중증 호흡기 질환과 관련된 바이러스 병원체 특성 및 실험실 감시

국립보건연구원
감염병센터
인플루엔자바이러스과
김기순

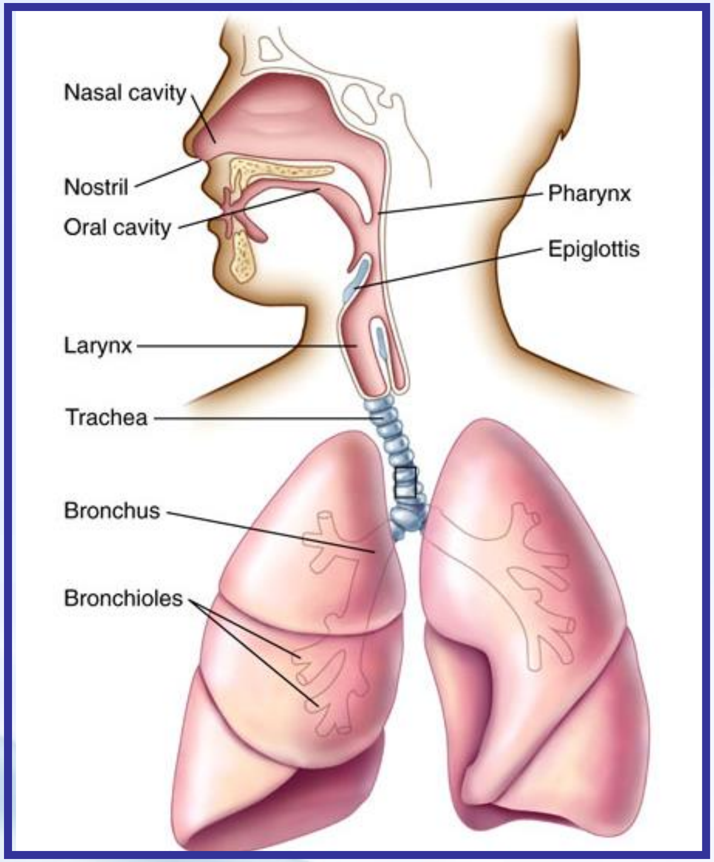
Contents

- Introduction of Respiratory viruses
  - Influenza virus (IFNV)
  - Human rhinovirus (HRV)
  - Human respiratory syncytial virus (HRSV)
- Viral factors related disease severity
- Surveillance system for respiratory viruses: KINRESS

(Korea Influenza and Respiratory Virus surveillance system)

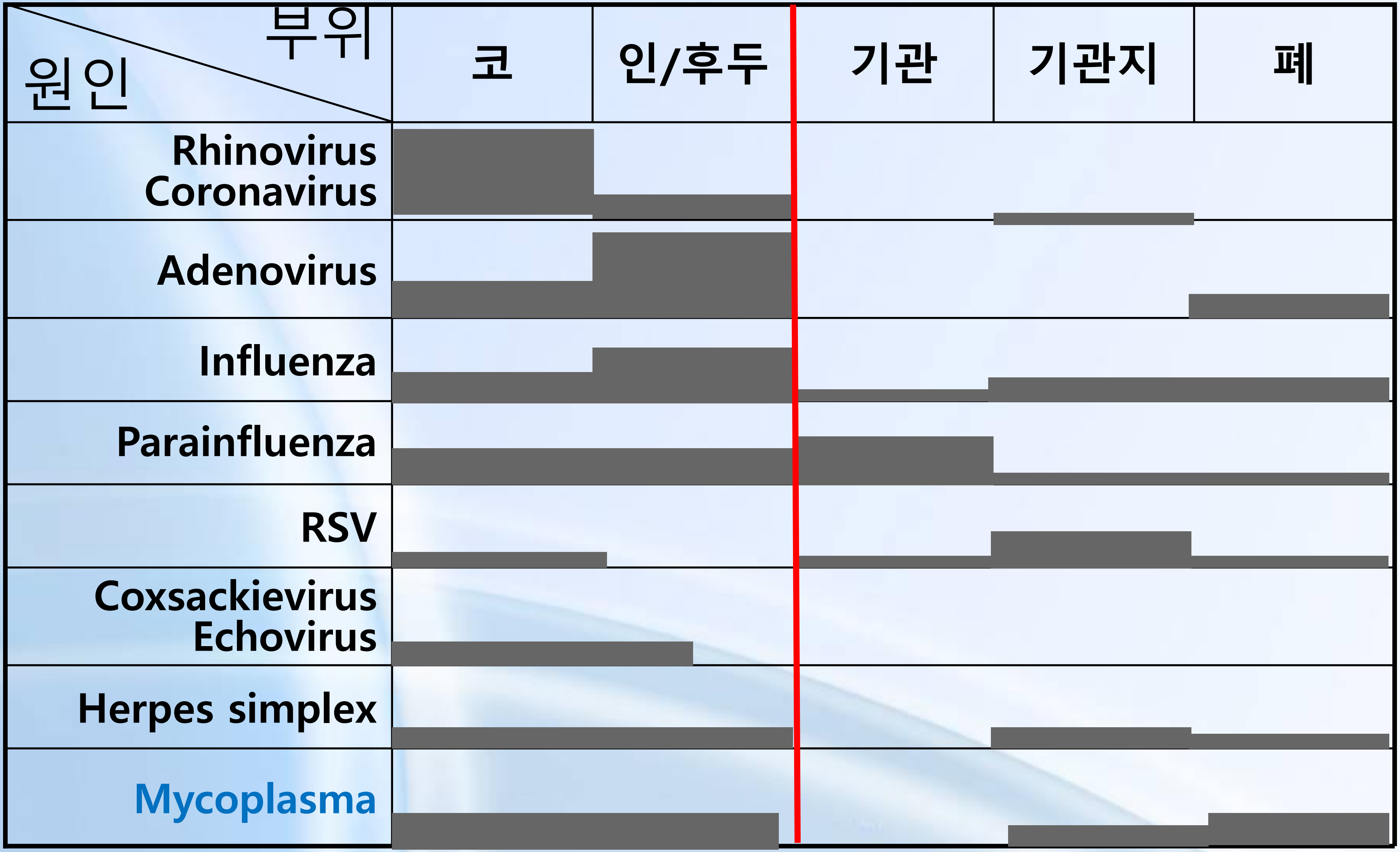
Infections of the Respiratory tract

- Most common entry point for infections
- <u>Upper respiratory tract</u>
  – nose, nasal cavity, sinuses, mouth, throat
  – Rhinitis, cough, cold…
- <u>Lower respiratory tract</u>
  – Trachea, bronchi, bronchioles, and alveoli in the lungs
  – Bronchiolits, pneumonia, COPD…



Commonly infected areas (tissues)

NIH 국립보건연구원 Korea National Institute of Health



Viruses Associated with Respiratory Infections

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Syndrome	Commonly Associated Viruses	Less Commonly Associated Viruses
Coryza	Rhinovirus, Coronavirus	Influenza virus, Parainfluenza virus
Influenza	Influenza virus	Parainfluenza virus, Adenovirus
Croup	Parainfluenza virus	Influenza virus, RSV, Adenovirus
Bronchiolitis	RSV	Influenza virus, Parainfluenza virus, Adenovirus
Bronchopneumonia	Influenza virus, RSV, Adenovirus	Parainfluenza virus

Influenza Viruses

인플루엔자 바이러스

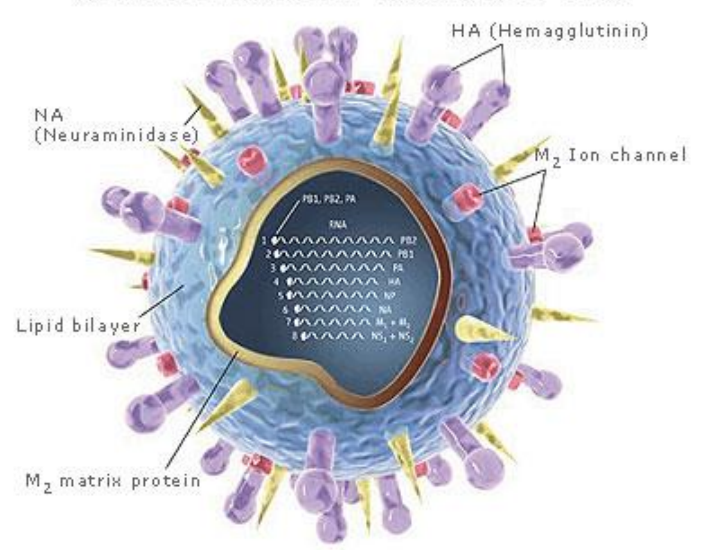
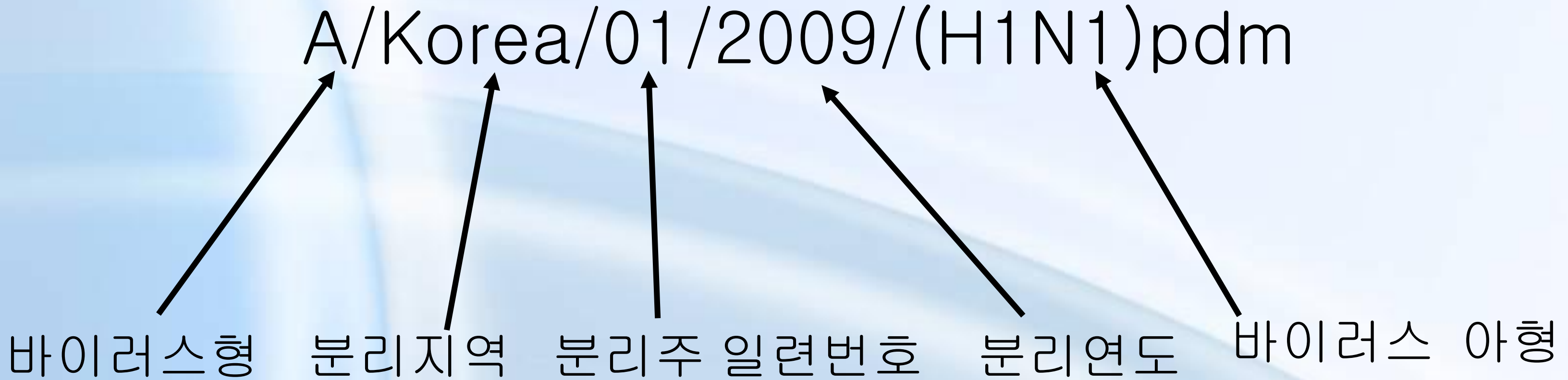


Illustration: Chris Bickel/Science. Reprinted with permission from Science Vol. 312, page 380 (21 April 2006) © 2006 by AAAS

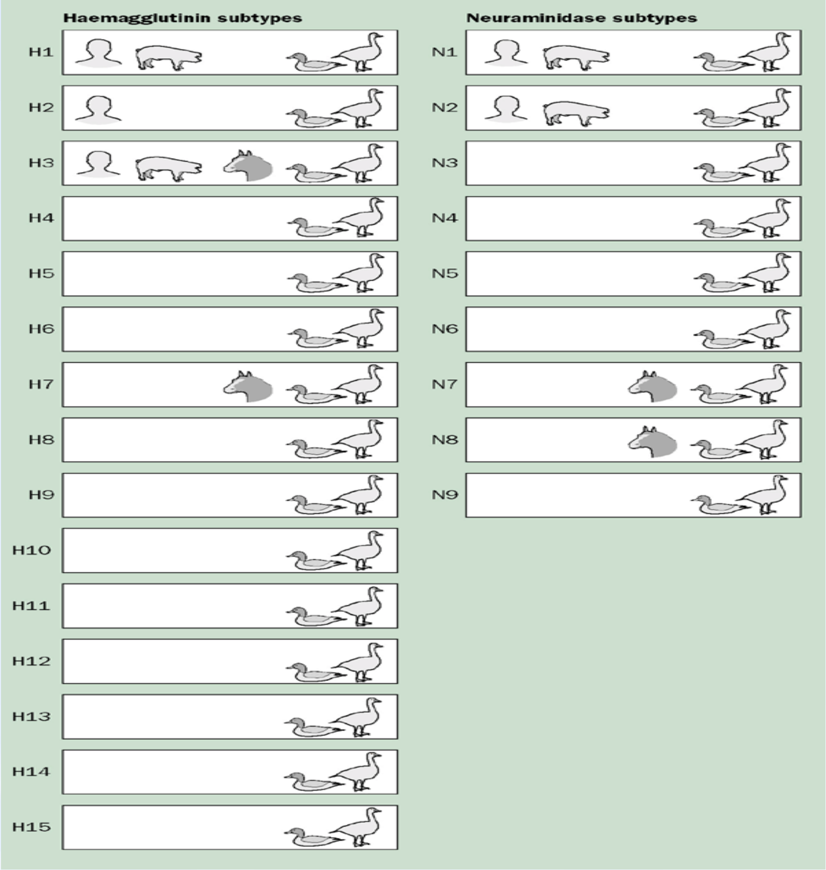
- *Orthomyxoviridae,* ss (-)RNA
- Type : A, B, C



동물인플루엔자 : A/swine/Korea/CY07/07 (H3N2)

General Characteristics

- ***Genomic diversity (Drift/Shift)***
  - 8 fragmented (-) RNAs
  - 198 combination of 18 HA and 11 NA
- ***Drug resistance***
  - NA inhibitor or M2 Inhibitor
  - Occurrence of drug resistant strain monitoring required
- ***Annual recommendation of Vaccine***
  - Antigenic change
  - Short-term immunogenic
  - Adjuvant required for enough immunity



