varied from 93% to 97% during 1993-2009, excluding 2007, when reported first-dose measles vaccine coverage was 83% because of a nationwide stock out. Measles vaccine first-dose is administered at 9 months of age, the second dose of measles vaccine was introduced in 2006 for children entering primary school and is administered at 6-7 years of age. Rubella vaccine was not introduced into the routine immunization.

Molecular epidemiologic data, when analyzed in conjunction with standard epidemiologic data, can help document viral transmission pathways, identify whether a virus is endemic or imported, and aid in case classification, thus enhancing control and elimination programs. Genetic analysis results showed that the H1 genotype virus was the predominant endemic measles virus in the North VietNam in 2006-2012. H1 genotype measles was also detected epidemic in Korea, China and in the centre of VietNam in 2000. In China, some studies showed that genotype H1 is the endemic genotype circulating in at least 16 years.

2B genotype of RV was circulating in some provinces in the North Viet Nam in 2008 - 2012. This genotype had a wide geographic distribution and were frequently found and were reported from 2 Middle Eastern countries, 5 European countries, 4 Southeast Asian, 4 South and Central American countries, 3 African countries and 2 Western pacific countries.

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8.12. Acknowledgement

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Research 9

6.13 Title: Laboratory diagnosis of enteroviruses from cases with Hand, Foot, and Mouth Disease in 2011-2012 in Vietnam North

6.14 Name of researcher: Nguyen Thi Hien Thanh
Tran Nhu Duong

6.15 Specific objectives:

1. Identification of causative agents and epidemiological characteristics of HFMD
2. Identification of molecular characteristics of EV71,

Virological Investigation of Hand, Foot, and Mouth Disease, Northern Vietnam, 2011

6.16 Abstract

603 / 912 clinical samples from patients with hand, foot and mouth disease in 2011 collected from Northern provinces of Vietnam are positive with enteroviruses including 275 positive samples with HEV71 (45,6%) and 328 positive samples with other enteroviruses (54,4%). As the result of 328 sequenced PCR products, 177 samples were identified as Coxsackievirus A6 (53,9%), 102 as Coxsackie A16 (31,3%), 4,6% as other CoxsackieA viruses including type 3, 10, 12 and 13; 0,6% as Coxsackievirus type B; 4,3% as echo viruses; and 0,9% as Polio-Sabin virus and enteroviruse typ 96, 15 samples were identified as Rhinovirus (4,6%). Phylogenetic analysis of 49 HEV71 strains showed 2 genogroups B and C with 3 subgenogroups B5, C4 and C5 that cocirculated in norththern Vietnam in 2011. However, viruses belonging to subgenogroup C4 predominated with 73.5% and after is 20.4 % as B5, only 6.1% as subgenogroup C5 that circulated during a long period before. Disease was mainly detected in children under 3 years of age

Keywords: Hand, Foot and Mouth Disease, enterovirus, human enterovirus 71, coxsackie virus A6, A16; coxsackie virusB5; Echo virus30

6.17 Introduction

Hand, foot, and mouth disease (HFMD) is a common febrile illness of early childhood, characterized by 3–4 days of fever and the development of a vesicular enanthem on the buccal mucosa, gums, and palate and a papulovesicular exanthem on the hands, feet, and buttocks (*1*). HFMD is caused by acute enterovirus infections, particularly by viruses belonging to the human enterovirus A (HEVA) species

The genus *Enterovirus* of the family *Picornaviridae* is divided into 9 species, 5 of which infect humans. These viruses include the prototype species poliovirus, as well as HEVA, HEVB, HEVC, and HEVD. Viruses belonging to the HEVA species include 11 serotypes of coxsackievirus A (CVA; serotypes 2–8, 10, 12, 14, and 16), and human enterovirus 71 (HEV71)

Since the discovery of HEV71 in 1969 , numerous outbreaks of this infection have occurred throughout the world, the prevalence of HEV71 infection in the Asia-Pacific region has greatly increased since 1997, concurrent with an increase in the prevalence of HFMD, HFMD outbreaks have been recorded in Japan, Malaysia, Singapore, South Korea, the People's Republic of China, and Australia, The most extensive epidemic of HEV71 occurred in Taiwan in 1998, with $\approx 1,3 \times 10^5$ cases of HFMD, 405 cases related to severe neurologic syndrome , and 78 deaths, The deaths were due primarily to the development of brainstem encephalitis and neurogenic pulmonary edema

In 2003, we isolated HEV71/C4 from 01 patient with HFMD and have relation to severe neurologic syndrome, In 2008, a HFMD outbreak happened in the north of Vietnam and we isolated HEV71/C5 from 7 patients in this outbreak, Although laboratory surveillance has been shown to provide adequate warning of impending outbreaks of enteroviruses associated HFMD, laboratory surveillance for enteroviruses from patient with HFMD has not yet been established

A reverse transcription-seminested PCR (RT-snPCR) assay was developed for the detection RNA of EVs and EV71 from clinical specimens. The VP1 RT-snPCR assay was slightly more sensitive, and was used to identify EVs in clinical specimens. A product of the expected size was successfully amplified and sequenced from clinical samples, The VP1 sequences derived from the RT-snPCR products allow rapid phylogenetic and molecular epidemiologic analysis of strains circulating during the EV season and comparison with EV sequences from past seasons or from different locations around the world,.

6.18 Materials and Methods

6.18.1 Specimen Collection

A total of 922 specimens were collected from the children with HFMD. Each child had at least 1 specimen collected from vesicle fluid, throat swab, or stool. 20% of specimens in PBS (+) were treateded with chloroform (1:10 in phosphate-buffered saline) before use for virus isolation in cell culture and molecular biology

Table1, Primer used for research

AN32	5-GTYTGCCA	cDNA	3009-3002	
AN33	5-GAYTGCCA		3009-3002	
AN34	5-CCRTCRTA		3111-3104	
AN35	5-RCTYTGCCA		3009-3002	
SO224-F	5-GCIATGYTIGGIACICAYRT	PCR1	2207-2226	762
SO222R	5-CICCIGGIGGIAYRWACAT		2969-2951	
AN89F	*5-CCAGCACTGACAGCA*GYNGARAYNGG	*PCR2/ EVs*	2602-2627	375
AN88R	*5-TACTGGACCACCTGG*NGGNAYRWACAT		2977-2951	
MAS01S	5'- ATAATAGCA(C/T)T(A/G)GCGGCAGCCCA -3')	*PCR2/ EV71*	2352-2375	376
MAS02A	5' – AGAGGGAG(A/G)TCTATCTC(C/T)CC -3')		2709-2728	

RNA extraction, Stool suspensions were prepared by adding 5 ml of phosphate-buffered saline (+), 1 g of glass beads (Corning Inc,, Corning, NY), and 0,5 ml of chloroform to 1 g of stool sample, shaking the mixture vigorously for 20 min in a mechanical shaker, and centrifuging at 1,500 _ *g* for 20 min at 4°C (33), the supernatant was transferred to a fresh tube, (10% stool suspensions), 140 _l of the specimen extract was combined with an equal volume of Vertrel XF (Miller-Stephenson Chemical Co,, Danbury, CT), shaken vigorously, and then centrifuged at 13,000 _ *g* for 1 min at room temperature, The aqueous phase was transferred to a fresh tube, Other specimen types (including blister fluid; throat swab samples) were processed without pretreatment, Twenty micrograms of proteinase K (Roche Applied Science, Indianapolis, IN) was added to 140 _l of each liquid specimen or fecal extract, and the mixture was then incubated for 30 min at 37°C, Nucleic acid was extracted from the digested specimen with a QIAamp Viral RNA mini kit (QIAGEN, Inc,, Valencia, CA), which was used according to the manufacturer's instructions, The eluted RNAs were passively dried in a benchtop desiccator under vacuum, The dried RNA was resuspended in 16 _l of sterile nuclease-free water and stored at _20°C until use,

RT-snPCR and sequencing.

