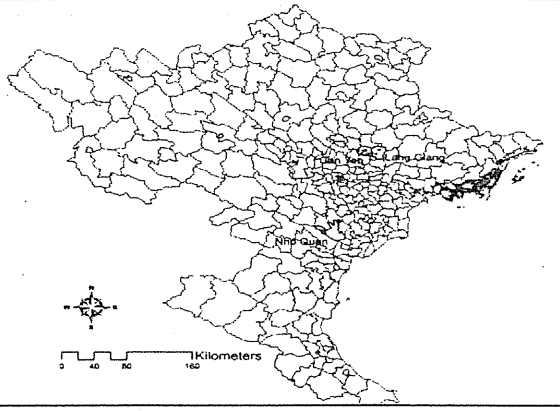
Figure 5. Distribution of rubella confirmed cases



! Ru2008 Rubella confirmed cases in 2008, ! Ru2009 rubella confirmed cases in 2009

8.11 Discussions

In Vietnam, measles vaccine was introduced into the routine immunization schedule in 1982. National coverage increased during the 1980s, reaching 89% by 1989 and 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 2006-2009. 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 provinces.

2B genotype of RV was circulating in 2 provinces: Ninh Binh and Bac Giang in the North Vietnam in 2008 - 2009. 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 conntries.

This study reports genotype H1 of MV is the endemic genotype circulating in the North Vietnam from 2006 to 2009.

8.12 Publications

None

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8.14 Acknowledgements

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Research 9

9.1 Project title: ***Epidemiology and molecular characteristics of the hand, foot and mouth disease in the North of Vietnam.***

9.2 Objectives:

a. To identify causative agents and epidemiological characteristics of HFMD
b. To estimate the asymptomatic rate (EV71, CA16 carrier proportion) in close contact group of HFMD.
c. To identify molecular characteristics of EV71.

9.3 Name of Researchers

National Institute of Hygiene and Epidemiology, Vietnam:

a. Nguyen Thi Hien Thanh
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a. National Institute of Hygiene and Epidemiology, Vietnam.
b. National Institute of Infectious Diseases, Japan.

9.5 Sub-project title: Virological Investigation of Hand, Foot, and Mouth Disease, Northern Vietnam, 2011

9.6 Summary

9.7 Purposes

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 (*1*).

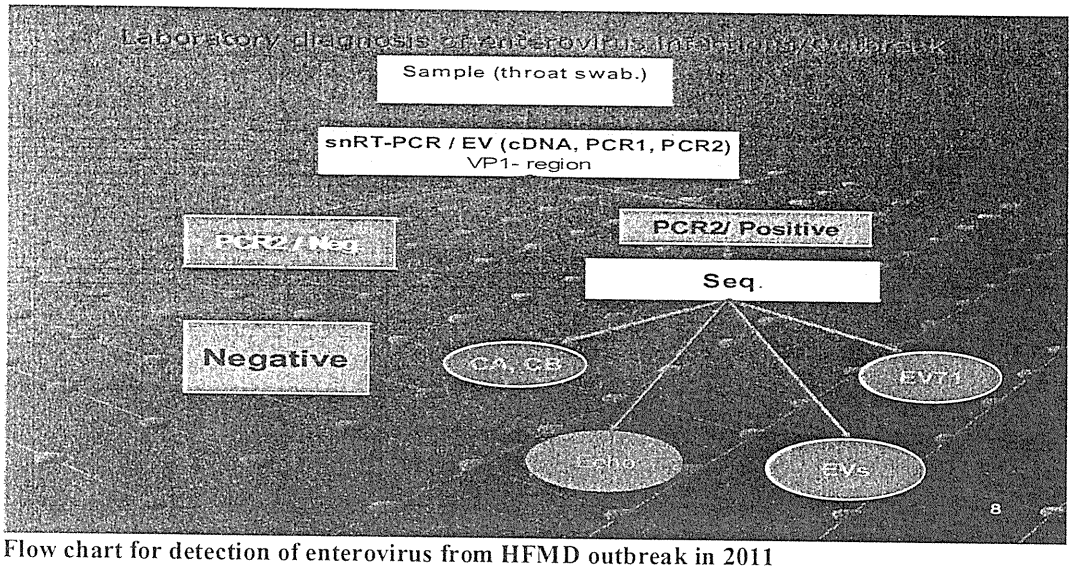
The genus *Enterovirus* of the family *Picornaviridae* is divided into 9 species, 5 of which infect humans. These viruses include the prototype species poliovirus, as well as HEVA, HEVB, HEVC, and HEVD. Viruses belonging to the HEVA species include 11 serotypes of coxsackievirus A (CVA; serotypes 2–8, 10, 12, 14, and 16), and human enterovirus 71 (HEV71) (*2,3*).