+ H16
H17
H18

+ N10
N11

(Karl N Nicholson, et al Lancet 2003; 362:1733-45

Antigenic changes

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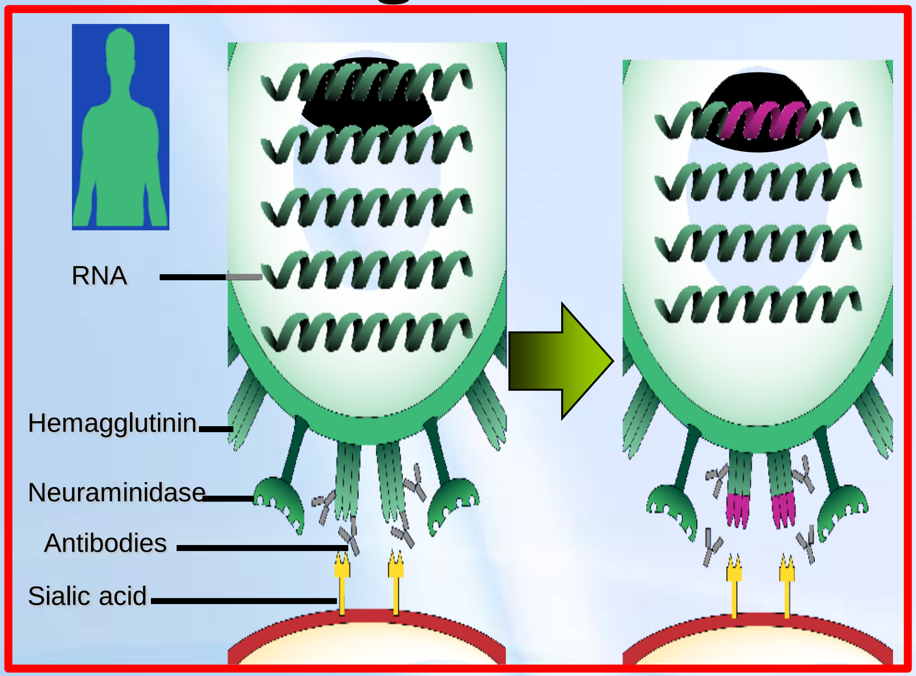
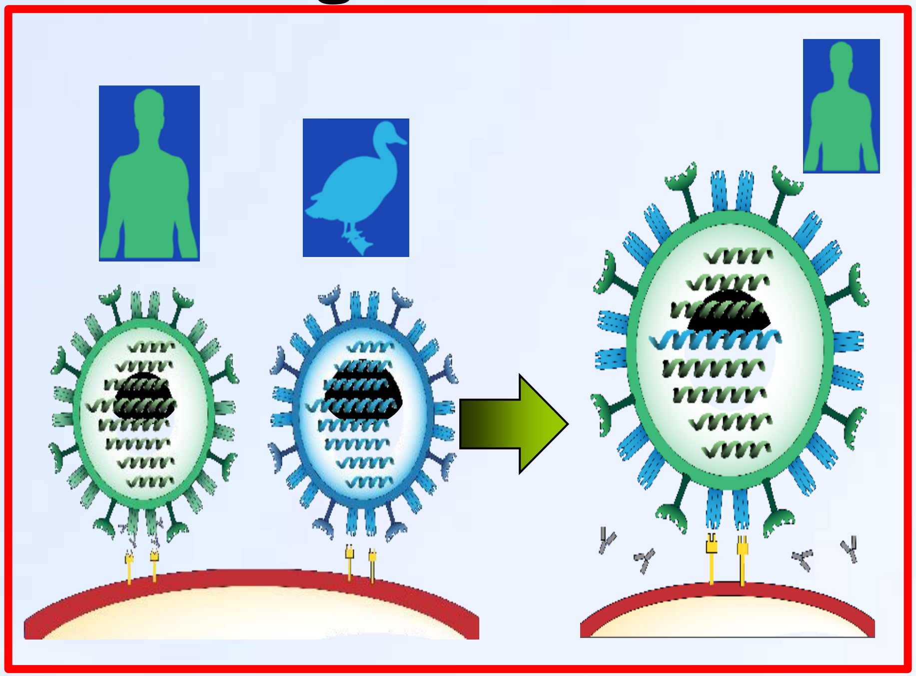
Korea National Institute of Health

Antigen Drift



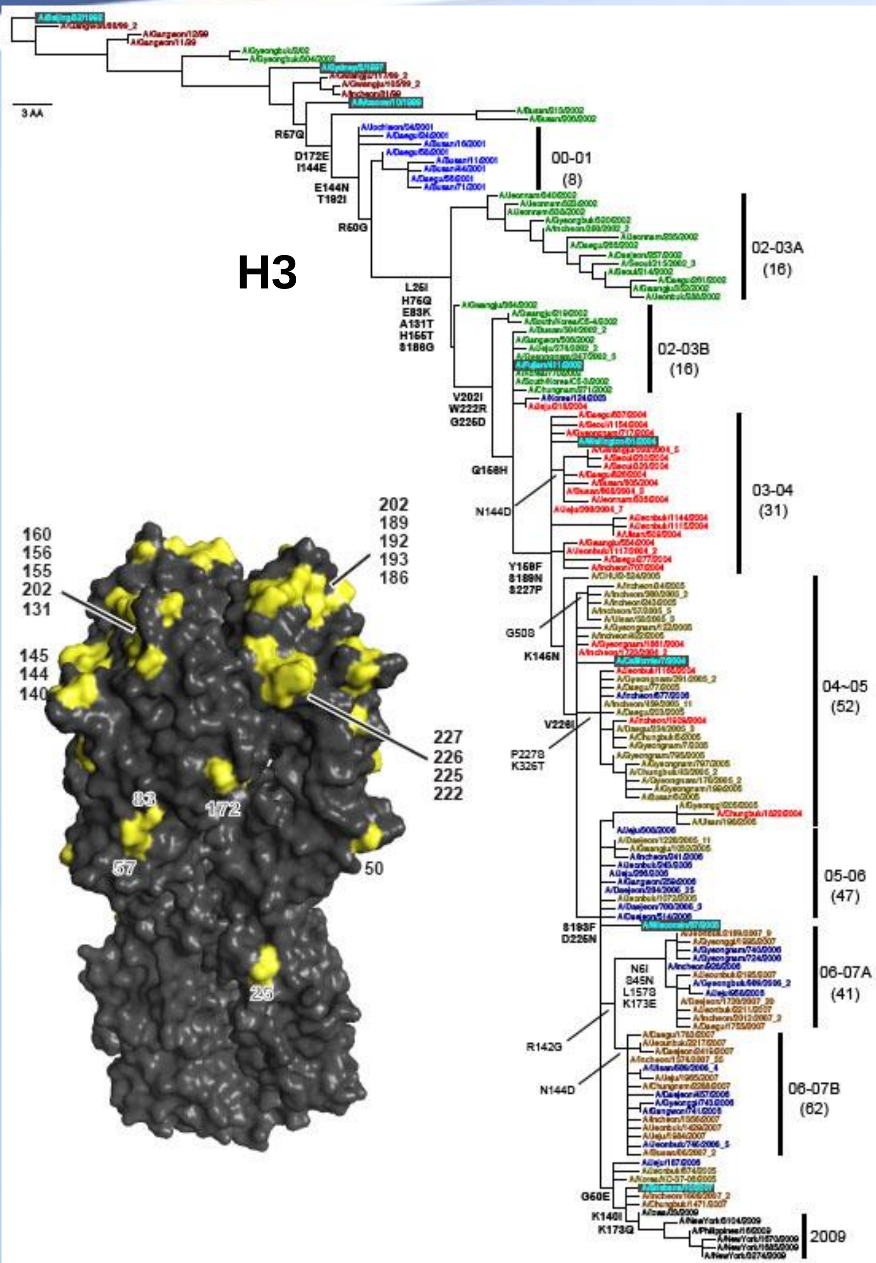
Antigen Shift

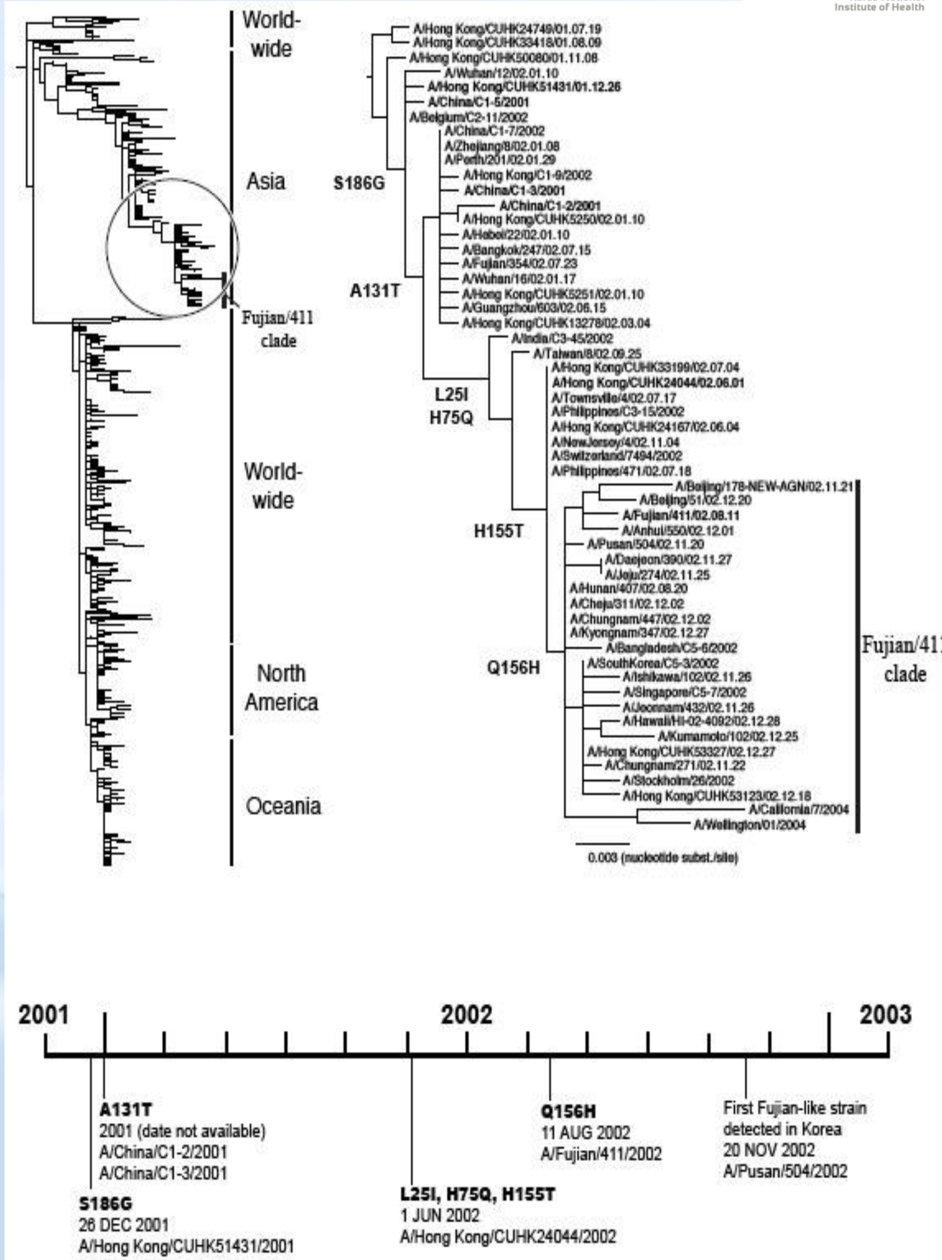


- Within Gene out of 8 segments
- point mutation
  as well as insertion, deletion

- Rearrangement of 8 segments
- One or more

Influenza HA gene diversity with one-way Evolution





Overall Evolution of the Influenza virus A(H5N1)