- Synthesis of cDNA was carried out in a 5 µl reaction mixture containing 4 µl of RNA and 1 pmol each cDNA primer (primers AN32, AN33, AN34, and AN35; Table 1), heat mix in a 70^{0}C hear block for 5 min and immediately chill in ice water for at least 5 min. In a 20 µl reaction mixture containing 05 µl RNA reaction mixture, 100 µM each deoxynucleoside triphosphate (dNTP; Invitrogen), 4 µl of 5 x reaction buffer (GoStript, Promega), 1.5 µl MgCl2, 20 U of RNasin (Promega Corp,, Madison, WI), and 100 U of GoScript reverse transcriptase (Rocke) , incubation at 25°C for 05 min, 42°C for 60 min, and 70°C for 15 min

- Synthesis of PCR1: Following incubation, the entire 05 µl RT reaction mixture was then used in the first PCR (final volume, 25 µl) (PCR1), consisting of 5 µl of 10 x PCR buffer (Roche Applied Science), 200 µM each dNTP, 50 pmol each of primers 224 and 222 (Table 1), and 2,5 U of *Taq* DNA polymerase (Roche Applied Science), with 40 cycles of amplification (95°C for 30 s, 42°C for 30 s, 60°C for 45 s),

One microliter of the first PCR was added to a second PCR (PCR2/EV71) for seminested amplification, PCR2/EV71 contained 40 pmol each of primers MAS01S and

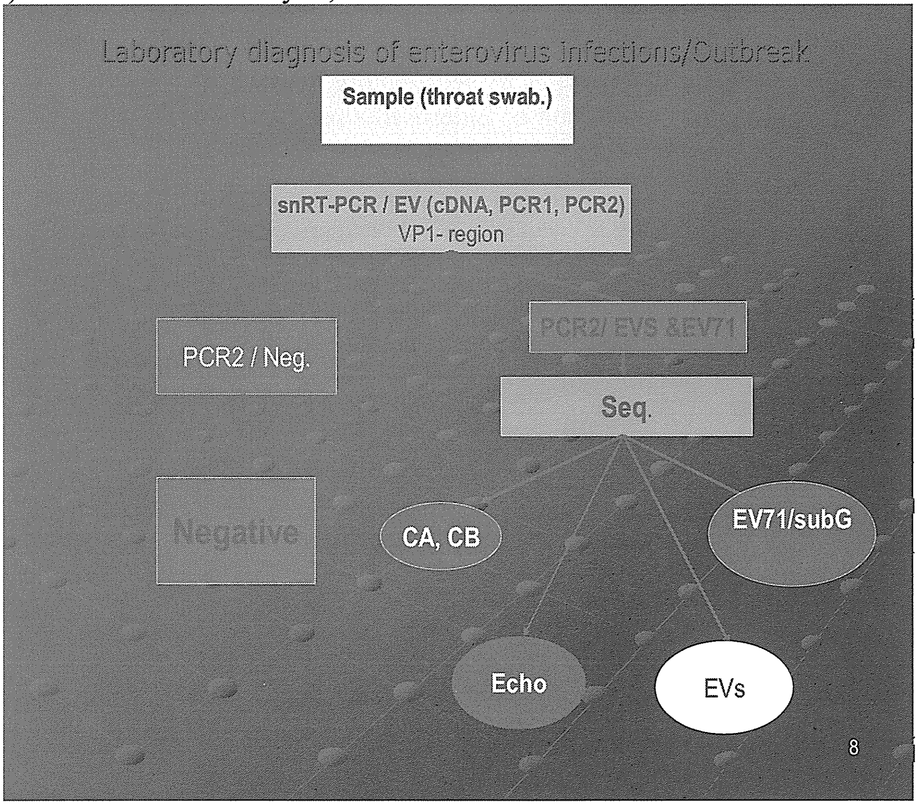
MAS02A (Table 1), 200 μM each dNTP, 5 μl of 10 x FastStart *Taq* buffer (Roche Applied Science), and 2,5 U of FastStart *Taq* DNA polymerase (Roche Applied Science) in a final volume of 50 μl, The FastStart *Taq* polymerase was activated by incubation at 95°C for 6 min prior to 40 amplification cycles of 95°C for 30 s, 60°C for 20 s, and 72°C for 15 s, The reaction products were separated and visualized on 2% agarose gels containing 0,5 μg ethidium bromide per ml

One microliter of the first PCR was added to a second PCR (PCR2/EV) for seminested amplification, PCR2 contained 40 pmol each of primers AN89 and AN88 (Table 1), 200 μM each dNTP, 5 μl of 10 x FastStart *Taq* buffer (Roche Applied Science), and 2,5 U of FastStart *Taq* DNA polymerase (Roche Applied Science) in a final volume of 50 μl, The FastStart *Taq* polymerase was activated by incubation at 95°C for 6 min prior to 40 amplification cycles of 95°C for 30 s, 60°C for 20 s, and 72°C for 15 s, The reaction products were separated and visualized on 1,2% agarose gels containing 0,5 μg ethidium bromide per ml and were purified from the gel by using a QIAquick gel extraction kit (QIAGEN), Slight variations in the sizes of the PCR products (350 to 400 bp) were observed due to VP1 gene length differences in the different serotypes, as described previously (12–14, 19), The resulting DNA templates were sequenced with a BigDye Terminator v1,1 ready reaction cycle sequencing kit on an ABI Prism 3100 automated sequencer (both from Applied Biosystems, Foster City, CA) by using primers AN89 and AN88 (Table 1),

Sequence analysis: The amplicon sequences were compared with the VP1 sequences of EV reference strains, including at least one representative of each recognized serotype, by script-driven sequential pair wise comparison with the program Gap (Wisconsin Sequence Analysis Package, version 10,2; Accelrys, Inc,, San Diego, CA), as described previously (15, 18, 19), In cases where the result was not unequivocal (highest score less than 75% or second-highest score greater than 70%), the deduced amino acid sequences were compared by a similar method,

6.18.2 Statistical Methods

Differences between proportions were tested by using the χ^2 test with Yates correction or Fisher exact test, Epi Info version 6 (Centers for Disease Control and Prevention, Atlanta, GA, USA) was used for the analysis,



Laboratory diagnosis of enterovirus infections/Outbreak

Sample (throat swab.)

snRT-PCR / EV (cDNA, PCR1, PCR2)
VP1- region

PCR2 / Neg.

PCR2/ EVS &EV71

Seq.