Since the discovery of HEV71 in 1969 (*5*), numerous outbreaks of this infection have occurred throughout the world (*4*). 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 (*12*), Malaysia (*7*), Singapore (*4*), South Korea (*6*), the People's Republic of China (*13*), and Australia (*14–16*). The most extensive epidemic of HEV71 occurred in Taiwan in 1998, with ≈1.3 i 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 (*8,17*).

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, we isolated HEV71/C5 from 7 patients and many Coxsackie-A16 virus in this outbreak. Although laboratory database for enterovirus surveillance from sample of patient with HFMD has been shown to provide adequate warning of impending outbreaks of enteroviruses associated HFMD(*18*) but laboratory need to set up SOP for. Nowadays, laboratory surveillance system on enteroviruses from patient with HFMD has not yet been established in Vietnam

Objective of this study is to identify the enterovirus agents caused Hand Foot and Mouth Disease in the north of Viet Nam in 2011.

9.8 Methods



Flow chart for detection of enterovirus from HFMD outbreak in 2011

Specimen Collection

Where, when and how to collect, what kinds of sample

In 2011, A total of 922 specimens were collected from the children with HFMD at 29 provinces of northern-Vietnam. Each child had at least 1 specimen collected from vesicle fluid, or throat swab, or stool.

- 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
SO222R	5-CICCIGGIGGIAYRWACAT		2969-2951
AN89F	*5-CCAGCACTGACAGCA*GYNGARAYNGG	*PCR2/EVs*	2602-2627
AN88R	*5-TACTGGACCACCTGG*NGGNAYRWACAT		2977-2951
MAS01S	*5-ATAATAGCA(C/T)T(A/G)GCGGCAGCCCA*	*PCR2/EV71*	

Reagents and method used for detection of enterovirus from clinical sample: A reverse transcription-seminested PCR (RT-snPCR) assay was used for the detection and identification of enterovirus (EV) RNA in clinical specimens.

The VP1 RT-snPCR assay was slightly more sensitive. the VP1 RT-snPCR assay 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.

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-PCR and sequencing.
Synthesis of cDNA was carried out in a 10àl reaction mixture containing 5 àl of RNA, 100 àM each deoxynucleoside triphosphate (dNTP; Amersham Biosciences, Piscataway, NJ), 2 àl of 5 x reaction buffer (Invitrogen, Carlsbad, CA), 0.01 M dithiothreitol, 1 pmol each cDNA primer (primers AN32, AN33, AN34, and AN35; Table 1), 20 U of RNasin (Promega Corp., Madison, WI), and 100 U of SuperScript II reverse transcriptase (Invitrogen), incubation at 22°C for 10 min, 45°C for 45 min, and 95°C for 5 min.

Following) incubation, the entire 10 àl RT reaction mixture was then used in the first PCR (final volume, 50 àl) (PCR1), consisting of 5 àl of 10 x PCR buffer (Roche Applied Science), 200 àM 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 àM each dNTP, 5 àl 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 àl. 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 àg 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 àM each dNTP, 5 àl 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 àl. 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 àg 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.

Statistical Methods
Differences between proportions were tested by using the $\hat{u}r^2$ 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.

9.9 Results

Table 1. Enteroviruses was protected by snRT-PCR

No. Samples	Positive Samples	Enterovirus were identified	
		(+) EV71	(+) other EVs
922	598	239	359
% of positive	64.8	40	60

In 2011, 922 samples was collected from patients with Hand Foot and Mouth Disease. These clinical samples were extracted and amplified to detect enteroviruses by EV specific primer pairs region VP1. Results showed that 25.9% was Enterovirus type 71, 38.9% was other enteroviruses as Coxsackievirus and echovirus

Table 2. Enterovirus Serotypes was confirmed by Sequencing

TT of PCR produts/ EVs	TT of PCR produts/ Seq.	Enterovirus Serotypes was confirmed by Sequencing					
		CA16	CA6	CA10	CA12	CB	Echo 30
359	62	30	24	3	1	2	2
%		48.4	39	4.8	1.6	3.2	3.2

62 PCR-2 products that negative with EV71 and positive with other enteroviruses were sequenced with primer pair at position of primer AN89F (2602-2627) and primer AN88R (2977-2951). The result showed with 48.4% of CoxsackieA virus type 16; 39% of of CoxsackieA virus type 6; a few of other entroviruses was CoxsackieA virus type 10, CoxsackieA virus type 12, CoxsackieB virus typ 3 & type4 and echovirus type 30

Table 3. Enterovirus type 71's Genotypes

TT of PCR produts/EV71	TT of PCR produts/EV71 sequenced	C4	C5
239	23	21	2
% of EV71 subgenotype		91.3%	8.7%