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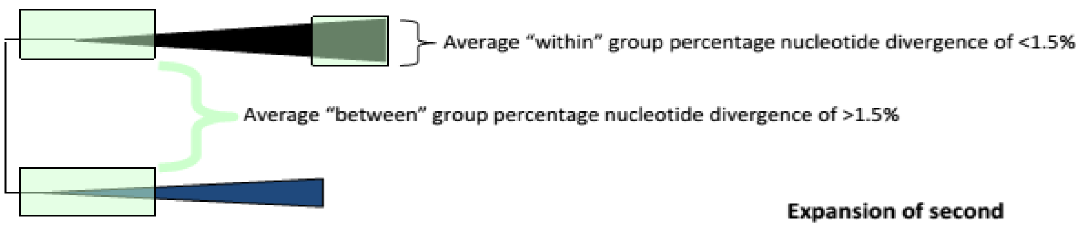
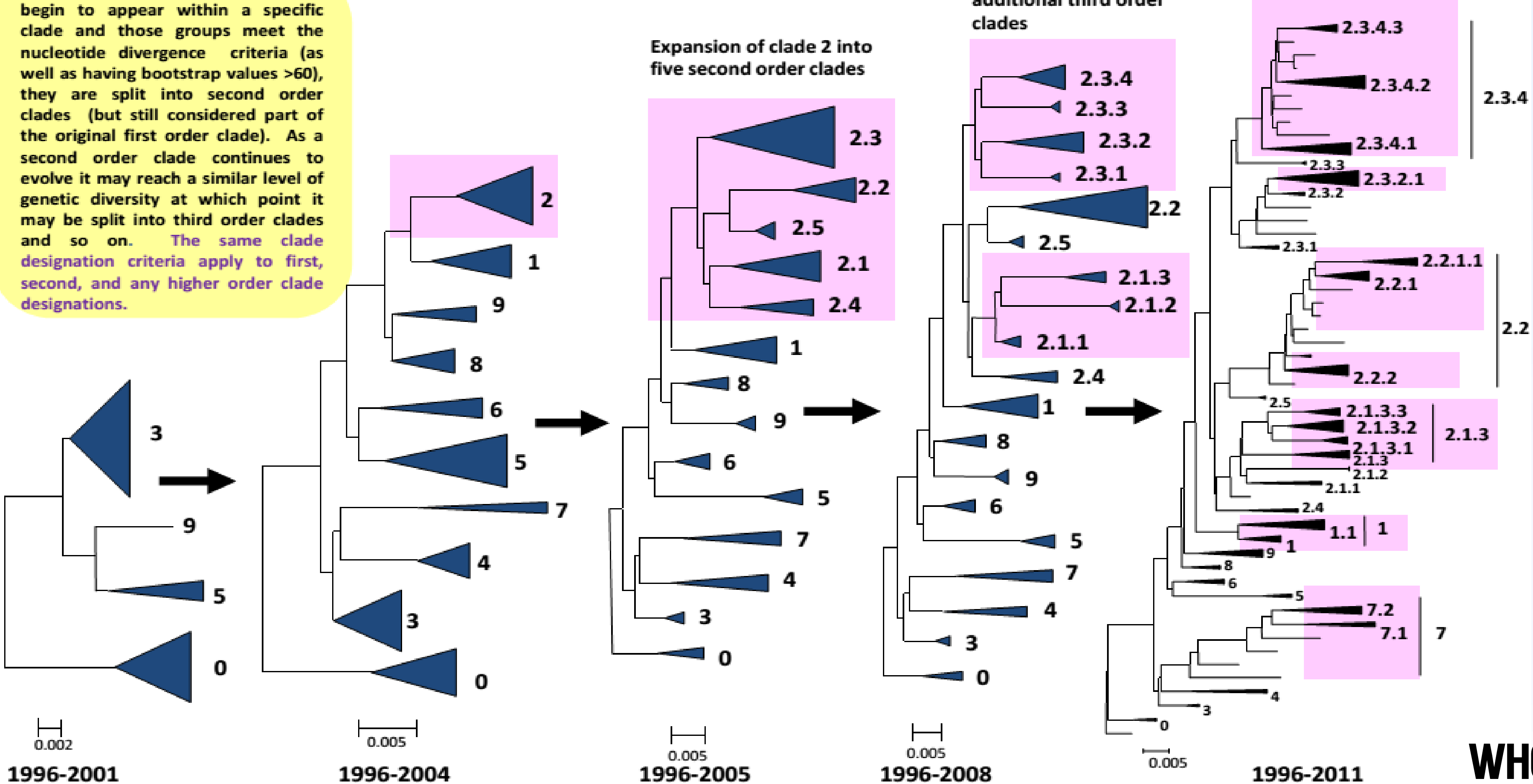
Evolution of the Asian H5 Hemagglutinin



When discrete monophyletic groups begin to appear within a specific clade and those groups meet the nucleotide divergence criteria (as well as having bootstrap values >60), they are split into second order clades (but still considered part of the original first order clade). As a second order clade continues to evolve it may reach a similar level of genetic diversity at which point it may be split into third order clades and so on. The same clade designation criteria apply to first, second, and any higher order clade designations.



WHO

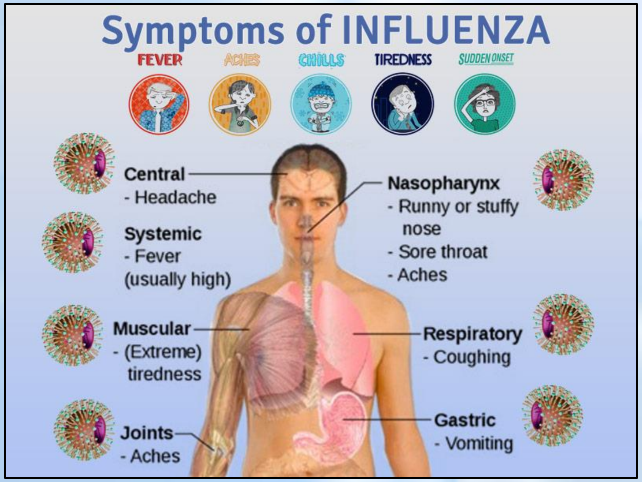
유전자 분석을 통한 인플루엔자 변이주 병원성 신속 규명 필요

◆ 다양한 병원성의 인플루엔자 바이러스 유행

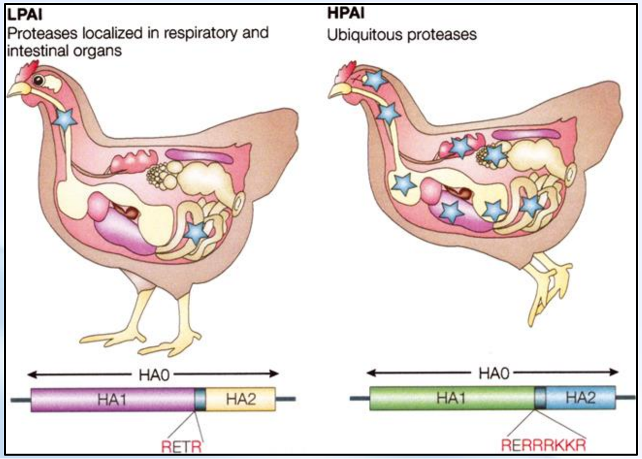
- 인플루엔자 바이러스 감염은 숙주 면역상태 및 바이러스 병원성에 따라 호흡기 증상 (고열, 근육통) 뿐만 아니라 장 관계 또는 신경계 증상 등 유발

- 동일한 형 (type), 아형 (subtype)이라도 바이러스마다 병원성 다름

- 특정 유전자에서의 단일 아미노산 변이에 의해서도 병원성이 달라질 수 있으며, 지속적인 변이로 신종 인플루엔자 출현 가능성이 높음.



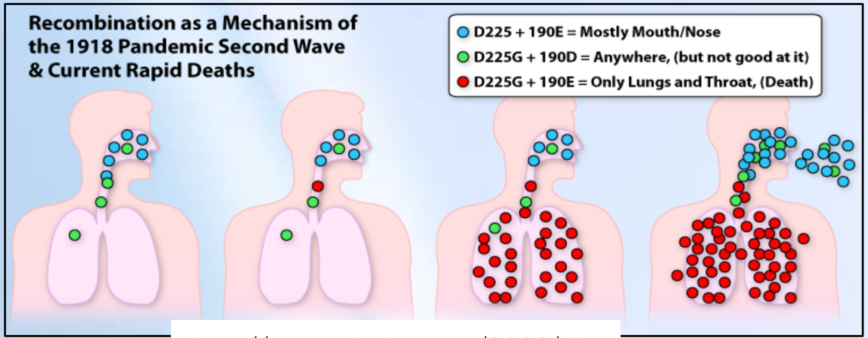
http://qsota.com/influenza-flu/



Influenza: lessons from past pandemics, warnings from current incidents, Nature Reviews Microbiology

유전자 변이와 병원성 관련 연구의 제한적 보고

- hemagglutinin (HA) 유전자 : D225G 변이 중증 발생 확률 높음
- HA1 유전자 :G218W 및 HA2 T156N : 마우스 감염 시 증식력 가속
- PB2 유전자 D701N E627K : polymerase activity 증가로 포유류 적응
- NS 유전자 : D92E



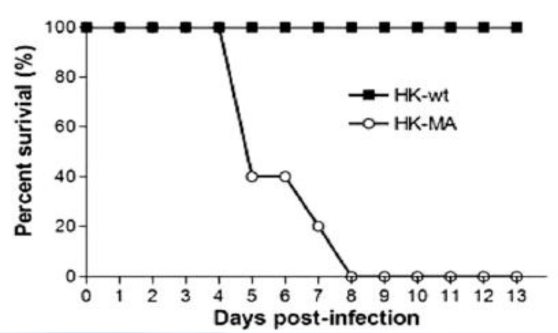
http://www.virology.ws/2009/11

Influenza proteins and pathogenicity determinants

Protein	Segment	Function	Pathogenicity determinant: High	Pathogenicity determinant: Low	Reference
PB2	1	Polymerase cofactor, binds most mRNA caps	627K[A]	627E[B]	80
PB2	1	Polymerase cofactor, binds most mRNA caps	701N	701D	89
PB1	2	Polymerase cofactor, RNA-dependent RNA polymerase	–	–	–
PB1-F2	2	Proapoptotic	66S	66N	5
PA	3	Polymerase cofactor endonuclease activity, elongation factor?	–	–	–
HA	4	Membrane glycoprotein, binding, and fusogenic functions	Multi-basic cleavage site	Single-basic cleavage site	79
NP	5	Component of RNP, encapsidates vRNA segments	–	–	–
NA	6	Membrane glycoprotein, sialidase	274Y[C]	274H	–
M1	7	Lies under the viral envelope	–	–	–
M2	7	Membrane protein, forms ion channel	–	–	–
NS1	8	Evasion of host immune response	92E	92D	102
NS1	8	Evasion of host immune response	C-terminal E-S-E-V motif	C-terminal deletion	105, 106
NEP	8	Nuclear export of vRNPs	–	–	–

TABLE 1. Amino acids mutations between HK/1/68 and MA viruses

Gene	Mutation site (residue no.)	Amino acid: HK/1/68	Amino acid: HK-MA
PB2	701	D	N
PB1	190	R	K
	578	K	T
PA	None		
HA	218[HA1]	G	W
	156[HA2]	T	N
NP	34	D	N
NA	468	P	H
M1	232	D	N
M2	None		
NS1	23	V	A
NS2	None		

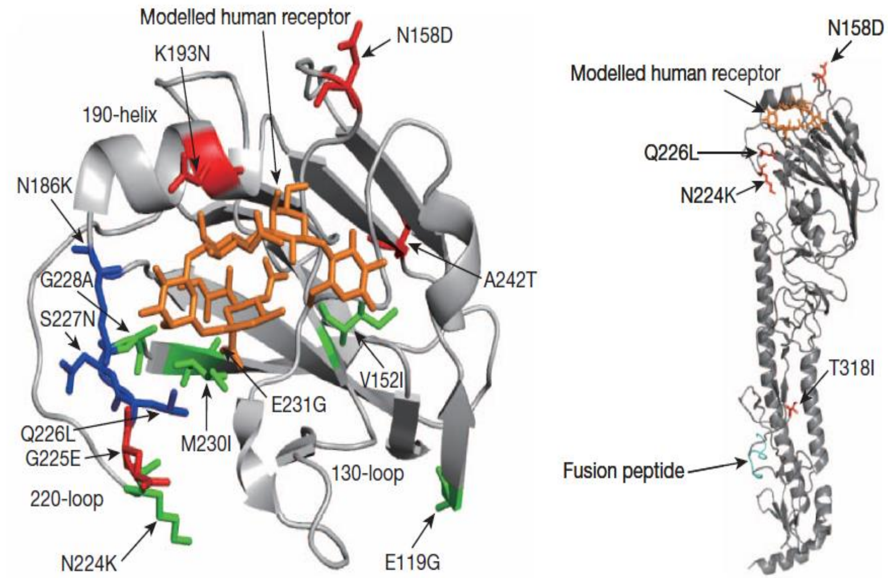


http://www.virology.ws/2009/11/24

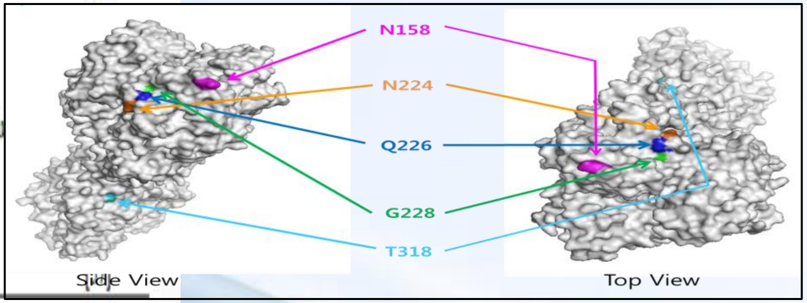
Mutation analysis related with mammalian pathogenesis

Viral components	Protein	Mutation	Adaptive Mechanism in Mammalian System	
Surface glycosylation protein	HA	N154S	Increase α2, 6 binding of H5N1 subtype	
		A158T	Decrease α2, 3 binding by oligosaccharide modification of H5N1 subtype	
		N182K	Increase α2, 6 binding and decrease α2, 3 binding of H5N1 subtype	
		Q192R	Increase α2, 6 binding and decrease α2, 3 binding of H5N1 subtype	
		Q222L	Increase α2, 6 binding and decrease α2, 3 binding of H5N1 subtype	
		S223N	Increase α2, 6 binding and decrease α2, 3 binding of H5N1 subtype	
		G224S	Increase α2, 6 binding and decrease α2, 3 binding of H5N1 subtype	
		Q226L	Increase α2, 6 binding and decrease α2, 3 binding of H2,H3,H5,H9 subtype	
		S227N	Increase α2, 6 binding and decrease α2, 3 binding of H5N1 subtype	
		G228S	Increase α2, 6 binding of H5N1 subtype	
	NA	Deletion in stalk	Functional balance with HA by decreasing enzyme activity of H5N1 subtype	
Polymerase	PB1	L473V	Increase polymerase activity of H5N1 and 2009pH1N1 subtype	[56]
		L598P	Increase polymerase activity of H5N1 subtype	[56]
	PB2	Q591K	Increase polymerase activity of H5N1 and 2009 pH1N1 subtype	
		E627K	Increase polymerase activity of H5N1 subtype	
		D701N	Increase polymerase activity by binding to beneficial importin α isoform in H5N1 and H7N7 su	
Non-structural protein	NS1	PDZ domain in C-terminus	Bind to host PDZ-carrying proteins to interfere host signal pathway	
		S103F, I106M	Bind to host CPSF30 to inhibit protein synthesis	
Nuclear export protein	NEP	M16I	Increase polymerase activity of H5N1 subtype	