Negative

CA, CB

EV71/subG

Echo

EVs

Flow chart for detection of all enterovirus serotypes from clinical samples

6.19 RESULTS

6.19.1 Enteroviruses were protected by snRT-PCR and Seq.

Table1. Result of Enteroviruses anf EV71 by snRT-PCR in 2011

N report	N collected samples	% tested	N (+)	% (+)	(+) EV71		(+) Enterovirus	
					N(+)	%(+)	N(+)	%(+)
20.529	912	4.4	603	63.5	275	45.6	328	54.4

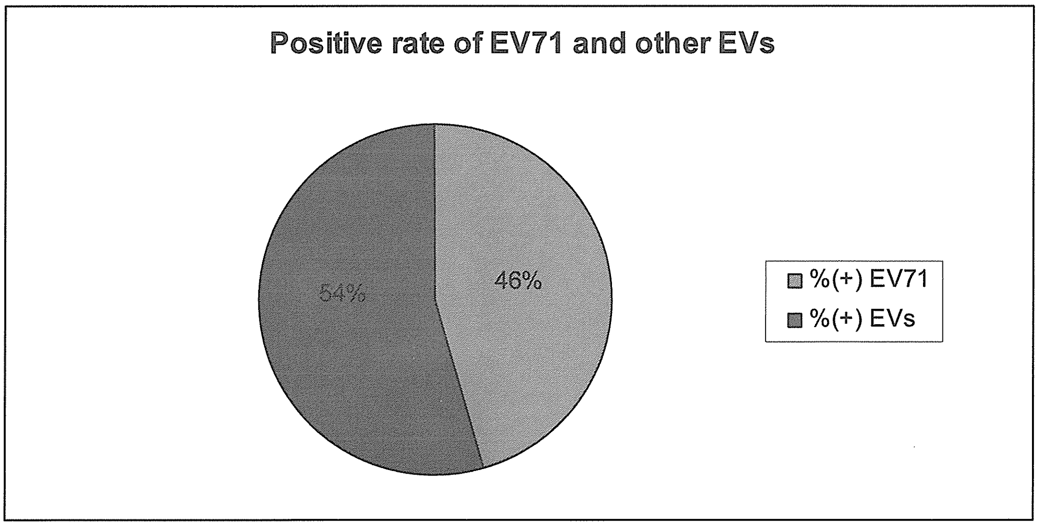


Figure 1. HEV-71 and EVs was protected by snRT-PCR

In 2011, 603 of 912 HFMD cases (63.5%) were found positive for HEV by using enterovirus general primers, and snRT-PCR method. HEV-71 and EVs occupied 46% (275) and 54% (328) respectively. (Figure 1).

Table2. Result of enterovirus serotypes by sequencing in 2011

N enterovirus by snRT-PCR	Result of Enterovirus serotypes by seq.						
	(+) Coxsackievirus - A			(+) CB	(+) Echo	(+) Other EV	Rhinovirus
	CAV- 6	CAV-16	Other CAV				
328	177 53,9%	102 31,3%	15 4,6%	2 0,6%	14 4,3%	3 (EV96 & Polio) 0,9%	15
	89,6%			5,8%			4,6%

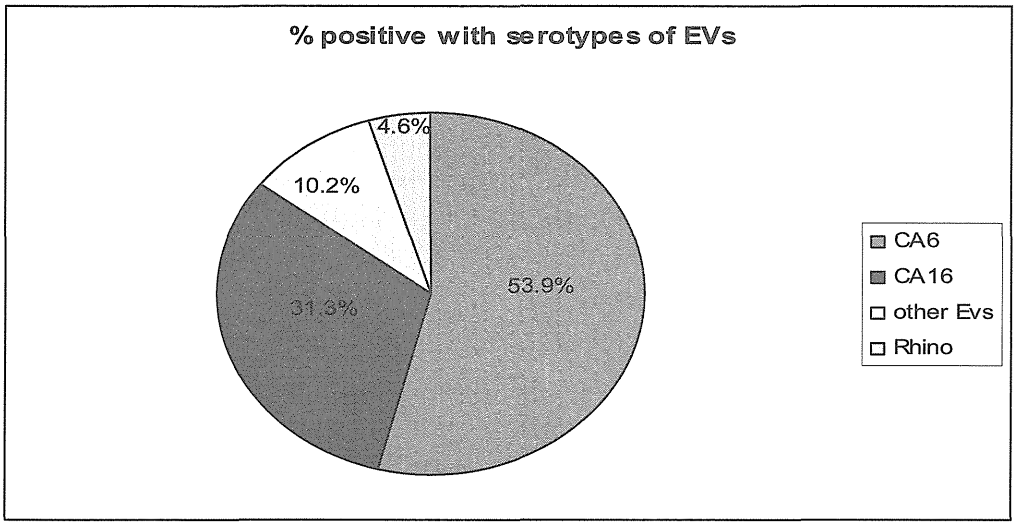


Figure 2. Enterovirus Serotypes was confirmed by Sequencing

328 of other EVs PCR-2 products were sequenced with primer pairs at position of primer F 2602-2627 or primer R 2977-2951 (375nt). The result showed with 53,9% of CoxsackieA 6; 31,3% of CoxsackieA16. The other entroviruses containing 10.2% with CV-A3(1), A10(4), A12 (8), và A13 (2); CV-B3, B4 (2);14 echovirus type 30 and 2 Polio, 1 EV96 were also detect (Figure 2)

Table 3. Enterovirus type 71's subgenogroups

TT of PCR produts/EV71	TT of EV71 PCR products sequenced	C4	C5	B5
275	49	36	3	10
% of each EV71 subgenogroup		73.5	6.1	20.4

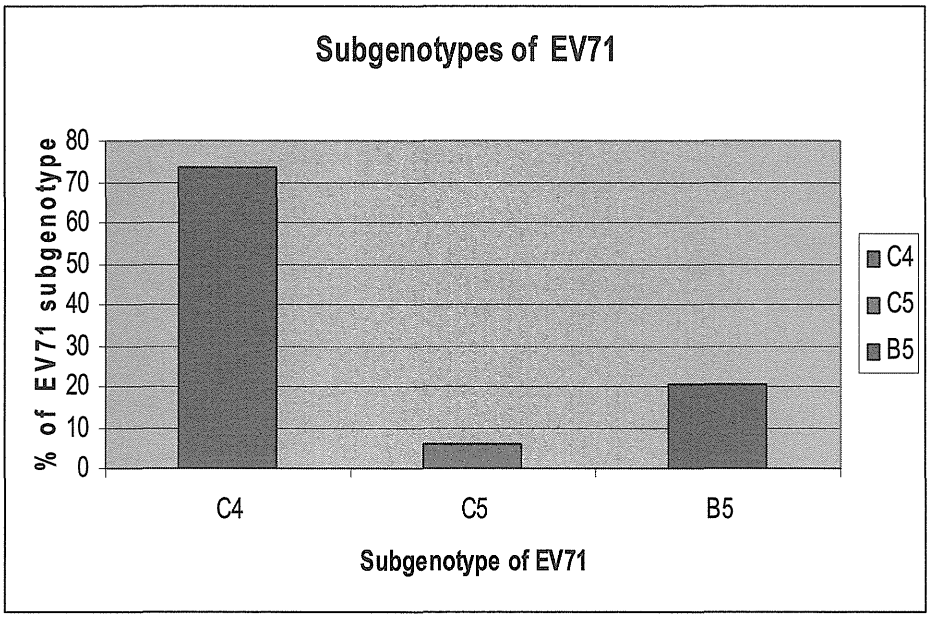


Figure 3. Enterovirus type 71's subgenogroups

49 PCR products of EV71 were amplified and sequenced. Result showed 73.5% as subgenotype C4, 6.1% as subgenotype C5 and 20.4 % as subgenotype B5