23 PCR products of enterovirus type 71 were amplified by primer pair with primer AN89F (2602-2627) and primer AN88R (2977-2951), and were sequenced by the same primer. The result showed 91.3% of subgenotype C4 and 8.7% of subgenotype C5

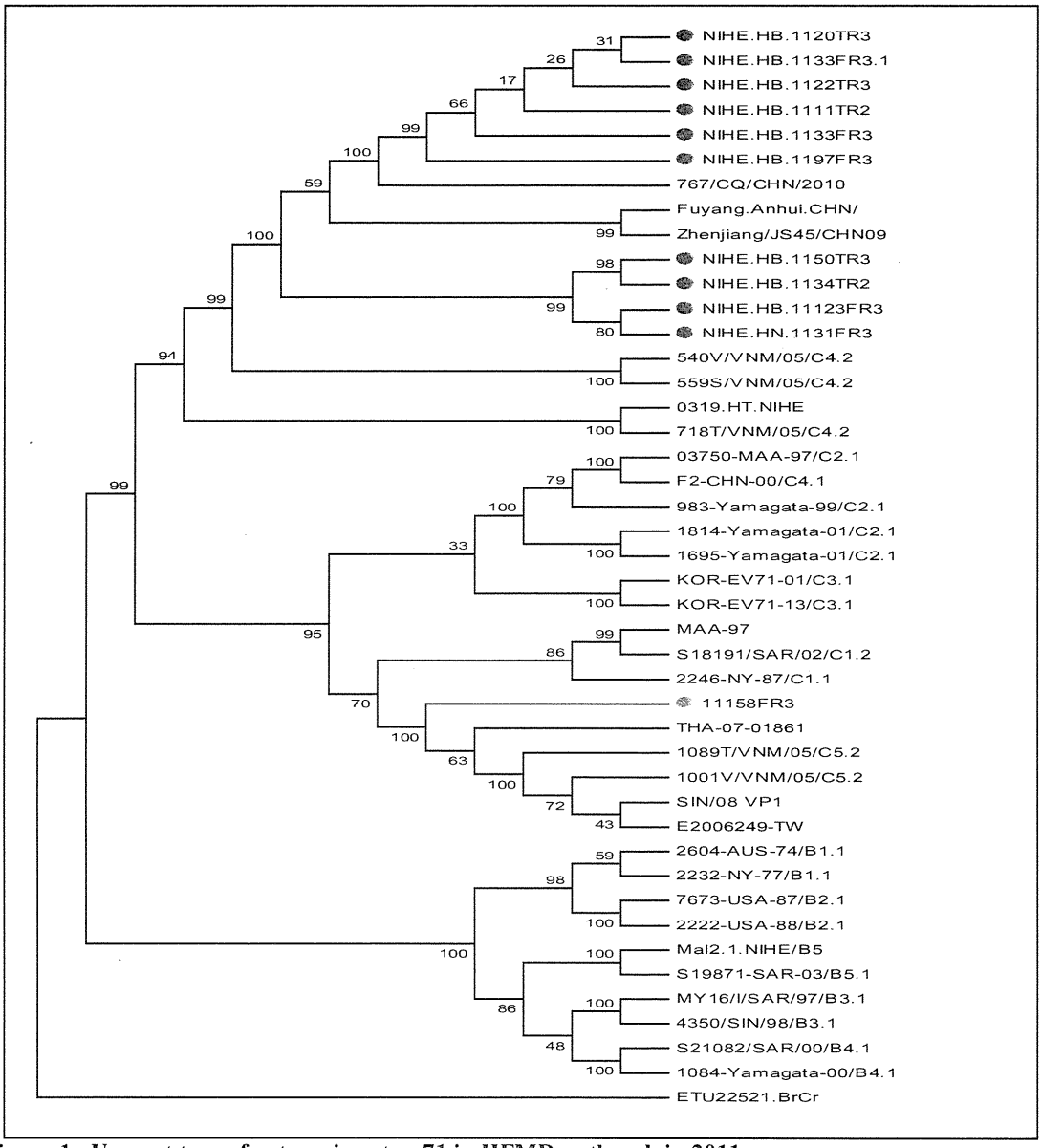


Figure 1 . Un-root tree of enterovirus typ 71 in HFMD outbreak in 2011

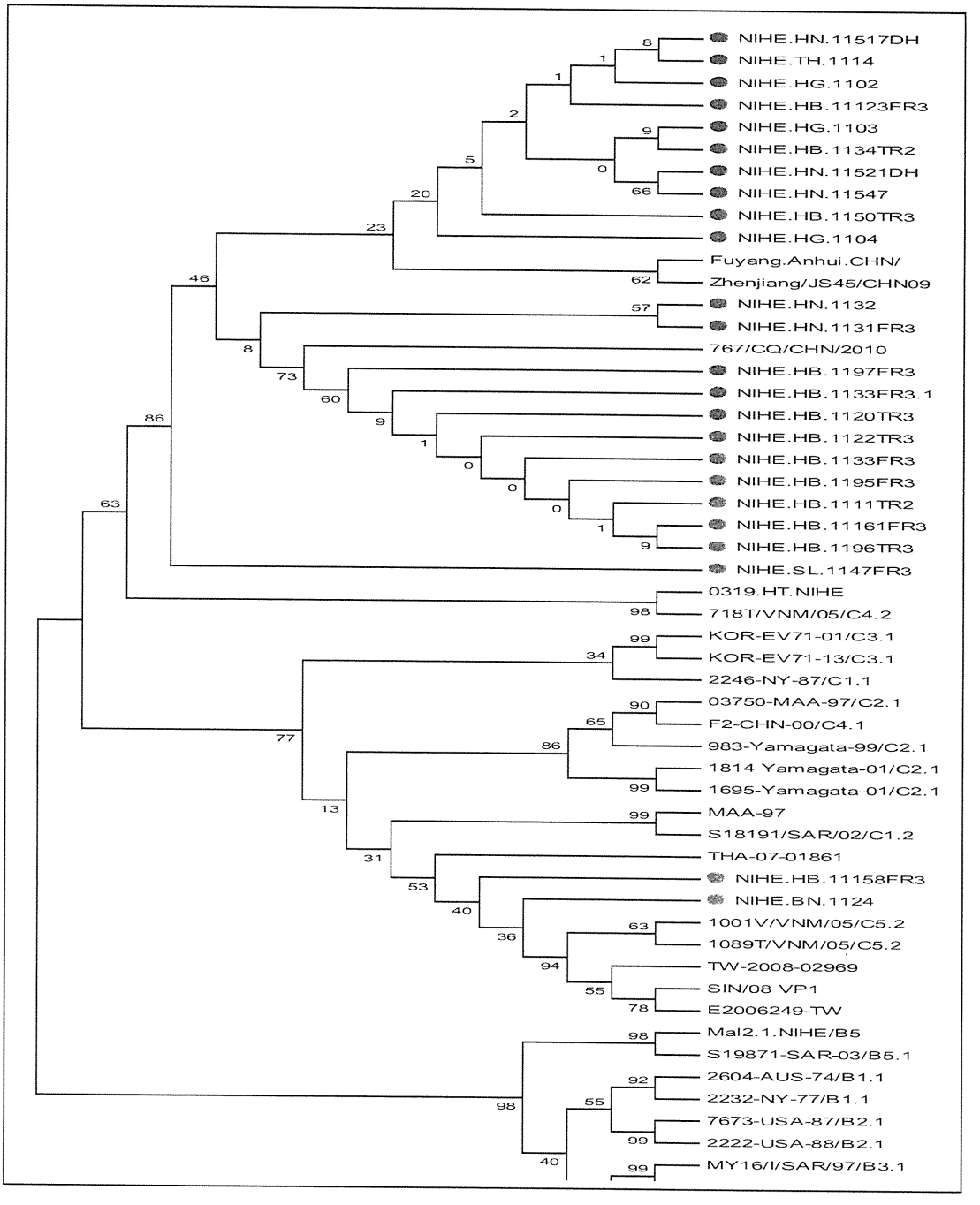
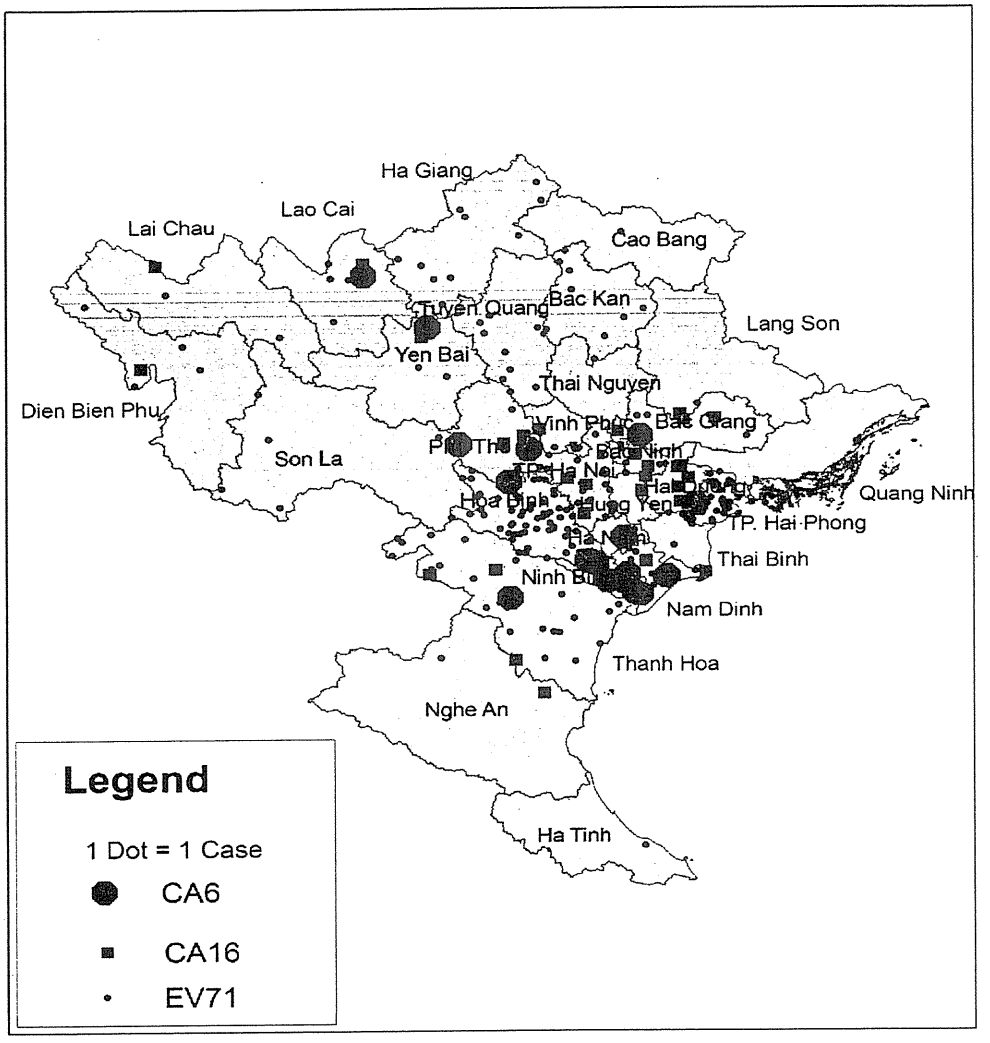