Zhang et al., Viruses, 2013(5):1431-1446

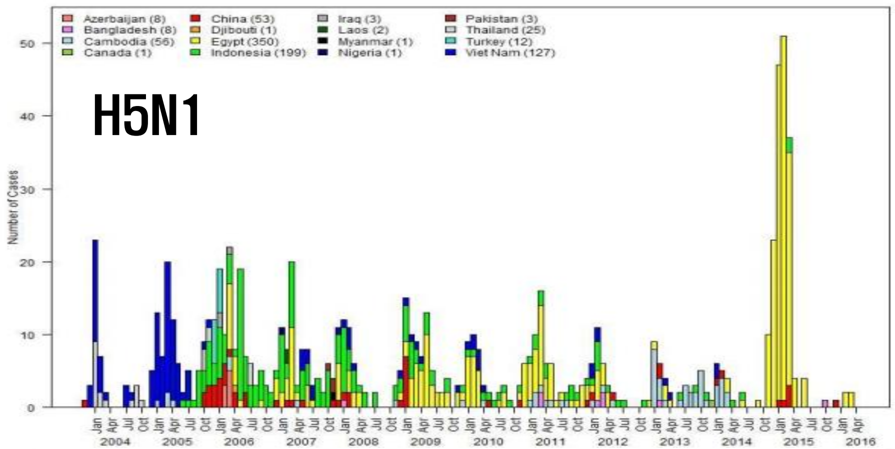


Imai et al., Nature, 2012(486):420-430

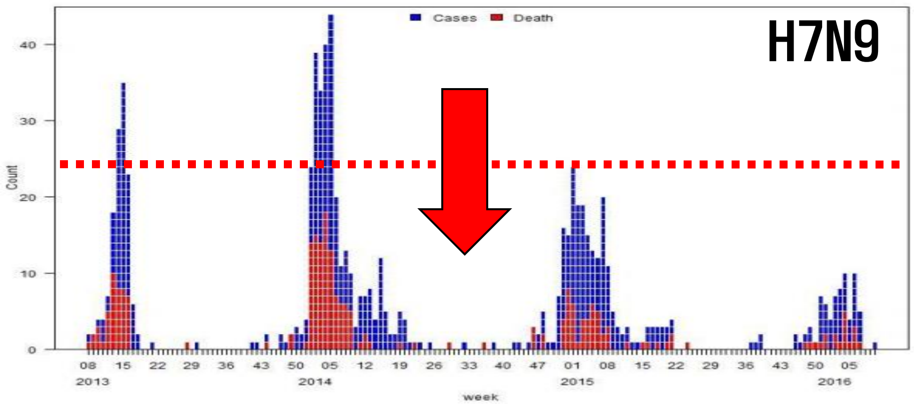


KISED, NIH, Korea

Avian Influenza human Infection (H5N1/H7N9/H5N6)

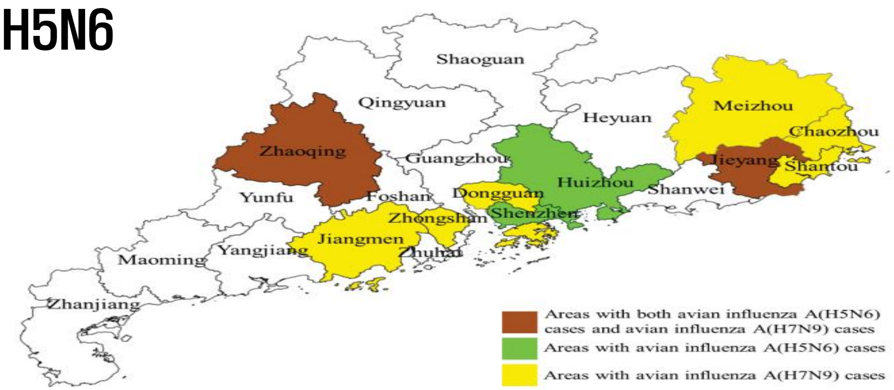


- 03년부터 16년 5월까지 16개국에서 총 850명 확진, 449명 사망 (WHO)



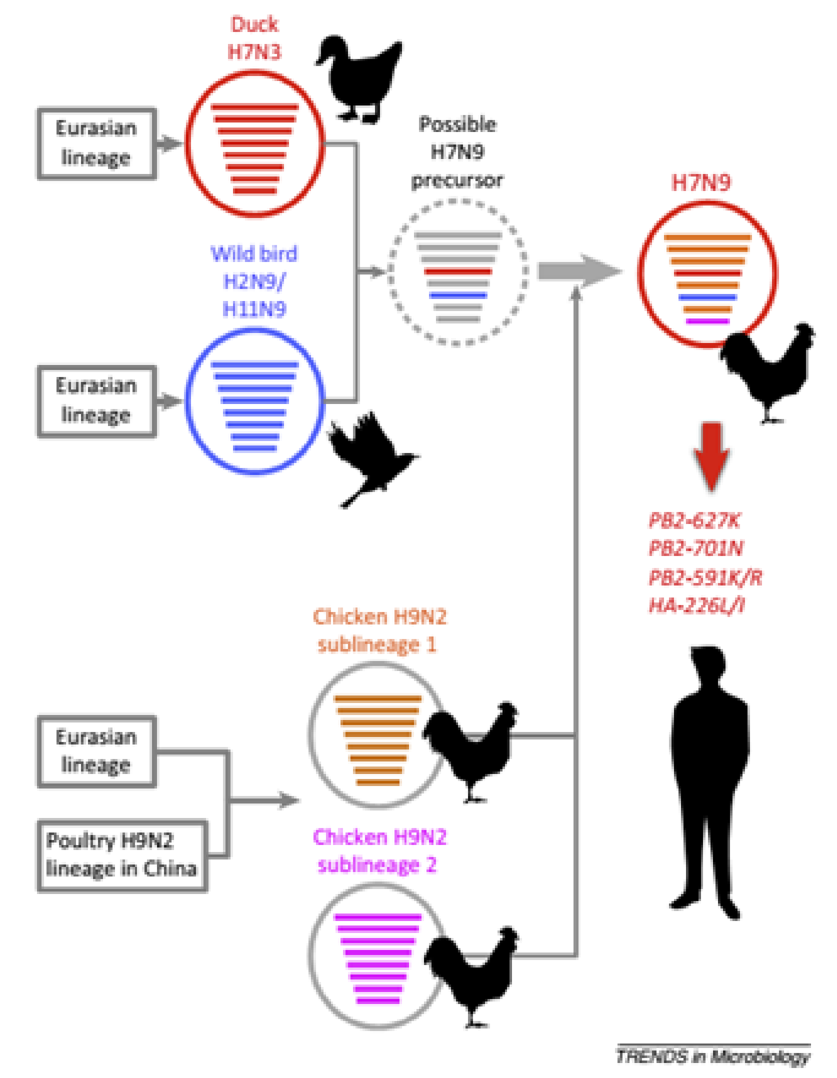
- 13.2월 최초발생 '16.04.04일까지 총 752명 확진, 295명 사망 (WHO)

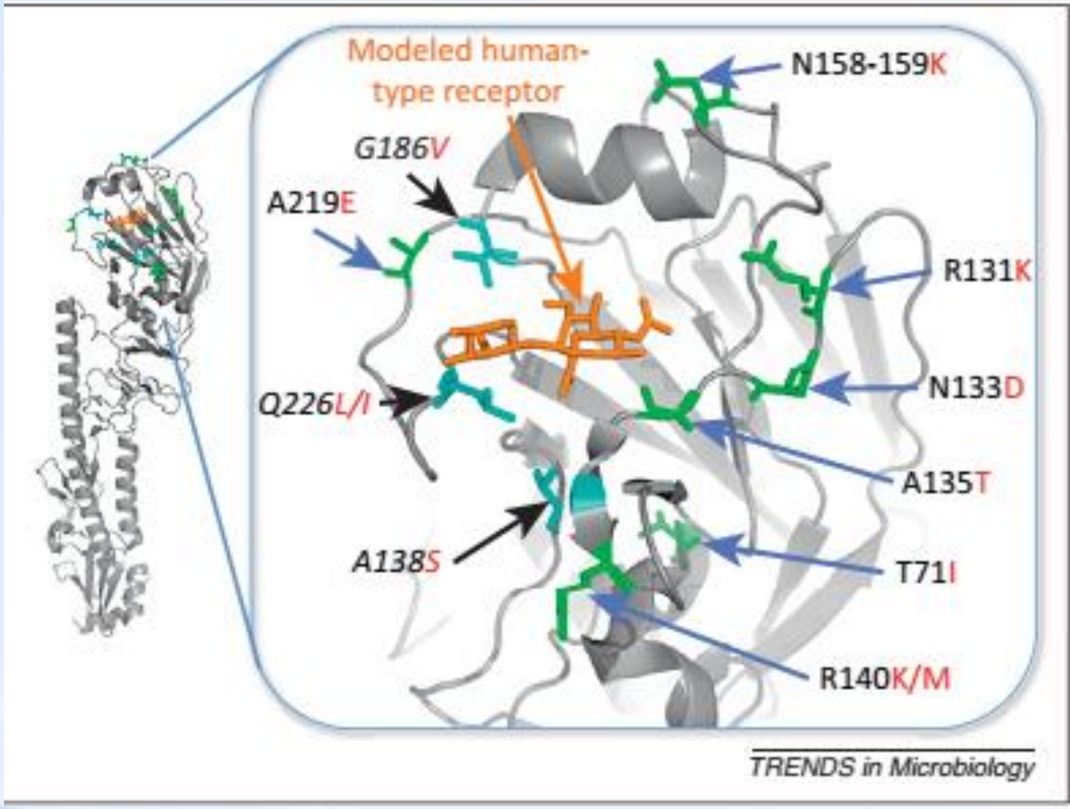
H5N6



- 13년 최초발생 이후 중국 내 총 13명 확진

The Genesis of H7N9

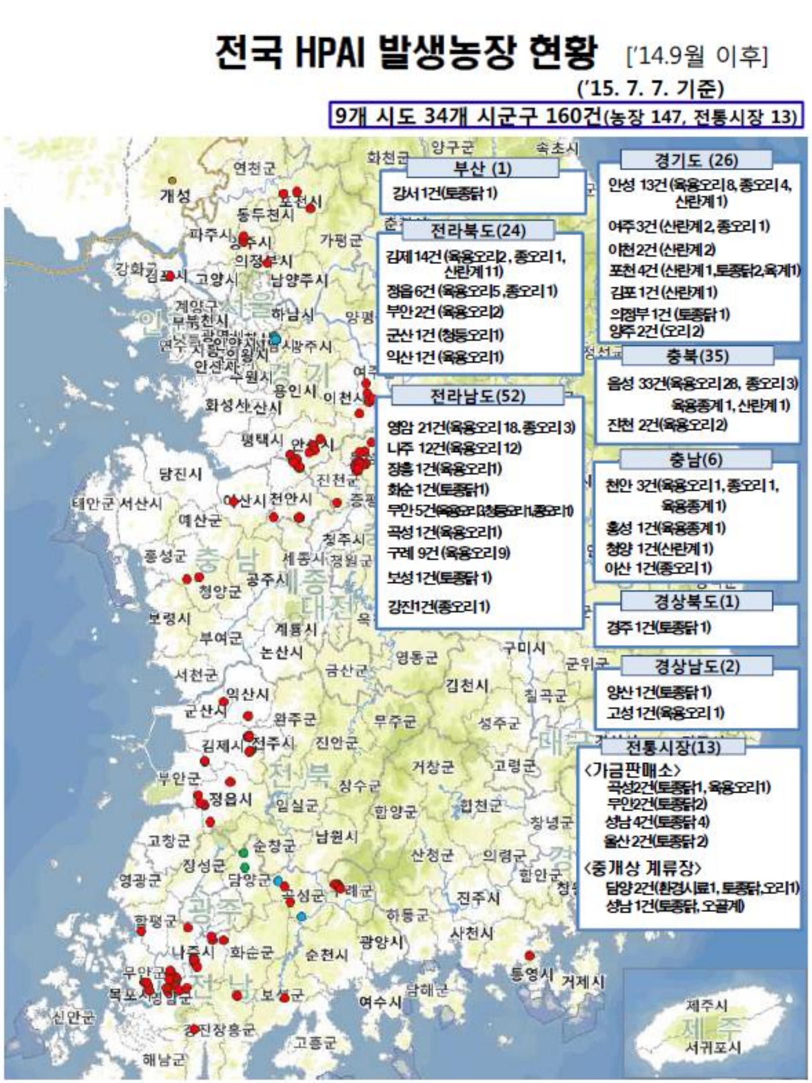




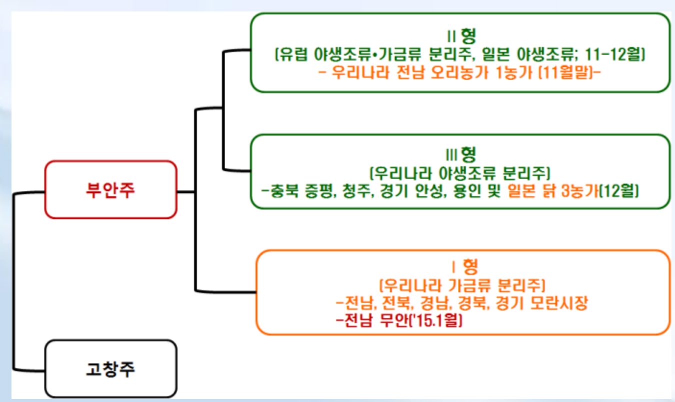
Amino acid changes in the hemagglutinin (HA) of viruses recovered from contact ferrets in the human-infecting H7N9 virus groups.