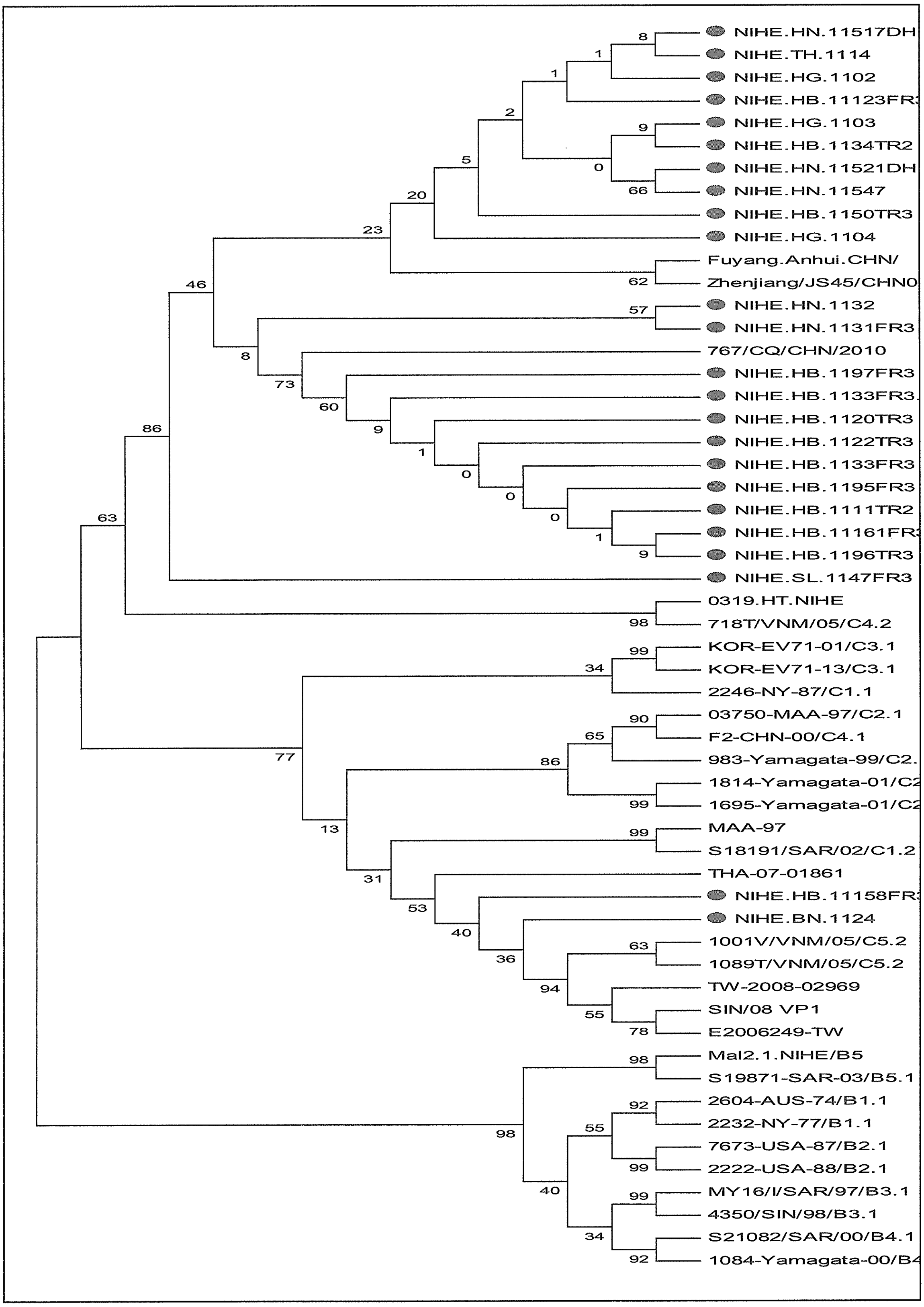


Figure 4. Phylogenetic relationships of human enterovirus 71 (HEV71) strains belonging to genogroup C

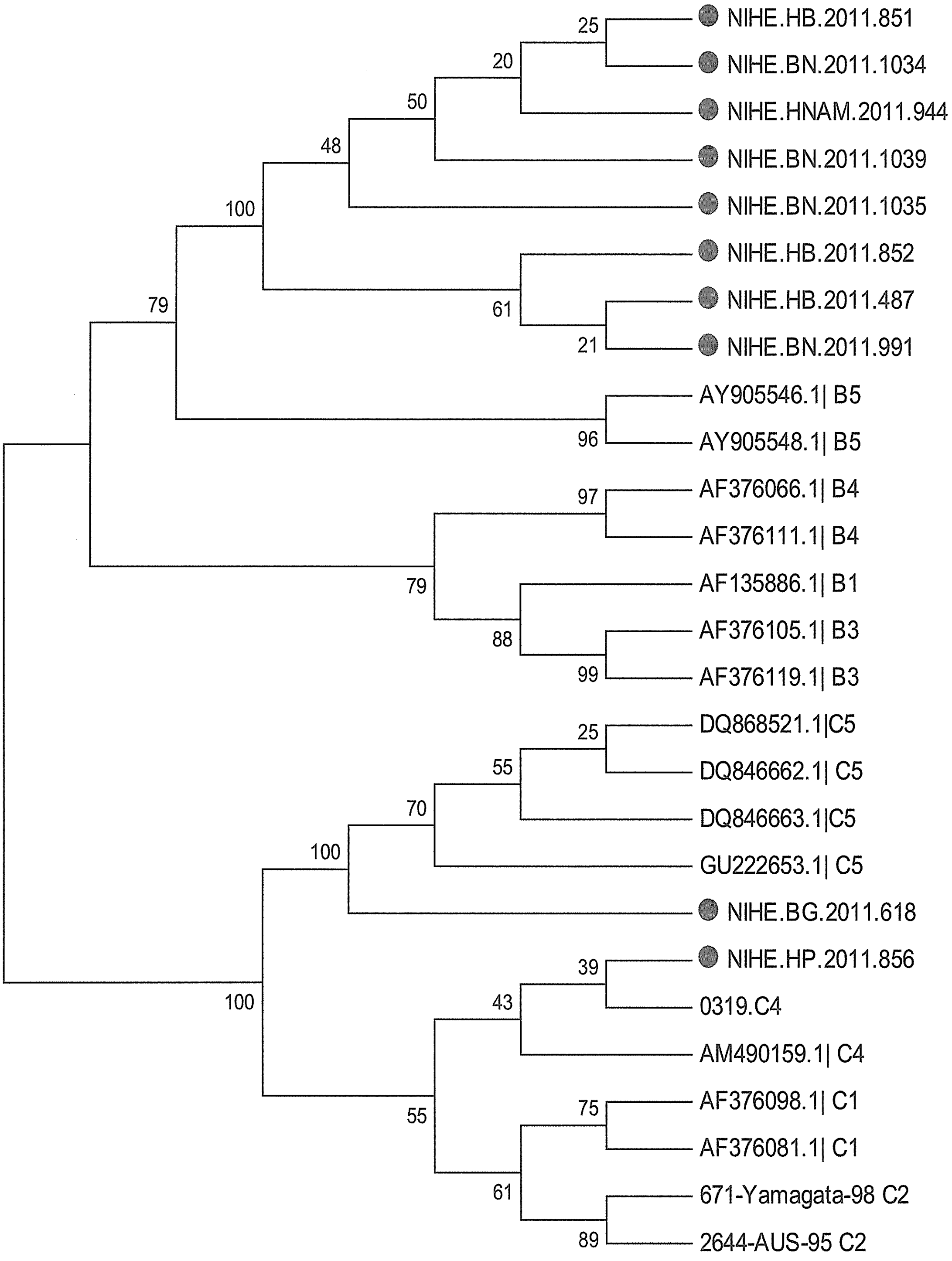


Figure 5. Phylogenetic relationships of human enterovirus 71 (HEV71) strains belonging to genogroup B and C