Figure 2. Un-root tree of enterovirus typ 71 in HFMD outbreak in 2011
Subgenotype C4 have circulated and caused HFMD outbreak in 2011 is the same Subgenotype C4 that circulated and caused HFMD outbreak in the south of Vietnam in 2005



Figue 3. Distribution of enterovirus that detected in patients with HFMD at provinces in the north of Viet Nam in 2011

7.10 Discussion

Pathogens and epidemiology of HFMD

HFMD was identified in northern Vietnam throughout the year 2011; HEV71, CVA16 and CA6 were also identified throughout the year. EV71 was the predominant virus during this time, accounting for 40% (239 cases) of HFMD compared to 60% (359 cases) for all other enteroviruses (table 1, 2). CA16 and CA6 were also the predominant viruses during this time, accounting for 48.4% of HFMD compared to 39% for CVA16 and CA6 respectively (table 2).

Hand foot and mouth disease happened in the most of the northern provinces in 2011 with 25/28 provinces where appeared HFMD outbreak (figure 3).

Molecular Epidemiology of HEV71

Phylogenetic analysis based on nucleotide sequence alignment of the complete VP1 gene of 23 representative strains of HEV71 from northern Vietnam showed that they belonged to 2 subgenogroups,

HEV71/C4 and HEV71/C5. Two genetic lineages of genogroup C, subgenogroups C4 and C5 have emerged recently in northern Asia. Viruses belonging to subgenogroup C4 were first identified in the People's Republic of China in 1998 and again in 2000 (*35*) before their identification in northern Vietnam during 2003. Furthermore, a new subgenogroup, HEV71/C5 circulated and caused HFMD outbreak in whole country throughout 2005-2008 but in 2011, predominant virus strain identified as subgenogroup C4 during the HFMD outbreak of year.

Our data indicate that the molecular epidemiology of HEV71 during the HFMD outbreak in northern Vietnam in 2011 belongs to genogroup C virus strains, sub-genogroup C4, with evidence of the ongoing evolution of new subgenogroups, similar to that observed for genogroup B-HEV71 strains in Southeast Asia (*6, 9, 33*). Furthermore, the year-round detection and circulation of multiple independent genetic lineages of HEV71 (*36*) suggested that this virus circulates endemically within the human population of northern Vietnam.

