagine have been found at position 123 in the JEV genotype I from patients. In this study, three more JEV samples were found containing asparagine (N) at the position of 123 amino acid, increasing the total number of JEV had this kind of amino acid. Changing the amino acid from serin (neutral amino acid) to asparagine (acidic amino acid) can alter the optimal pH for changing the spatial structure of the virus prior to membrane integration.

4.2.2. Several molecular biological characteristics of EV

In this study, we determined the serotype of 19.2% of samples positive for EV causing AES. The viral load in the cerebrospinal fluid of AES patients is low with short survival time, so the direct sequence identification taken from clinical samples is often ineffective. Therefore, the isolation virus was carried out with confirmed positive samples for EV by realtime RT-PCR results to amplify the genetic material of the virus in the cultured sample, increasing the chances of successful sequencing, the entire VP1 gene region of the EV strain. Based on molecular characteristics analysis of intestinal virus, the EV strains isolated in Bac Giang belong to group B intestinal virus, the results of the research are argreed with other researchs on molecular epidemiology of EV in

the world determining group B being the dominant group causing AES. The serotypes detected were E6, E30, E33 and E4, of which E30 was the most dominant with 46.7%, following E6 with 33.3%. Compared with the results of the EV study, 2003 in cerebrospinal fluid of patients with AES, E30 accounts for almost absolute dominance (96.6%), the rest was E6, our research results show an increase of E6. The results of our study are similar to other researches in the world, showing that E30 and E6 are the most common AES types globally. In addition, this study also discovered the circulation of E33, E4 which cause AESs. This is the first time E4 cause AES in Vietnam was discovered, adding more the data of Genbank with only 10 strains published previous. Analyzing the molecular characteristics of the entire region of VP1 gene, which plays an important role in the mechanism of immunogenicity with EV, showing that the intestinal virus E30 strains in Bac Giang from 2004-2017 have similar levels of nucleotide sequence to be 94.1%-94.5%. This genetic difference is not so much different from the one in other countries in the world, corresponding to two subtypes of 91.6% and 95%.

This shows that the types of EV circulating in Vietnam or especially in Bac Giang in general are not different from the other strains in the world, they do not evolve into an independent sub-group specific to Vietnam but have a close relationship with the strains of other countries around the world.

Although this study only focused on case studies of local government studies, it is one of the few studies analyzing the molecular characteristics of the serotypes of EV causing AES. The results of this study will contribute greatly to the control of AES and the prediction of EV serotype for future as type E18 has been detected in China but not yet detected in Vietnam so far.

CONCLUSION

1. Identify a number of Arbo virus, Enterovirus, Herpes virus causing acute encephalitis syndrome in Bac Giang, 2004-2017

- In the period of 2004-2017, AES cases appeared in 10/10 districts and cities of Bac Giang province with the incidence was 2.67/100,000 population. There were 50.84% AES patients in total of 594 AES patients, The study has

identified 4 agents causing AES, which were Enterovirus, Japanese encephalitis virus (JEV), Banna virus and Nam Dinh virus, with positive rate to be 13.13%, 20.20%, 18.86% and 4.71%, respectively. None of AES case due to HSV was detected.

- Several characteristics of AES caused by 4 kind virus agents have been identified, mainly in the age group below 15 years old, the incidence rate in men was higher than female, mainly occursed in the summer season from April to August, the peak in June and recorded in 10/10 districts and cities of Bac Giang province.

- About 64.17% patients with JE cases were not vaccinated against disease; 35.83% was not fully injected, or not booster of vaccinations to boost immunity. The study identified a shift in the age group of JE cases in the direction of the higher age group.

2. Several molecular characteristics of Japanese encephalitis virus, Enterovirus causing acute encephalitis syndrome in Bac Giang, 2004-2017.

- In the period of 2004-2017, it was determined that there were circulating JEV GI and GIII in Bac Giang causing AES. The strains of JEV virus isolated in 2004 belong to genotype III and 3 JEV samples were detected from cerebrospinal fluid of JE patients in 2007 and 2017 are JEV genotype I, sub-group GI-b. The difference in nucleotide sequence between JEV genotype III in Bac Giang and other regions of Vietnam is about 2.5%. The difference in nucleotide sequence between samples of JEV GI in Bac Giang compared with other strains of JEV GI in Vietnam and GI-b strains in the region ranging from 3.5% to 6.1%. Comparison of amino acid sequences of 3 JEV samples in Bac Giang, there were 8 amino acid position changed. The characterization of all amino acide substitutions are non-conservation. Analysis of the haloptype of the three strains at the 4 amino acid sites was 123, 209, 227 and 408 respectively, all three of which had the haloptype NKSS.

- Molecular characteristics of Enterovirus: The analysis of molecular characterization of enteroviruses causing encephalitis in Bac Giang province

during 2004- 2017 were belonged B group. There were 4 serotypes dected including E4, E6, E30 and E33. And E4 was firstly detected in Vietnam (2009) in cerebroispinal fruid of AES patient. The most predemonant serotype circulating was E30, which was detected in many years and high identity with global strains (91.6-95%). E6 serotype was accounted for 33.3%, detected in only two year, 2006 and 2012 with the identity of 97.6%. E33 serotype was accounted for 13.3% and detected in only year of 2012 and the rest was E4 (6.7%). The identity between E6, E33, E4 strains in Bac Giang and the most identical strains in the database were 89.9%; 85.3% và 87.2%, respectively.

RECOMENDATIONS

1. In this study, only 4 kind of virus agents have been detected cause AES with only 50.84 %, but nearly 50% patients with AES has not been identified, so further research is needed to identify the unknown etiology causing AES in Bac Giang. Among the causative agents of AES in Bac Giang, the JEV has had a prophylactic vaccine, but cases diagnosed with JEV were mainly attributed to insufficient vaccination. It is necessary that the increasing use of JEV vaccines should be put into practice to proactively prevent and combat diseases effectively.

2. Research on the molecular characteristics of some AES-causing viruses shows that the intestinal viruses detected in Bac Giang have high similarities with regional and neighboring viruses, indicating the need to strengthen prevention of diseases which can be easily infected or transmitted when there are no preventive vaccines.