49 HEV-71 PCR products from 21 isolates and 28 clinical samples were sequenced. Result showed 79.6 % belonging to genogroup C (Figure 3,4) with 73.5% of subgenogroup C4 predominantly responsible for almost all HEV-71 infections in 2011 in the north of VN and 6.1% of subgenogroup C5, and 20.4% belonging to genogroupB, subgenogroup B5 (Figure4). However, the analysis of recent and previous HEV71 isolates in the Western Pacific Region showed that several subgenogroups, B1, B2, B3, B4, C1, C2, C3 and C4 were cocirculating in Australia, Malaysia, Singapore, Taiwan and Japan respectively.

Table 4. Ages distribution of 603 cases with HFMD confirmed as EV71 and other enteroviruses

Age	N tested	N(+)	N (+) VRĐR	(+) EV71		(+) VRĐR khác	
				N(+)	% (+)	N(+)	%(+)
<1	97	62	10.3%	22	8.0%	40	12.8%
1	329	232	38.5%	97	35.3%	135	43.1%
2	261	183	30.3%	87	31.6%	96	30.7%
3	103	57	9.5%	33	12.0%	24	7.7%
4	37	22	3.6%	13	4.7%	9	2.9%
5	30	15	2.5%	7	2.5%	8	2.6%
6 -30	39	20	3.3%	12	4.4%	8	2.6%
No infn	16	12	2.0%	4	1.5%	8	2.6%
TT	912	603	100%	275	100%	313	100%

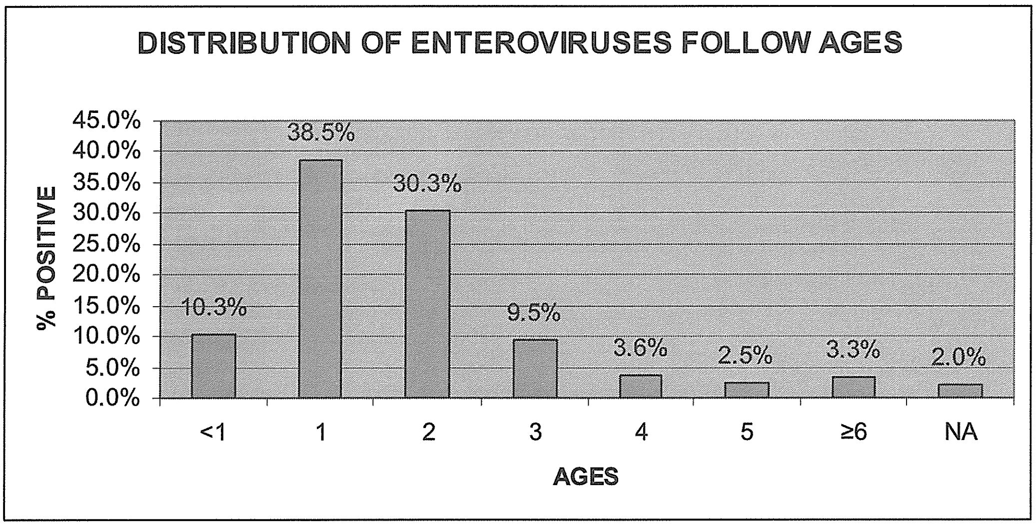


Figure 6, Ages distribution of 603 cases with HFMD confirmed as enteroviruses

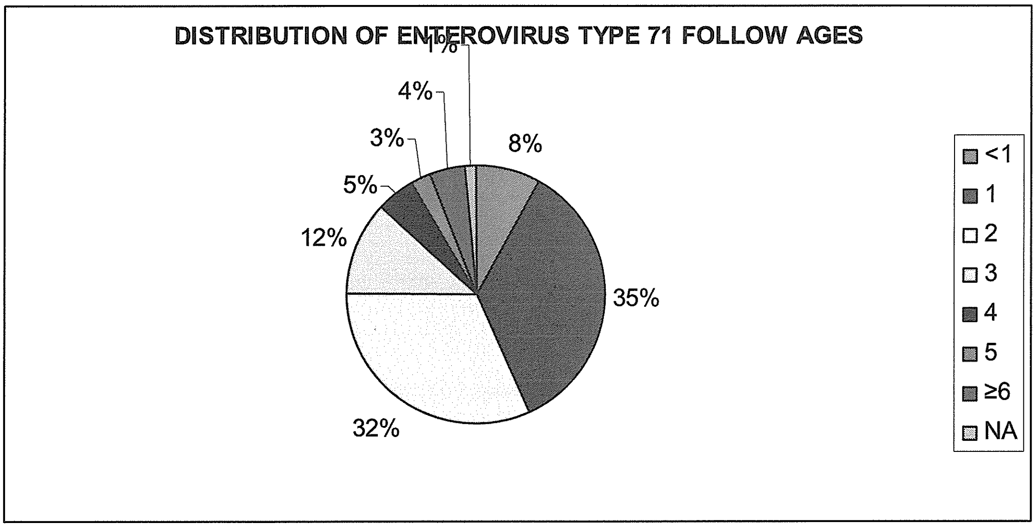


Figure 7, Ages distribution of 275 cases with HFMD confirmed as enterovirus TYPE 71

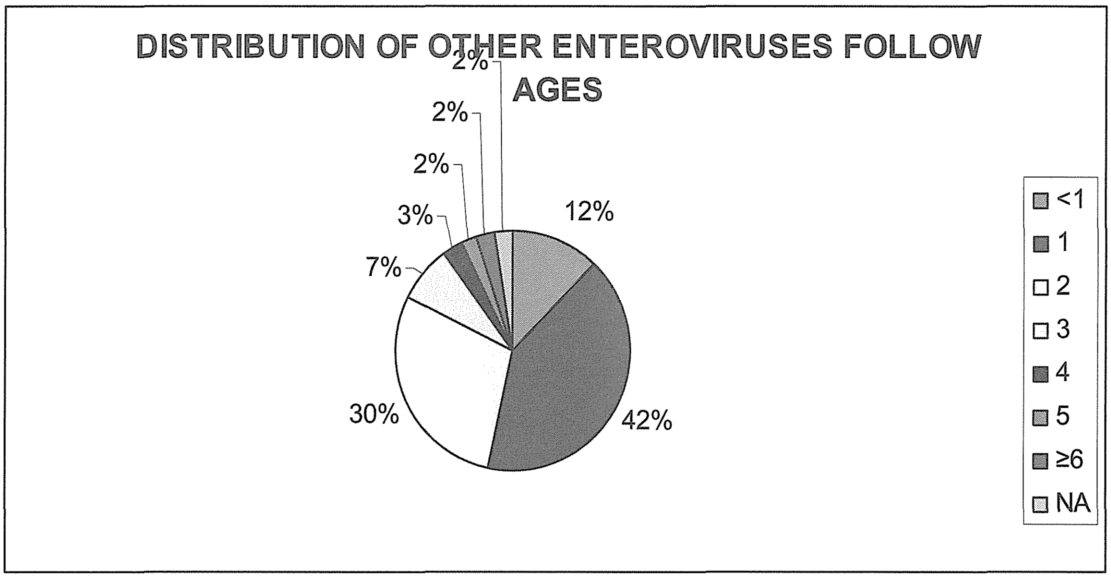


Figure 8, Ages distribution of 328 cases with HFMD confirmed as other enteroviruses