9.10 Publications

None

9.11 Reference

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9.12 Acknowledgements

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平成 23 年度分担研究報告書

「アジアの感染症担当研究機関とのラボラトリーネットワークの促進と共同研究体制の強化に関する研究」 (H23－新興―指定―020)

研究分担者 寺嶋 淳	国立感染症研究所 細菌第一部
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研究要旨 アジア太平洋地域における細菌性下痢症起因菌のサーベイランスとして PulseNet Asia Pacific (PNAP)が稼働している。当該地域では、PNAP を構成する国・地域の感染症研究機関においても解析機器・技術に著しい格差が存在する現実がある。一方、PNAP における正確なデータ収集を確保するためには解析方法の標準化とともに、標準化方法を使用した解析技術の精度管理を徹底することが重要である。PNPA では、Pulse-field Gel Electrophoresis (PFGE)及び解析アプリケーションである BioNumerics を使用してデータの収集と解析が行われている。本ネットワーク機能を強化するための活動として、当該地域の PNAP 構成国あるいは参加を希望する国・地域を対象とした Pulse-field Gel Electrophoresis (PFGE)及び解析に使用するアプリケーションである BioNumerics のワークショップを計画・実行した。

A. 研究目的

アジア・太平洋地域において細菌性下痢症起因菌の分子解析ネットワークとして PNAP が日本をはじめとして当該地域の 14 カ国の感染症研究機関等において構成され稼働している。ネットワーク内でのデータの互換性を確保するために、PFGE 解析手技と解析アプリケーション BioNumerics 使用法の標準化を目的とした。

B. 研究方法

PNAP に参加している国・地域における 14 機関にワークショップの開催を周知し参加者を募った。PNAP では、BioNumerics に米国 CDC が開発した PulseNet Scripts (version 5.1)をインストールして共通の解析アプリケーションとして使用している。4 日間のワークショップでは、BioNumerics + PulseNet Scripts を用いた PFGE 画像解析を中心として研修を実施した。また、PFGE 解析手技の標準化を確認するために、実験室において PFGE 実施デモンストレーションを行った。研修の行程は別添1により、参加者によるワークショップの評価を回収した(別添2)。

C. 研究結果及び考察

PNAP 参加機関及び関連研究機関から 8 名(4 カ国、表 1)が参加した。マレーシア及びシンガポールからの参加者は、直接的な PNAP 参加機関からではないが、アジア・太平洋地域における近隣各国とのデータ交換を実施するうえでPNAPでの標準化解析方法の入手を希望したものである。当該地域におけるPNAPの活動が浸透している一端を示すものと考えられる。本ワークショップは 9 年間に及ぶ実績があり、主要な参加国では手技・手法が確立されてきていると言えるが、それらを除く関連機関においては、設備や解析手法の標準化がなされているとは言い難い。参加者による本ワークショップの評価が概して高いことも情報を求める関連研究者の需要があることを反映しているのであろう(別添 2)。本ワークショップでは、PFGE 解析を基本とした研修となっているが、参加者からも要望があるように、MLVA 導入に対応した内容のワークショップとすることも今後の課題と考えられる。

E. 結論

アジア・太平洋地域では、PFGE 解析手技、BioNumerics による解析でまだサポートを必要とする研究機関があることから、将来的に導入が予想される MLVA 手法の研修等を含めたワークショップの継続が必要と考えられる。

F. 健康危険情報

なし

G. 研究発表

特になし

H. 知的財産権の出願・登録状況

特になし

表 1

Participants List for PulseNet Asia Pacific PFGE Workshop 2012 (7-10 FEB 2012)

		Name	Sex	Email	From	Arrival Date	Departure Date
1	Trainer	Dr. Jun Terajima	M	terajima@nih.go.jp	Department of Bacteriology National Institute of Infectious Diseases Japan	6 Feb	11 Feb
2	Trainee	Mohammad Tarequl Islam	M	tareqislam62@yahoo.com	Laboratory Sciences Divisi ICDDR,B Bangladesh	6 Feb	10 Feb
3	Trainee	Mst. Mahmuda Akter	F	mahmuda@icddrb.org	Laboratory Sciences Divisi ICDDR,B Bangladesh	6 Feb	10 Feb
4	Trainee	Miss Cao Yanhong	F	delphinecao@gmail.com	Diagnostic Bacteriology Department of Pathology Singapore General Hospit Singapore	4 Feb	12 Feb
5	Trainee	Dr. Phua Kia Kien	M	kkphua@kb.usm.my	Institute for Research in Molecular Medicine Universiti Sains Malaysi Malaysia	6 Feb	11 Feb
6	Trainee	Ying Huang (黄瑛)	F	huangying819@163.com	Beijing Centers for Diseas Control and Prevention, PR China	6 Feb	11 Feb
7	Trainee	Guirong Liu (刘桂荣)	F	lgr420@sohu.com	Beijing Centers for Diseas Control and Prevention, PR China	6 Feb	11 Feb
8	Trainee	Yun Luo (罗芸)	F	amanda_lv@163.com	Zhejiang Provincial Cente for Disease Control and Prevention, PR China	6 Feb	11 Feb
9	Trainee	Xiaoli Du (杜小莉)	F	duxiaoli@icdc.cn	National Institute for Communicable Disease Control and Prevention, Chinese Center for Diseas Control and Prevention, P China	6 Feb	11 Feb