Mutation→cyan (A138S, G186 V, and Q226L/I) are known to increase the binding of avian H5 and H7)

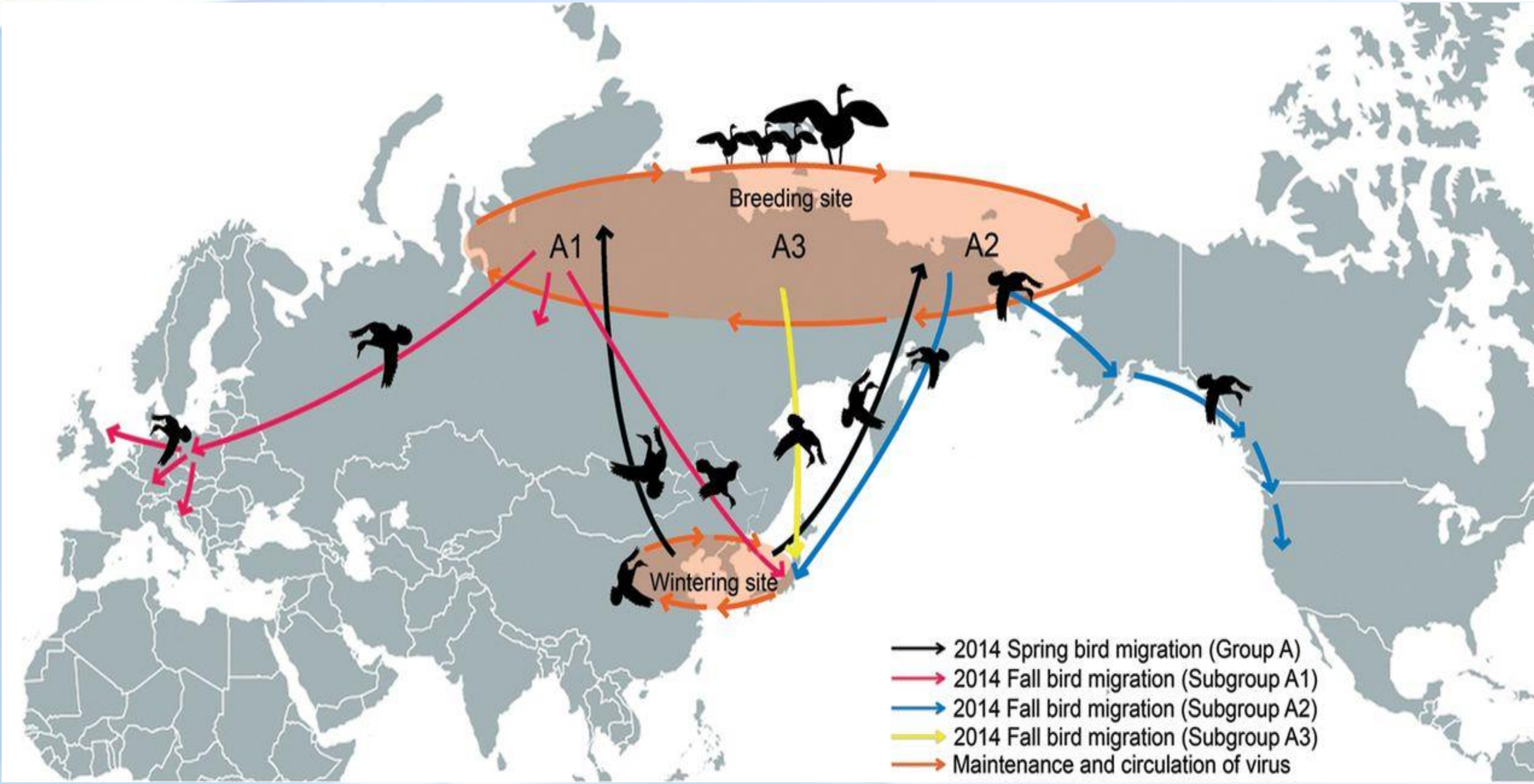
AI (H5N8) Incidence Summary as of 2016.3.26.



- ◆ Primary case reported in Jan-2014.
- ◆ Total 4th wave were encountered
- ◆ Declared AI free a month ago, but rebounded this year (March and April).
- ◆ Two sub-classified H5N8 viruses were related (GC, BA : H5 clade 2.3.4.6)
- ◆ Buan strain was classified into 3 subtype



Movement of H5N8 Asia, Europe and America

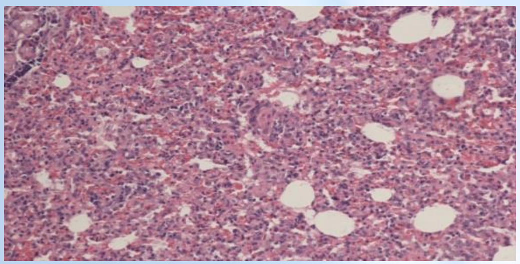


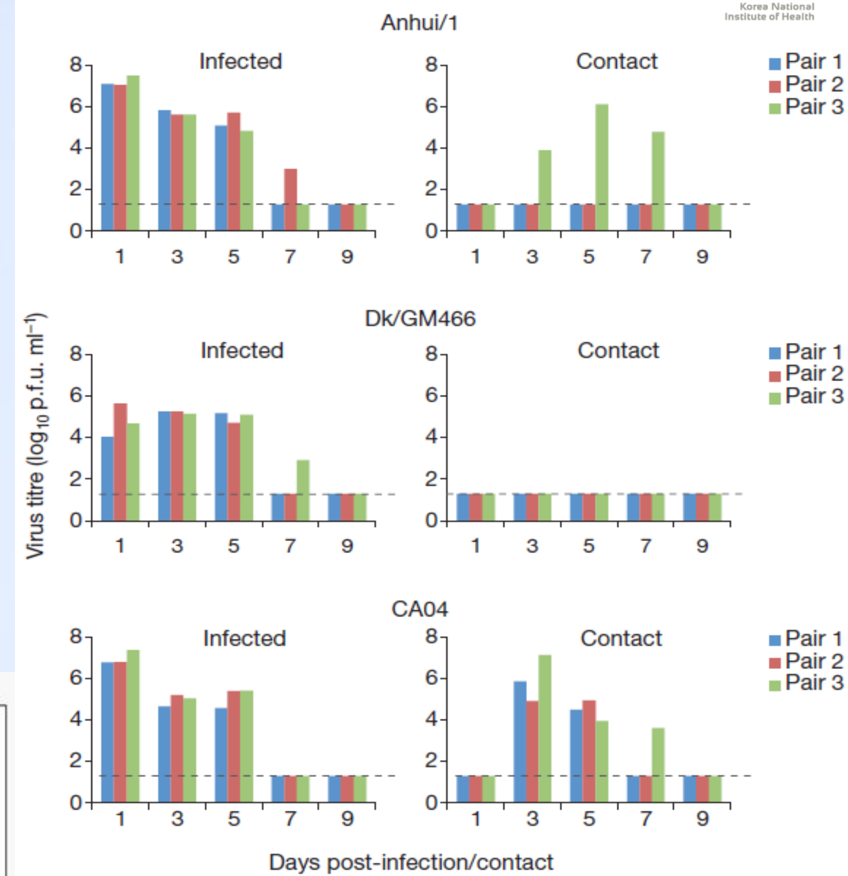
Cited from JVI, 2015 (89):6521-6524, DH Lee et al.

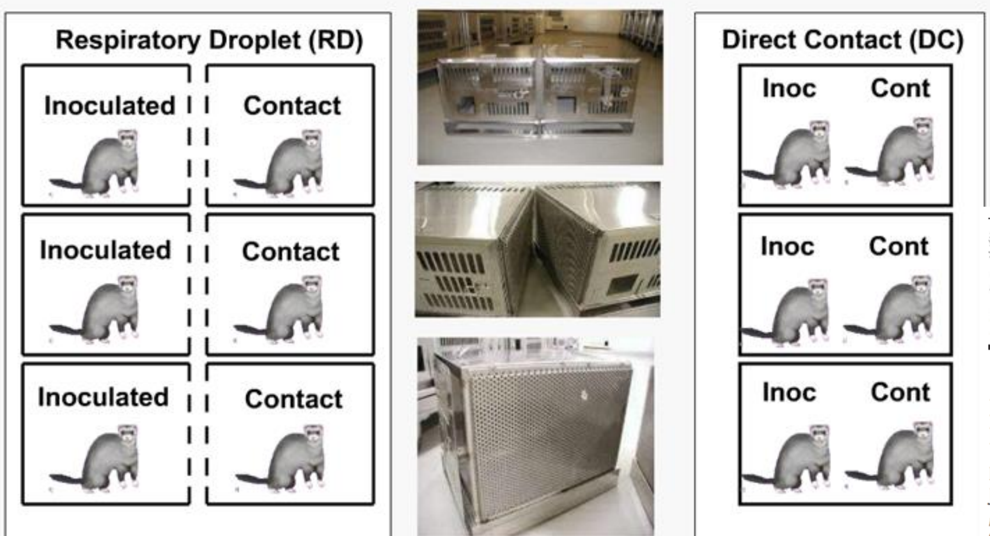
People also migrate from countries to countries.
Approximately, 8M of Chinese tourists visit Korea annually and so do in Korea.

Pathological study using Ferret

국립보건연구원
Korea National Institute of Health







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ELSEVIER

Pathogenesis of novel reassortant avian influenza virus A (H5N8) Isolates in the ferret☆

CrossMark

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Division of Influenza Virus, Center for Infectious Diseases, National Institute of Health, Korea Centers for Disease Control and Prevention, 187, Osongsaengmyeong2-ro, Osong-eup, Cheongju, Chuncheongbuk-do 363–951, South Korea

신종인플루엔자 중증감염 기전 관련 연구

◎ 신종인플루엔자 중증감염 용인의 기전 규명 필요

- 신종 및 조류인플루엔자의 인체 감염은 중증 증상과 함께 높은 치사율을 나타냄
- 현재 사용되는 NA/M2 저해제 기반 치료제는 경증의 인플루엔자에 효과적
- 중증인플루엔자의 기전 연구와 실험동물 모델개발이 필요

* 2009년 신종인플루엔자 중증 사망사례

Anatomic Pathology / Autopsy Findings in Fatal H1N1 Influenza

Autopsy Findings in Eight Patients With Fatal H1N1 Influenza

Paul W. Harms, MD, PhD,[1] Lindsay A. Schmidt, MD,[1] Lauren B. Smith, MD,[1] Duane W. Newton, PhD,[1] Maria A. Pletneva, MD, PhD,[1] Laura L. Walters, MD, PhD,[1] Scott A. Tomlins, MD, PhD,[1] Amanda Fisher-Hubbard, MD,[1] Lena M. Napolitano, MD,[2] Pauline K. Park, MD,[2] Mila Blaivas, MD, PhD,[1] Joseph Fantone, MD,[1] Jeffrey L. Myers, MD,[1] and Jeffrey M. Jentzen, MD, PhD[1]

Abstract

A novel H1N1 influenza A virus emerged in April 2009, and rapidly reached pandemic proportions. We report a retrospective observational case study of pathologic findings in 8 patients with fatal novel H1N1 infection at the University of Michigan Health Systems (Ann Arbor) compared with 8 age-, sex-, body mass index–, and treatment-matched control subjects. Diffuse alveolar damage (DAD) in acute and organizing phases affected all patients with influenza and was accompanied by acute bronchopneumonia in 6 patients. Organizing DAD with established fibrosis was present in 1 patient with preexisting granulomatous lung disease. Only 50% of control subjects had DAD. Peripheral pulmonary vascular thrombosis occurred in 5 of 8 patients with influenza and 3 of 8 control subjects. Cytophagocytosis was seen in all influenza-related cases. The autopsy findings in our patients with novel H1N1 influenza resemble other influenza virus infections with the exception of prominent thrombosis and hemophagocytosis. The possibility of hemophagocytic syndrome should be investigated in severely ill patients with H1N1 infection.

Cytophagocytosis (hemophagcytosis)

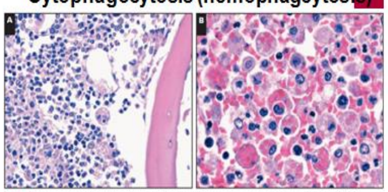

Image 3 Hemophagocytosis in deaths of novel H1N1 influenza. A, Cytophagocytosis in bone marrow (H&E, ×600). B, Hemophagocytosis in spleen (H&E, ×400).

Histopathologic Lung Findings

Fibrin Thrombi	Necrosis	Other	Sites of Hemophagocytosis
Bilateral	Bilateral	—	Spleen; BM; LN
Bilateral	Right	—	BM
Right	Absent	—	BM
Left	Bilateral	Sarcoidosis	Spleen; BM
Bilateral	Bilateral (LUL infarct)	Necrotizing acute inflammation at trach site with fungal hyphae (Candida sp)	BM; LN
Absent	Bilateral	—	BM; LN
RLL	RLL	—	Spleen; BM
Absent	Absent	—	BM

➔ Cytophagocytosis was seen in all influenza related cases.

혈구탐식성 림프조직구증, HLH by severe influenza infection

EXPERIENCE & REASON

Hemophagocytic Lymphohistiocytosis Complicating Influenza A Infection

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Departments of [a]Anesthesiology, [b]Pediatrics, [c]Pathology, and [d]Cardiothoracic Surgery, Wake Forest University School of Medicine and Brenner Children's Hospital, Winston-Salem, North Carolina

ABSTRACT

During the influenza A (H3N2) season of 2003–2004, several influenza-related complications and deaths were reported in children. Hemophagocytic lymphohistiocytosis complicating influenza A infection is very rare. We report a 3-year-old girl who presented with severe pneumonia and hemophagocytic lymphohistiocytosis associated with influenza A infection. Clinicians should be aware of hemophagocytic syndrome as a serious complication of influenza A infection.