Table 4 showed the results of HFMD following age group, the disease is highly concentrated in children within 3 years, there is no difference of the incidence of diseases caused by enterovirus type 71 (46.7%) and other enterovirus (53.3%) in each age group.

Table 5. Geographic distribution of hand, foot, and mouth disease cases

IT	Provinces	N-report	N-tested	(+) Enteoviruses	% (+) EVs	(+) EV71	% (+)	(+) other EVs	% (+)
1	LẠNG SƠN	662	2	1	0.2%	0	0,0	1	100,0
2	HƯNG YÊN	154	3	2	0.3%	0	0,0	2	100,0
3	T, NGUYÊN	224	6	2	0.3%	0	0,0	2	100,0
4	LAI CHÂU	50	6	3	0.5%	2	66,7	1	33,3
5	NGHỆ AN	561	10	4	0.7%	1	25,0	3	75,0
6	SƠN LA	174	6	5	0.9%	5	100,0	0	0,0
7	ĐIỆN BIÊN	28	10	6	1.0%	5	83,3	1	16,7
8	THÁI BÌNH	814	12	6	1.0%	1	16,7	5	83,3
9	HÀ NAM	270	16	7	1.2%	2	28,6	5	71,4
10	CAO BẰNG	257	10	9	1.5%	1	11,1	8	88,9
11	HÀ TĨNH	118	13	9	1.5%	1	11,1	8	88,9
12	LÀO CAI	88	10	9	1.5%	4	44,4	5	55,6
13	YÊN BÁI	495	11	9	1.5%	2	22,2	7	77,8
14	NINH BÌNH	1,028	33	15	2.6%	1	6,7	14	93,3
15	QUẢNG NINH	473	25	16	2.7%	2	12,5	14	87,5
16	HÀ GIANG	285	33	20	3.4%	10	50,0	10	50,0
17	NAM ĐỊNH	133	25	21	3.6%	9	42,9	12	57,1
18	BẮC CẠN	380	31	22	3.7%	9	40,9	13	59,1
19	VĨNH PHÚC	872	38	23	3.9%	2	8,7	21	91,3
20	HẢI DƯƠNG	536	45	24	4.1%	1	4,2	23	95,8

21	BẮC NINH	158	42	25	4.3%	11	44,0	14	56,0
22	T, QUANG	561	24	26	4.4%	15	57,7	11	42,3
23	PHÚ THỌ	967	77	31	5.3%	1	3,2	30	96,8
24	THANH HÓA	3,744	49	33	5.6%	25	75,8	8	24,2
25	BẮC GIANG	508	60	46	7.8%	8	17,4	38	82,6
26	HẢI PHÒNG	3,046	80	63	10.7%	56	88,9	7	11,1
27	HÀ NỘI	1,579	116	70	11.9%	32	45,7	38	54,3
28	HÒA BÌNH	2,364	119	81	13.8%	69	85,2	12	14,8
	Tổng	20,529	912	588		275	46,8	313	53,2

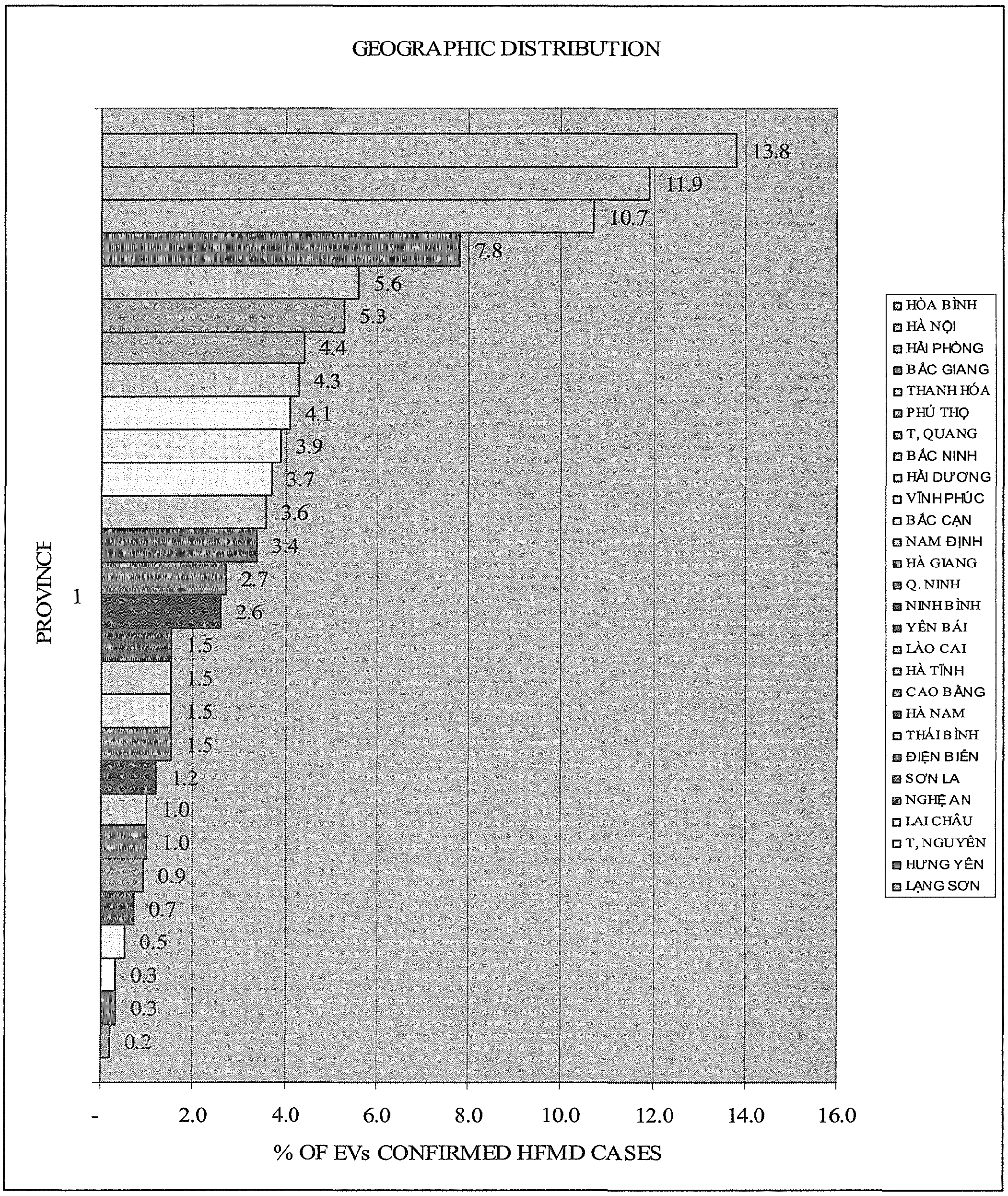


Figure 9. Geographic distribution of hand, foot, and mouth disease cases

The results showed 28/28 Northern provinces had HFMD cases. However, the HFMD cases in delta area was higher than mountain area; 24/28 provinces with the co-circulation of EV71 and other EVs. One only province (Son La province) only detected EV71 and three provinces (Hung Yen, Lang Son and Thai Nguyen province) only detected enterovirues