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Agenda for PulseNet Asia Pacific PFGE Workshop Hong Kong 2012

Date: February 7- 10, 2012
Venue: Conference Room at Public Health Laboratory Centre (PHLC), Hong Kong

February 7, 2012 (Tuesday)

Chairperson of the day: Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrive at PHLC	
9:00 – 9:15 am	Registration	
9:15 – 9:30 am	Welcome remarks, expectations of the Workshop	Kai Man Kam, PHLC, HK Jun Terajima, NIID, Japan
9:30 – 9:45 am	Overview of Workshop	Danny Cheung, PHLC, HK
9:45 – 10:20 am	Installation and Overview of BioNumerics/ PulseNet MasterScripts	Cindy Luey, PHLC, HK
10:20 – 10:30 am	Group Photo	
10:30 – 11:00 am	Coffee Break	
11:00 – 11:40 am	Analysis of PFGE Gel (TIFF), Linking Lanes, and Adding Text Data	Alf Chu, PHLC, HK
11:40 – 1:00 pm	Exercise 1: Analyze a PFGE Gel (TIFF) and Link Entries to a Database	Alf Chu, PHLC, HK
1:00 – 2:00 pm	Lunch	
2:00 – 2:30 pm	Creation and File Location of PulseNet Bundle Files	Cindy Luey, PHLC, HK
2:30 – 3:30 pm	Exercise 2: Prepare and Create a PulseNet Bundle File for Distribution	Cindy Luey, PHLC, HK
3:30 – 4:00 pm	Coffee Break	
4:00 – 4:15 pm	Laboratory Experience Sharing- Bangladesh	Participant presentation
4:15 – 4:30 pm	Laboratory Experience Sharing - (Beijing Regional Central Laboratory of PulseNet China)	Participant presentation
4:30 – 5:00 pm	Q and A	
5:00 pm	End of Day 1 – Shuttle back to Hotel	

February 8, 2012 (Wednesday)

Chairperson of the day: Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrive at PHLC	
9:00 – 9:20 am	Data Importing into and Exporting from BioNumerics	Alf Chu, PHLC, HK
9:20 – 10:30 am	Exercise 3: Analyze a PFGE Gel Image; Data Import into and from BioNumerics	Alf Chu, PHLC, HK
10:30 – 11:00 am	Coffee Break	
11:00 – 11:45 am	Querying and Performing Comparisons with Exporting Data from BioNumerics	Alf Chu, PHLC, HK
11:45 – 1:00 pm	Exercise 4: Performing Queries and Performing Comparisons	Alf Chu, PHLC, HK
1:00 – 2:00 pm	Lunch	
2:00 – 2:20 pm	Advanced Tools	Cindy Luey, PHLC, HK
2:20 – 3:00 pm	Exercise 5: Query the Database Using the Advanced Query Tool	Cindy Luey, PHLC, HK
3:00 – 3:30 pm	PFGE Experience in Japan	Jun Terajima NIID, Japan
3:30 – 3:45 pm	Coffee Break	
3:45 – 4:15 pm	Identify the Problems	Cindy Luey, PHLC, HK
4:15 – 4:30 pm	Laboratory Experience Sharing – (PulseNet China Central laboratory)	Participant presentation
4:30 – 4:45 pm	Laboratory Experience Sharing – Zhejiang Regional Central Laboratory of PulseNet China)	Participant presentation
4:45 – 5:00 pm	Q and A	
5:00 pm	End of Day 2 – Shuttle back to Hotel	

February 9, 2012 (Thursday)