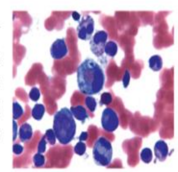


FIGURE 1

CLINICAL AND LABORATORY OBSERVATIONS

J Pediatr Hematol Oncol • Volume 33, Number 2, March 2011 www.jpho-online.com | 135

Hemophagocytic Lymphohistiocytosis Associated With 2009 Pandemic Influenza A (H1N1) Virus Infection

Halil Özdemir, MD, Ergin Çiftçi, MD,* Elif Ünal İnce, MD,† Mehmet Ertem, MD,† Erdal İnce, MD,* and Ülker Doğru, MD**

Summary: Hemophagocytic lymphohistiocytosis (HLH) has not been described earlier in the context of 2009 pandemic influenza A (H1N1) virus infection, although certain populations are thought to be at risk for complicated pandemic influenza A disease. Here, we report the second case of HLH after infection with the influenza A H1N1 virus treated with peroral oseltamivir successfully.

Key Words: children, hemophagocytic lymphohistiocytosis, influenza A (H1N1)

(*J Pediatr Hematol Oncol* 2011;33:135–137)

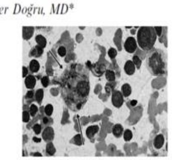


FIGURE 1.

Case Report

Hemophagocytic Lymphohistiocytosis Induced by Severe Pandemic Influenza A (H1N1) 2009 Virus Infection: A Case Report

Xiang-Yan Zhang,[1,2] Xian-Wei Ye,[1,2] Duan-Xing Feng,[1,2] Jing Han,[1,2] Dan Li,[1,2] and Cheng Zhang[1,2]

Hindawi Publishing Corporation
Case Reports in Medicine
Volume 2011, Article ID 951910, 3 pages
doi:10.1155/2011/951910

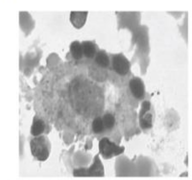


Figure 1: Bone marrow smear showed clasmatocytes engulfing blood cells (Wright's staining, 10 × 100).

Beutel *et al. Critical Care* 2011, 15:R80
http://ccforum.com/content/15/2/R80

CRITICAL CARE

RESEARCH Open Access

Virus-associated hemophagocytic syndrome as a major contributor to death in patients with 2009 influenza A (H1N1) infection

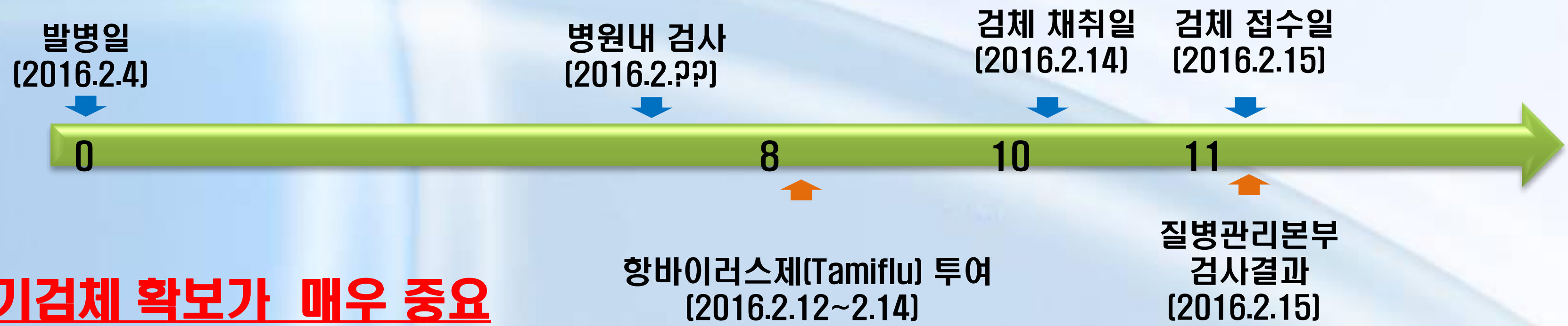
Gernot Beutel[1*], Olaf Wiesner[2], Matthias Eder[1], Carsten Hafer[3], Andrea S Schneider[4], Jan T Kielstein[3], Christian Kühn[5], Albert Heim[6], Tina Ganzenmüller[6], Hans-Heinrich Kreipe[7], Axel Haverich[5], Andreas Tecklenburg[8], Arnold Ganser[1], Tobias Welte[2] and Marius M Hoeper[2]

최근 결핵호흡기학회 의뢰 결과

NIH 국립보건연구원 Korea National Institute of Health

항목		내용
인플루엔자 바이러스 검사	검사개요	대한결핵 및 호흡기 학회를 통하여 고려대 · 한림대 등 다수병원에서 인플루엔자 관련 중증호흡부전사례 검사 의뢰 (‘16.2)
	검사대상	대한결핵 및 호흡기 학회에서 요청한 3개 병원에서의 인플루엔자 감염으로 인한 중증급성 호흡부전 환자 6명
	검사항목	인플루엔자바이러스 4종[A, B, A(H3N2), A(H1N1)pdm09]
	검사결과	2명에서 계절인플루엔자 **A(H1N1)pdm09 양성 확인***
인플루엔자 바이러스 주요 유전자 변이 분석	분석 대상	양성 검체 2건에 대한 주요 유전자 (HA, NA, M, NS1) * ‘13–’14 및 ‘14–’15절기 국내 H1N1, ‘15–’16절기 국외 H1N1 분리주 포함 비교
	분석결과	– 백신유사성: 당해연도 백신(A/California/7/2009, Clade 6b)과 유사 – 치료제 내성: NA억제제(타미플루) 감수성, M2억제제(아만타딘)내성 – 병원성 관련 유전자 : 사이토카인 관련 NS1 주요 유전자 부위 (R38A, D92E) 변이 없음

•타 호흡기 바이러스 및 호흡기 세균 검사 모두 음성 /기관 간 (의뢰병원 및 NIH) 검사 결과 차이
- NIH 양성 검체 (2인)는 항바이러스제 <u>투여 4일 이내,</u>
- NIH 음성 검체 (4인)는 11일 이상 경과된 것)



☞ **초기검체 확보가 매우 중요**

최근 결핵호흡기학회 의뢰 1례의 중증관련인자 분석 결과

; Sari 는 clade 6b.1 에 속함

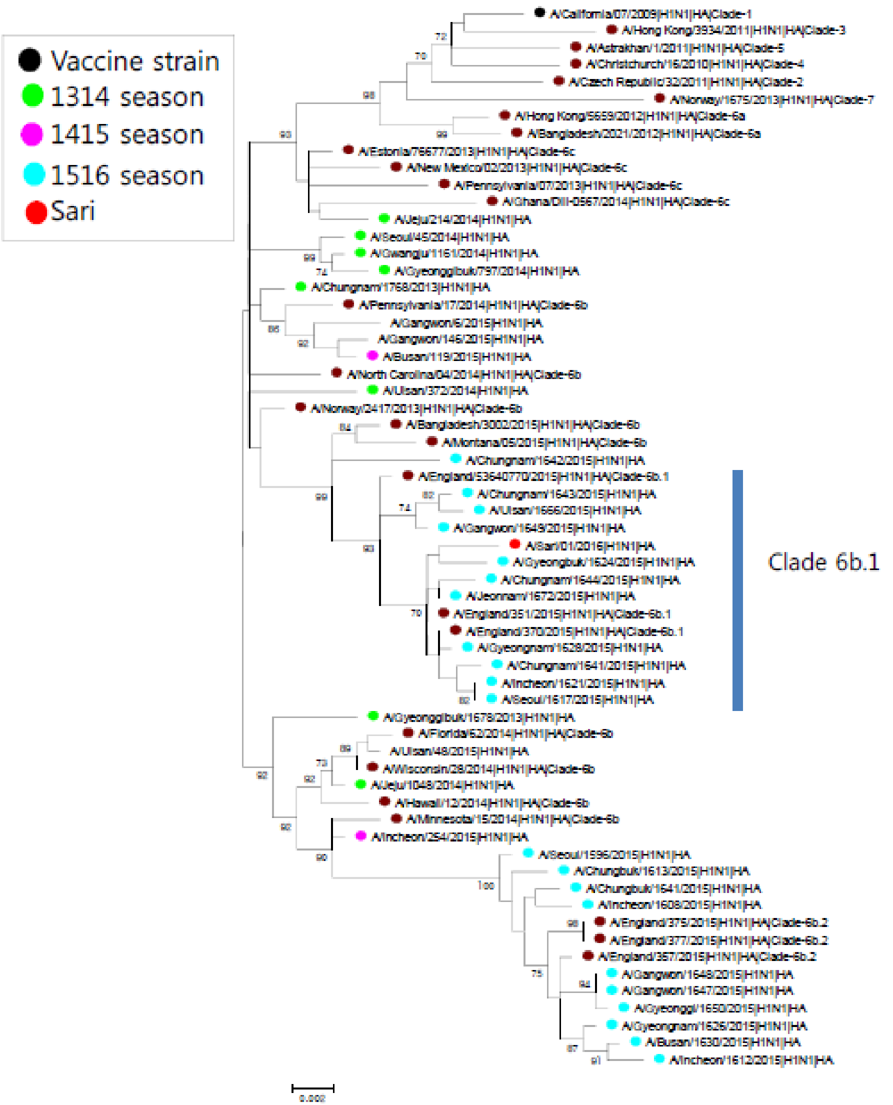


표1. HA

Position	158	193	224	226	228	318
1516-Foreign	N(700),S(2),X(1)	L(689),X(7)I(7)	R(702),X(1)	Q(689),R(10),X(4)	G(700),X(2),A(1)	T(703)
1314-Kor-season	N(10)	L(10)	R(9),W(1)	Q(10)	G(10)	T(10)
1415-Kor-season	N(10)	L(10)	R(9),W(1)	Q(10)	G(10)	T(10)
1516-Kor-samples	N(2)	L(2)	R(2)	Q(2)	G(2)	T(2)

표2. NA

H1N1 NA pos	119	222	223	246	274	294
1516-Foreign	E(491),X(10),K(2)	N(499),D(1),S(1),K(1),H(1)	I(502),T(1)	P(503)	Y(502),C(1)	D(503)
1314-Kor-season	E(10)	N(10)	I(10)	P(10)	Y(10)	D(10)
1415-Kor-season	E(10)	N(10)	I(10)	P(10)	Y(10)	D(10)
1516-Kor-samples	E(2)	N(2)	I(2)	P(2)	Y(2)	D(2)

표3. M1

H1N1 M1 pos	15	30	101	166	215
1516-Foreign	I(221),V(6)	S(219),D(6),N(2)	K(221),R(6)	A(220),V(6),X(1)	A(227)
1314-Kor-season	I(10)	S(10)	K(10)	A(10)	A(10)
1415-Kor-season	I(10)	S(10)	K(10)	A(10)	A(10)
1516-Kor-samples	I(2)	S(2)	K(2)	A(2)	A(2)

표4. M2

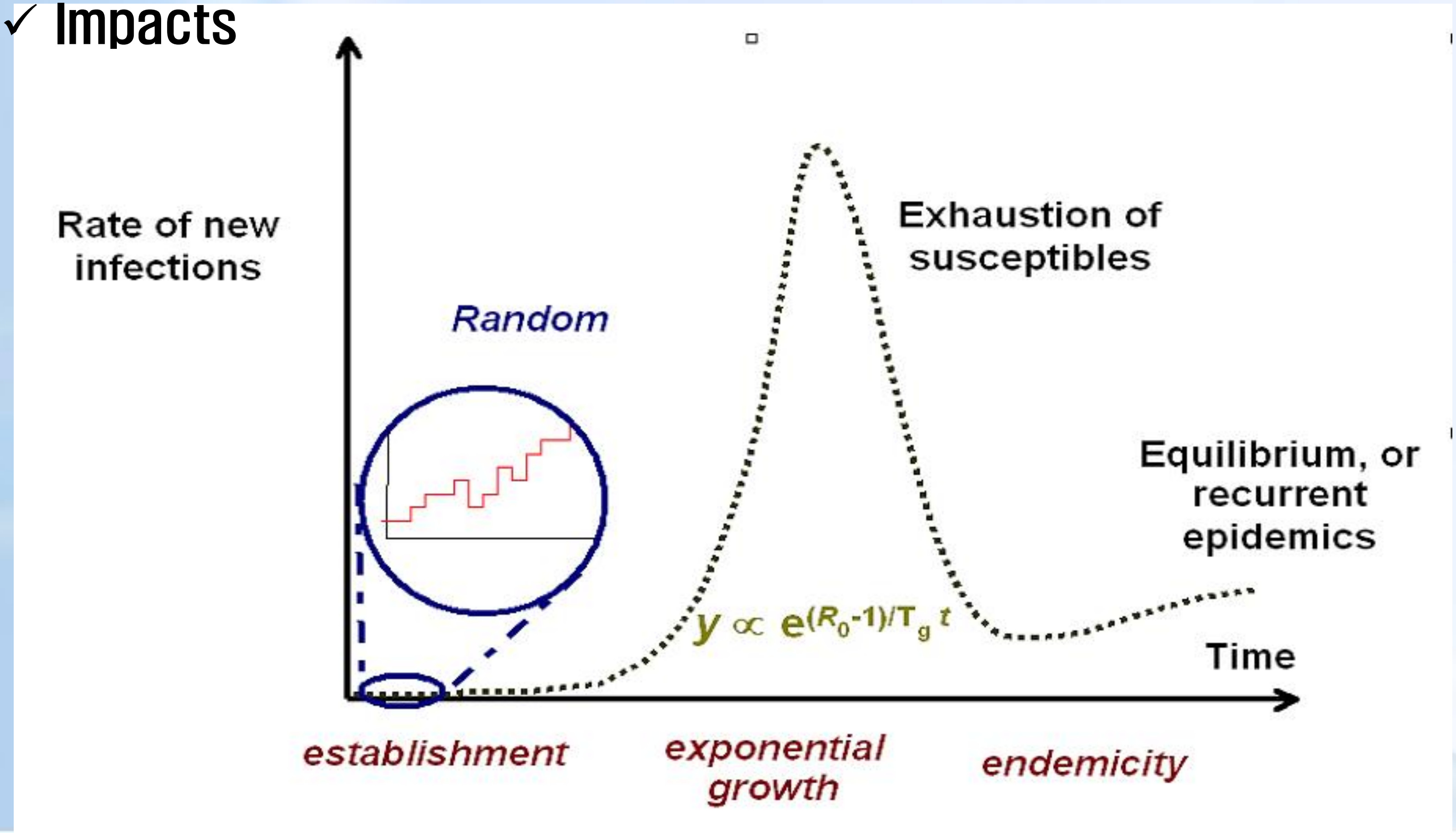
H1N1 M2 pos	28	31
1516-Foreign	I(221),V(6)	N(226),S(1)
1314-Kor-season	I(10)	N(10)
1415-Kor-season	I(10)	N(10)
1516-Kor-samples	I(2)	N(2)