Table 6. Geographic distribution of hand, foot, and mouth disease cases by EV71, CAV-6 and CAV-16

PROVINCE	% (+)/ EV71	% (+)/ CA16	% (+)/ CA6	% (+)/ Other EVs
BẮC CẠN	40.9%	9.1%	50.0%	0.0%
BẮC GIANG	18.2%	22.7%	47.7%	9.1%
BẮC NINH	50.0%	18.2%	22.7%	4.5%
CAO BẰNG	11.1%	88.9%	0.0%	0.0%
ĐIỆN BIÊN	83.3%	16.7%	0.0%	0.0%
HÀ GIANG	55.6%	0.0%	38.9%	5.6%
HÀ NAM	28.6%	42.9%	28.6%	0.0%
HÀ NỘI	48.5%	20.6%	25.0%	2.9%
HÀ TĨNH	10.0%	0.0%	80.0%	0.0%
HẢI DƯƠNG	0.0%	56.5%	26.1%	17.4%
HẢI PHÒNG	100.0%	0.0%	0.0%	0.0%
HÒA BÌNH	93.3%	0.0%	4.0%	0.0%
HƯNG YÊN	0.0%	50.0%	50.0%	0.0%
LAI CHÂU	40.0%	20.0%	0.0%	0.0%
LẠNG SƠN	0.0%	100.0%	0.0%	0.0%
LÀO CAI	33.3%	50.0%	0.0%	16.7%
NAM ĐỊNH	42.9%	14.3%	42.9%	0.0%
NGHE AN	20.0%	20.0%	20.0%	0.0%
NINH BÌNH	7.7%	7.7%	69.2%	7.7%
PHÚ THỌ	3.3%	23.3%	46.7%	26.7%
QUẢNG NINH	13.3%	6.7%	73.3%	6.7%
SƠN LA	100.0%	0.0%	0.0%	0.0%
T. NGUYÊN	0.0%	0.0%	100.0%	0.0%
T. QUANG	47.6%	0.0%	38.1%	9.5%
THÁI BÌNH	14.3%	0.0%	85.7%	0.0%
THANH HÓA	64.5%	12.9%	9.7%	3.2%
VĨNH PHÚC	8.3%	79.2%	12.5%	0.0%
YÊN BÁI	33.3%	33.3%	33.3%	0.0%

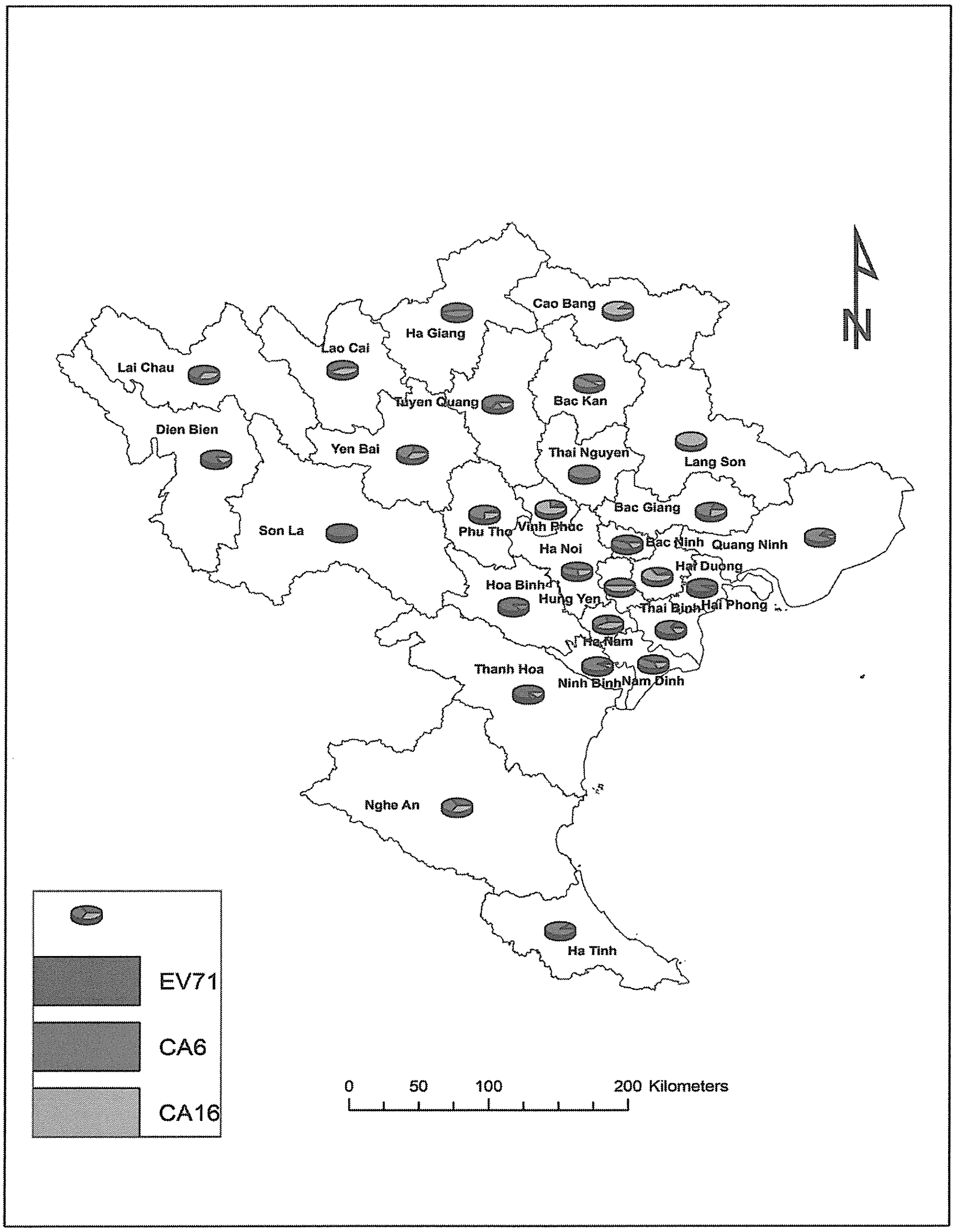


Figure 10. Geographic distribution of hand, foot, and mouth disease cases associated with infection of human enterovirus 71, coxsackievirus A6 and coxsackievirus A6 in Northern Vietnam, 2011.