Chairperson of the day: Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrive at PHLC	
9:00 – 9:35 am	Update on Platform for Interlaboratory Comparison Work Group (PIC WG) Activities	Edman Lam, PHLC, HK
9:35 – 9:55 am	Database Management Tools	Cindy Luey, PHLC, HK
9:55 –10:15 am	Exercise 6: Database Settings and Layouts, Pick List and Printing Reports	Cindy Luey, PHLC, HK
10:15 – 10:45 am	Coffee Break	
10:45 – 11:00am	Working with Subsets	Patricia Leung, PHLC, HK
11:00 – 11:40 pm	Exercise 7: Create Subsets for serotype in Salmonella Database	Patricia Leung, PHLC, HK
11:40 – 12:00 pm	Naming Patterns and Creating Local Unique Pattern Lists	Cindy Luey, PHLC, HK
12:00 – 1:00 pm	Exercise 8: Identifying and Naming Unique Patterns in the database	Cindy Luey, PHLC, HK
1:00 – 2:00 pm	Lunch	
2:00 – 2:20 pm	Using the Chart and Statistics Tool and Groups	Patricia Leung, PHLC, HK
2:20 – 3:00 pm	Exercise 9: Create Charts and Graphs to Create Reports	Patricia Leung, PHLC, HK
3:00 – 3:30 pm	Composite Data Sets	Alf Chu, PHLC, HK
3:30 – 4:00 pm	Exercise 10: Cluster analysis using a composite data set for *Salmonella*	Alf Chu, PHLC, HK
4:00 – 4:15 pm	Coffee Break	
4:15 – 4:30 pm	Laboratory Experience Sharing –Singapore	Participant presentation
4:30 – 4:45 pm	Laboratory Experience Sharing – Malaysia	Participant presentation
4:45 – 5:00 pm	Q and A	
5:00 pm	End of Day 3 – Shuttle back to Hotel	

February 10, 2012 (Friday)

Chairperson of the day: Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrive at PHLC	
9:00 – 9:30 am	Analysis of recent Enterohemorrhagic *E. coli* and *Shigella sonnei* isolates in Japan by the use of PFGE and MLVA	Jun Terajima NIID, Japan
9:30– 10:00 am	Coffee Break	
10:00 – 12:00 pm	Demo on PFGE protocols	Cindy LUEY, PHLC, HK
12:00 -1:00 pm	Lunch	
1:00 – 1:30 am	Development of PulseNet Standardized Protocol for Subtyping *Shigella flexneri* by PFGE	Kai Man Kam, PHLC, HK
1:30 – 2:00 pm:	Troubleshooting PFGE Gels	Cindy LUEY, PHLC, HK
2:00 – 4:00 pm	Practical Session on BioNumerics	PHLC, HK NIID, Japan
4:00 – 5:00 pm	Discussion Summary Certificate presentation	All participants Kai Man Kam, PHLC, HK Jun Terajima, NIID, Japan
5:00 pm	End of Workshop – Shuttle back to Hotel	

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WORKSHOP EVALUATION

Course name: The Ninth PulseNet Asia Pacific PFGE Workshop

Location: Public Health Laboratory Centre (PHLC), 382 Nam Cheong Street
Shek Kip Mei, Kowloon, Hong Kong

Dates: February 7-10, 2012

Offered by:
- Public Health Laboratories Centre (PHLC), Department of Health, Hong Kong
- Association of Public Health Laboratories (APHL)
- National Institute of Infectious Diseases (NIID), Department of Bacteriology, Japan
- PulseNet Program, Enteric Diseases Laboratory Branch (EDLB), Centers for Disease Control and Prevention (CDC), USA

Please complete this evaluation so that we can improve this workshop when it is given again.

1. What is your overall evaluation of this course?

Excellent 8 Good_____ Satisfactory_____ Unsatisfactory_____

2. Were the objectives of the course clearly defined? Yes 8 No____

3. Were the objectives of the course met? Yes 8 No____

4a. Please rate the quality and usefulness of handouts.

Excellent 8 Good_____ Satisfactory_____ Unsatisfactory_____

4b. Please rate the quality and usefulness of the practices.

Excellent 7 Good 1[1] Satisfactory_____ Unsatisfactory_____

5. Please rate how this course will influence your ability to perform and interpret molecular subtyping of *Salmonella* serotypes, *E. coli, Shigella, Vibrio* and other organisms in the future.

Very positively 6 Positively 2 Not much_____ Not at all_____

6. Would you recommend this course to others in public health laboratories? Yes 7 No 1

Please explain:

1. **It was excellent**
2. **Not answered**
3. **Not answered**
4. **Not answered**
5. **Not answered**
6. **Not answered**
7. **Very useful for users in the laboratory. Allows exchange of information/technique. There's more room for further improvement in terms of protocols/techniques.**[2]
8. **Not answered**

7. Please rate each of the following lectures:

"Subject Matter": **1 = material was not at all pertinent; 5 = it was very pertinent**
"Presentation": **1 = material was not at all clear; 5 = it was very clear**

"Time Allotted": **TS = lecture was too short; S = short; R = right amount of time; L = long;**

TL = lecture was too long

Date: Feb 7, 2012

	Subject Matter Time Allotted					Presentation			
	1	2	3	4	5	1	2	3	4
A. Installation and Overview of BioNumerics/ PulseNet MasterScripts					5 TS S R L TL				