표5. NS1

H1N1 NS1 pos	38	42	92	207
1516-Foreign	R(222)	S(222)	D(222)	D(219),N(3)
1314-Kor-season	R(10)	S(10)	D(10)	D(10)
1415-Kor-season	R(10)	S(10)	D(10)	D(10)
1516-Kor-samples	R(2)	S(2)	D(2)	D(2)

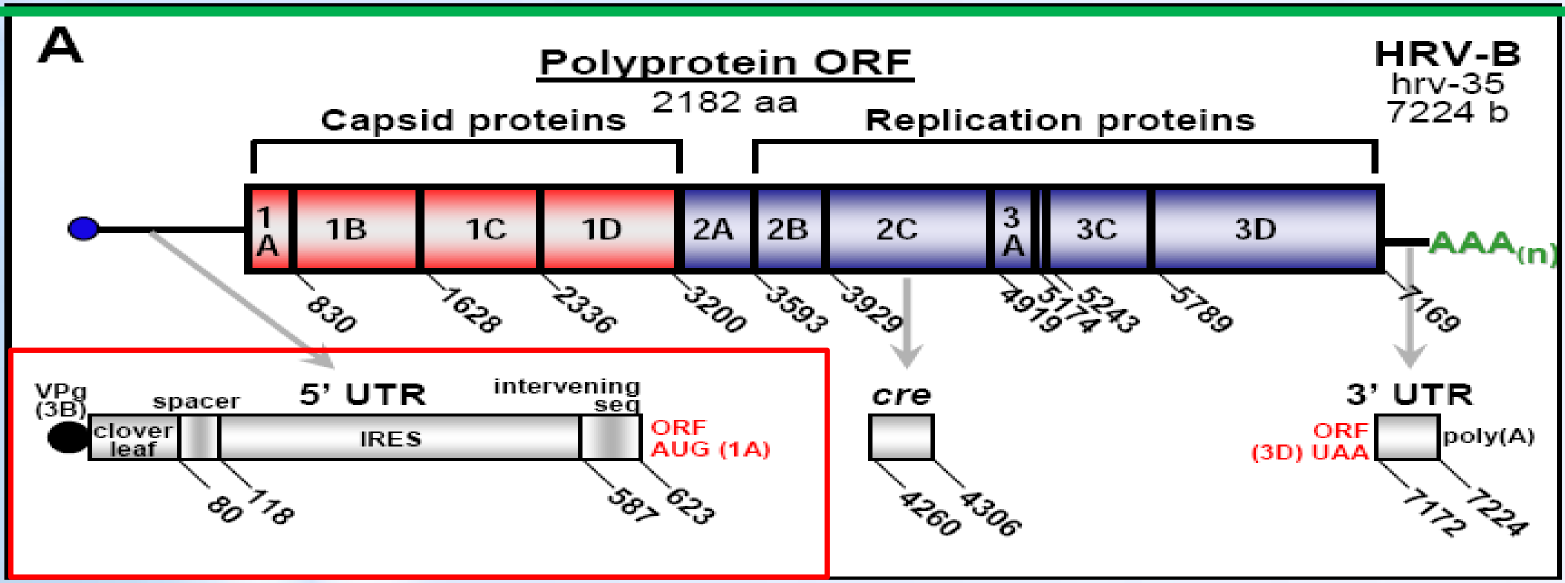
National Severity Estimation

- ✓ Transmissibility
- ✓ Seriousness of the Disease
  - ❖ (Antiviral/genetic marker/immune status)
  - ❖ (CFR/Severe abnormal cases)
  - ❖ Death related with Influenza viruses
- ✓ Impacts

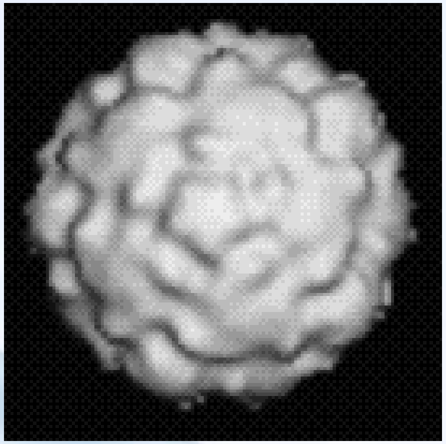


Human Rhinovirus
(사람 라이노바이러스)

Genomic Organization of the HRV



- Plus sense single stranded RNA genomes (~7200 bases).
- RNA 5' end has a covalently attached VPg (~20aa) and 3' end is polyadenylated.
- The 5' end contains a highly structured (~620nt) untranslated region that contains several AUG's.
- Downstream of the 5-UTR has structural / functional protein genes.



SCIENCE. 324 :55-59, 2009 Dr. Ann P. UWM, USA

Comparison of URI and LRI surveillance

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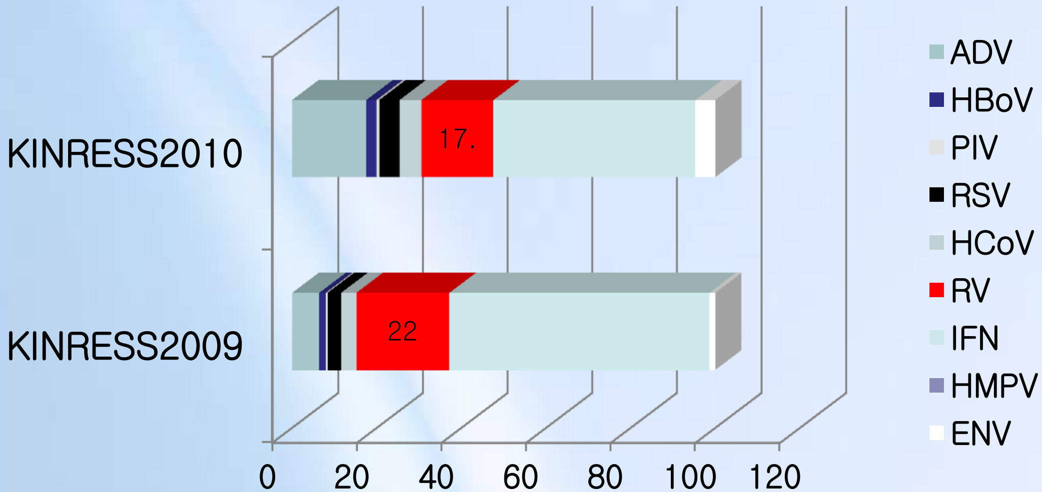
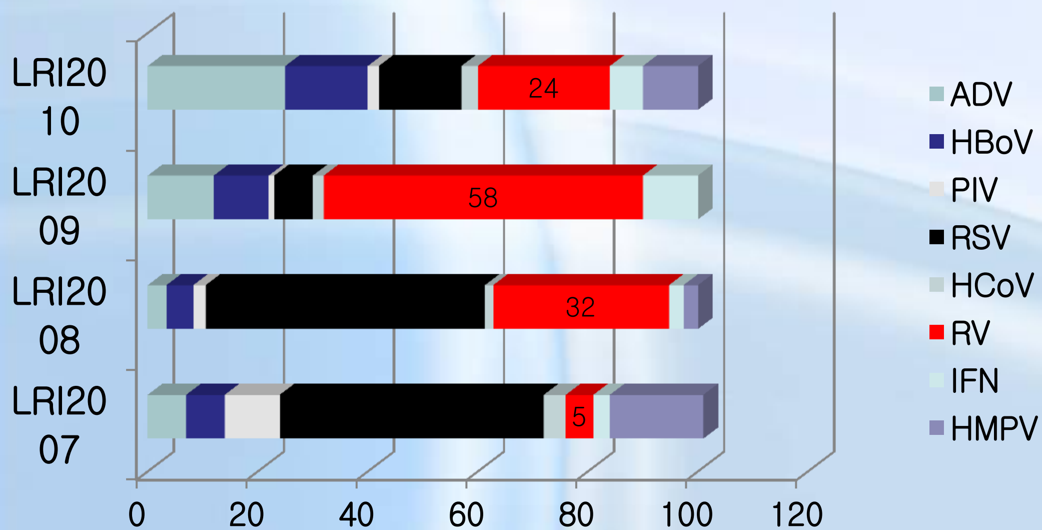
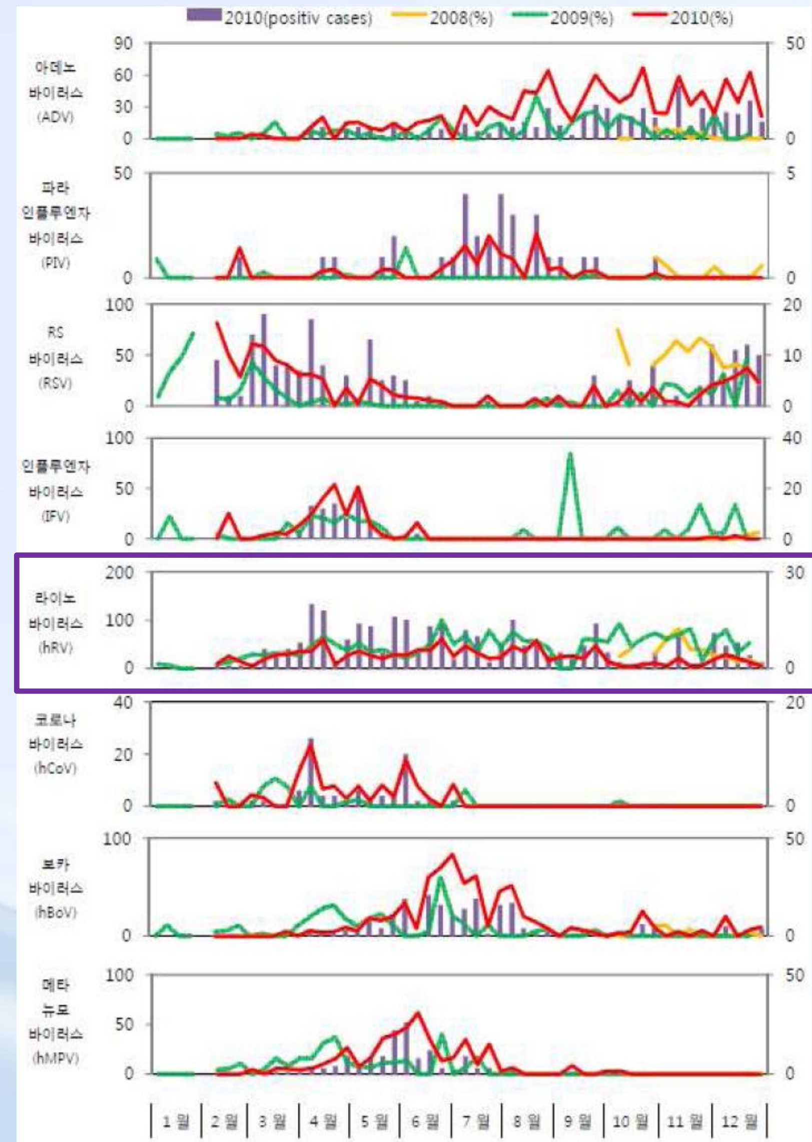