After circulation of EV71 is CAV 6 and CAV 16. There are difference for circulating of serotypes of Coxsackievirus-A in the northern provinces, some provinces with only circulation of Coxsackievirus-A6 (Ha Tinh, Thai Nguyen, Ha Giang, Hoa Binh, Tuyen Quang), and some provinces have only Coxsackievirus A16 (Cao Bang, Dien Bien, Lai Chau, Lang Son and Lao Cai), the remaining provinces have co-circulation of both Coxsackievirus-A6 and CAV16

Some provinces detected Coxsackievirus A3 (Bac Giang), Coxsackievirus A10 (Bac Giang, Lao Cai, Hai Duong, Phu Tho), Coxsackievirus A12 (Bac Giang, Phu Tho, Hanoi, Hai Duong, Quang Ninh), Coxsackievirus A13 (Tuyen Quang), echovirus type 30 in Phu Tho and

Ninh Binh, Ha Giang, Bac Ninh; especially with appearance of enterovirus type 96 in Tuyen Quang province.

Table 7. Geographic distribution of hand, foot, and mouth disease cases by EV71 subgenogroup

IT	PROVINCE	N - EV71	C4	C5	B5
1	BẮC GIANG	2		1	1
2	BẮC NINH	5	1		4
3	HÀ GIANG	4	4		
4	HÀ NAM	1			1
5	HÀ NỘI	6	6		
6	HẢI PHÒNG	6	6		
7	HOÀ BÌNH	17	14		3
8	SƠN LA	1	1		
9	THANH HOÁ	5	4	1	
	TT	47	36	2	9

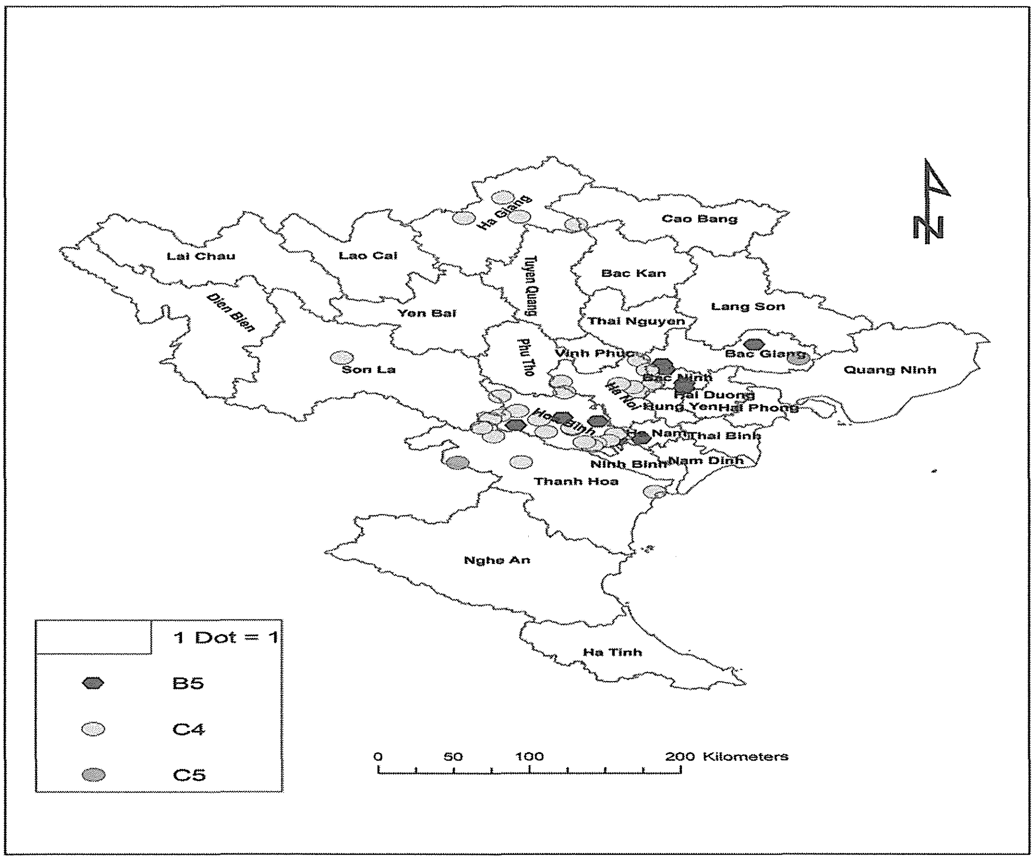


Figure 11. Geographic distribution of hand, foot, and mouth disease cases associated with infection of subgenogroup/HEV-71, in Northern Vietnam, 2011.

46 EV71 from 9 provinces in the north of Viet Nam were sequenced and analyzed. Results showed, they belonged to 2 genogroups with 3 subgenogroups B5, C4 and C5. Bac Ninh and Hoa Binh province had co-circulation of 2 genogroups B and C with subgenogroup B5 and C4; Bac Giang province had also co-circulation of 2 genogroups B and C with subgenogroup B5 and C5; Thanh Hoa province had circulation of genogroup C with 2 subgenogroups C4 and C5

6.20 DISCUSTION

6.20.1 Epidemiology of HFMD

HFMD was identified in northern Vietnam in 2011; HEV71, CVA16 and CA6 were also identified throughout the year, EV71 was the predominant virus during this time, accounting for 46% (275 cases) of HFMD compared to 54% (328 cases) for all other enteroviruses (table 1). After EV71 were CA6 and CA16, they were also the predominant viruses during this time, accounting for 53.9 % of HFMD compared to 31.3% for CVA6 and CA16 respectively (table 2).

Enteroviruses have circulated and caused HFMD outbreak in 2011 at all 28 northern provinces (table 5 and figgue 9)

6.20.2 Molecular Epidemiology of HEV71

Phylogenetic analysis based on nucleotide sequence alignment of 18 representative strains with the complete VP1 gene and 28 PCR products from clinical samples of HEV71 at 9 provinces of northern Vietnam. Result showed 2 genogroups that belonged to genogroup C with 2 subgenogroups C4, C5 and genogroup B with subgenogroup B5. Predominant EV71 strain in 2011 identified as subgenotype C4 during the HFMD outbreak of year and after subgenotype C4 was subgenogroup B5. Subgenogroup C5 was sporadic

Genogroup C with subgenogroup C4 and C5 have emerged recently in Southeast Asia, Viruses belonging to subgenogroup C4 were first identified in the People's Republic of China in 1998 and again in 2000 before their identification in northern Vietnam in 2003 (only one case), Furthermore, a new subgenogroup C5, circulated in northern Vietnam throughout 2008-2010 but decreased in 2011 and subgenogroup C4 reappeared and replaced for subgenogroup C5, and a new genogroup (genoproup B) with subgenogroup B5 appeared and caused HFMD in 2011

With evidence of the ongoing evolution of new subgenogroup and appearance of new genogroup similar to that observed for genogroup B HEV71 strains in Southeast Asia . Furthermore, the year-round detection and circulation of multiple independent genetic lineages of HEV71 suggested that this virus have circulated endemically within the human population of northern Vietnam.

平成２４年度業績

＊研究成果の刊行に関する一覧表

＊学会発表一覧表

研究成果の刊行に関する一覧表　(平成２４年度)

執筆者氏名	刊行書籍又は雑誌名（雑誌のときは雑誌名、巻号数、論文名）	刊行書店名	巻名	ページ	刊行年
プロジェクト１：中国					
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