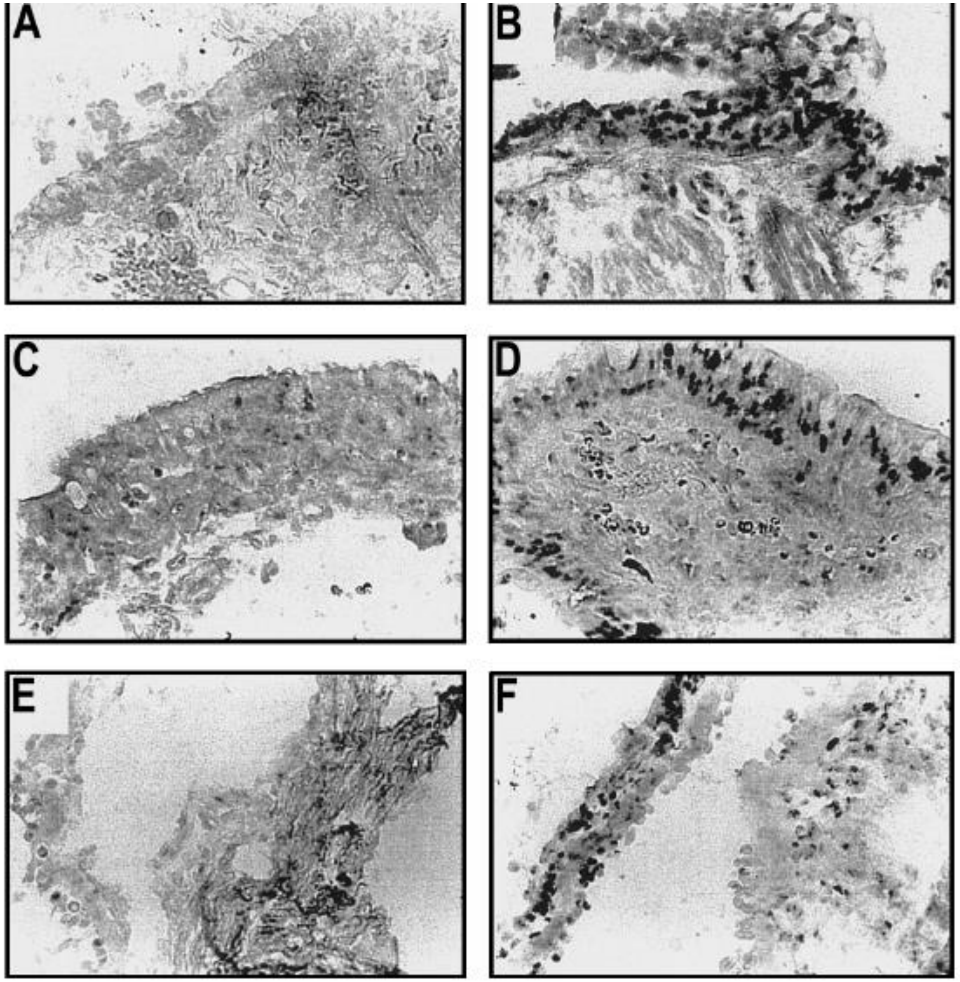


LRI Surveillance

HRV related with LRI

Rhinoviruses Infect the Lower Airways

Nikolaos G. Papadopoulos,[a] Philip J. Bates,
Philip G. Bardin,[a] Alberto Papi, Shih H. Leir,
David J. Fraenkel, Jon Meyer, Peter M. Lackie,
Gwendolyn Sanderson, Stephen T. Holgate,
and Sebastian L. Johnston[a]



NG Papadopoulos, SL Johnston. Rhinoviruses as pathogens of the lower respiratory tract. Can Respir J 2000;7(5):409-414.

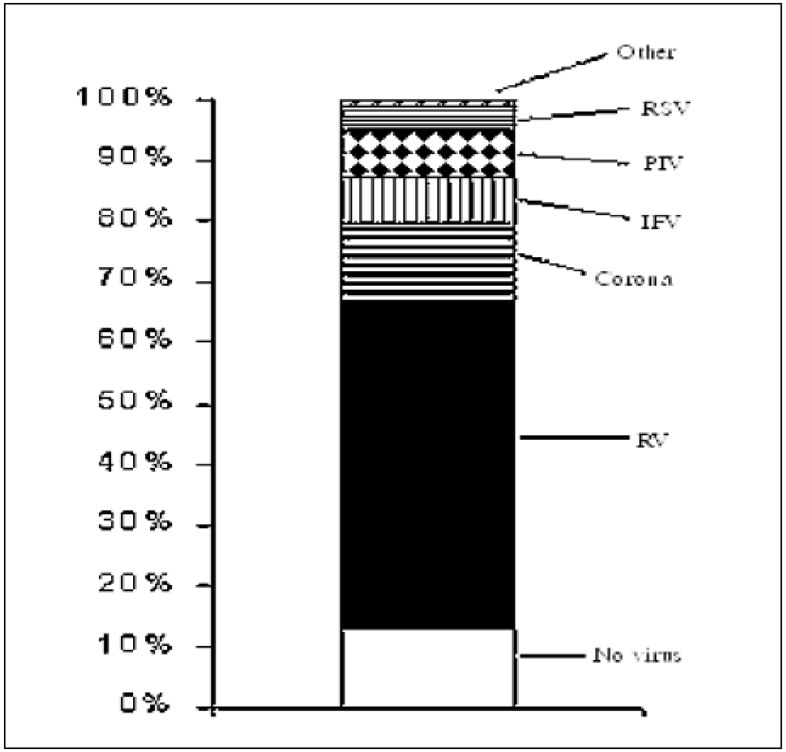


Figure 1) *Detection rates of respiratory viruses in children aged nine to 11 years with exacerbations of asthma. Rhinoviruses (RV) predominate, followed by coronaviruses (Corona). Influenza (IFV), parainfluenza (PIV) and respiratory syncytial (RSV) viruses are also present. Viruses were absent in only 15% of exacerbations*

hRV-C15 culture in sinus mucosa

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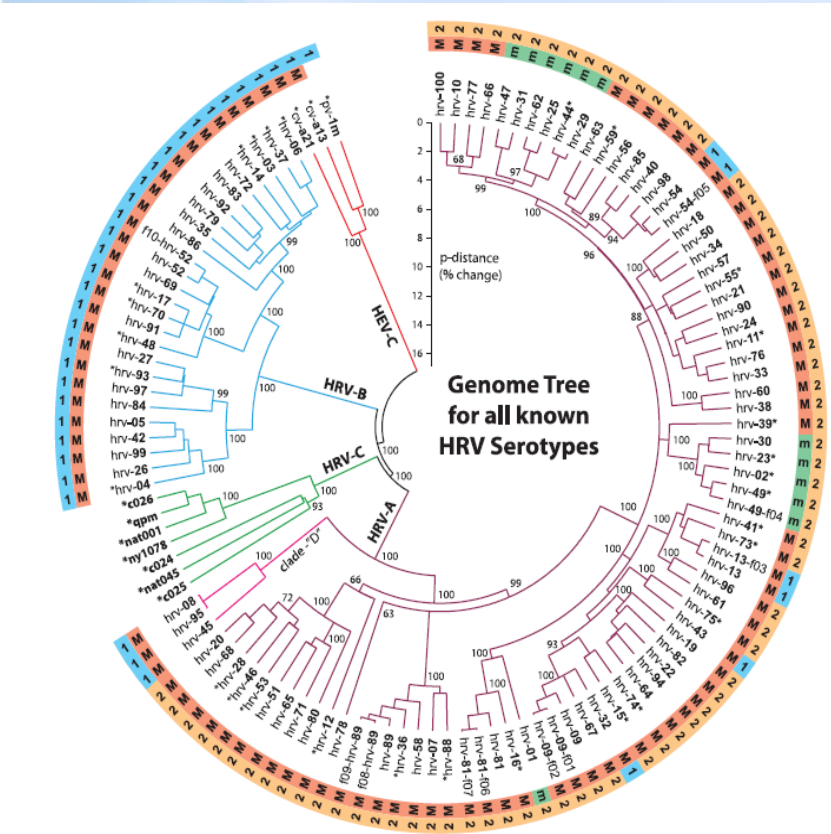
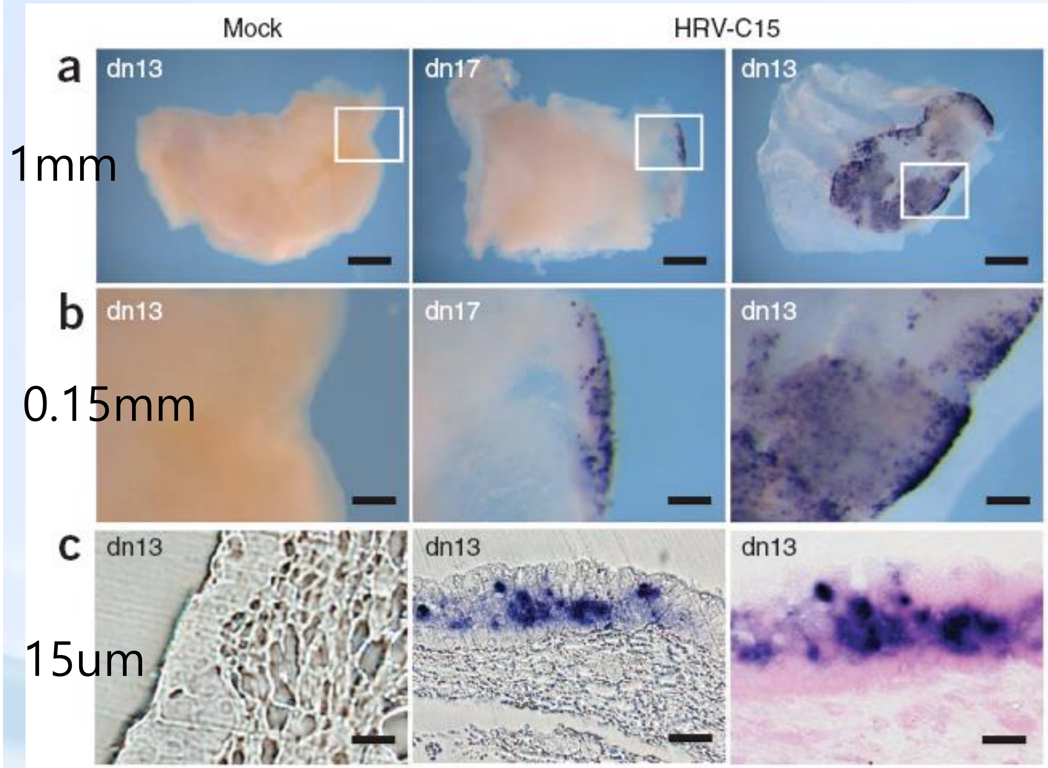
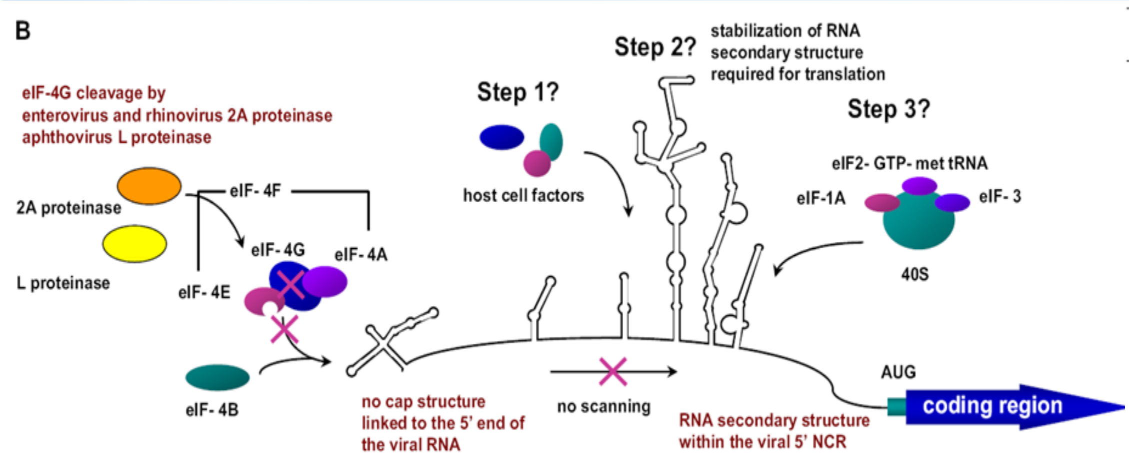




Nature medicine; published online 10 April 2011

Importance of 2nd Structures in IRES



Proteins	Binding sites/associated viral proteins	Viruses
PTB	IRES, 3C	PV, HRV, EMCV, FMDV, TMEV, HAV
nPTB	IRES	PV, TMEV
PCBP1	Cloverleaf, IRES	PV, HRV
PCBP2	Cloverleaf, IRES, 2A, 3CD, 3C	PV, HRV, HAV, CVB3
Unr	IRES	PV, HRV
La	IRES, 3C	PV, HRV, HAV, CVB3, EMCV
ITAF45	IRES	FMDV
hnRNP A1	IRES	HRV2, EV71
Nucleolin/C23	IRES	PV, HRV
DRBP 76: NF45 heterodimer	IRES	HRV2
GAPDH	IRES	HAV
FBP2	IRES	EV71
PABP	IRES	PV, EMCV

Translation mechanism of poliovirus belonging to Picornavirus

Host proteins interacting with IRES to viral translation in Picornavirus

Cap-independent translation modulated by IRES(internal ribosomal entry site)

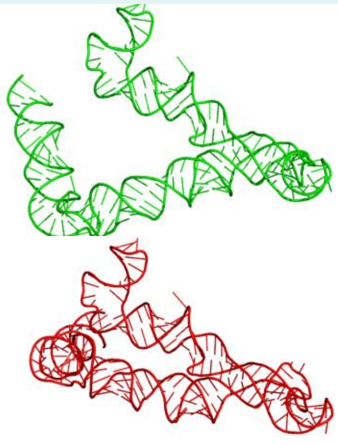
Major site of these function is known to be determined at the 5' NCR

That's why we are focusing on 5'-NCR of the HRV

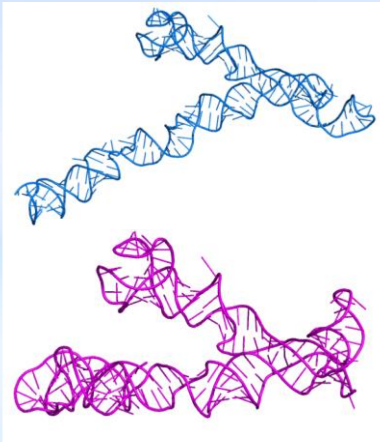
➡ **We determined to investigate the structure-based domain characters.**

Recombination break-points in IRES

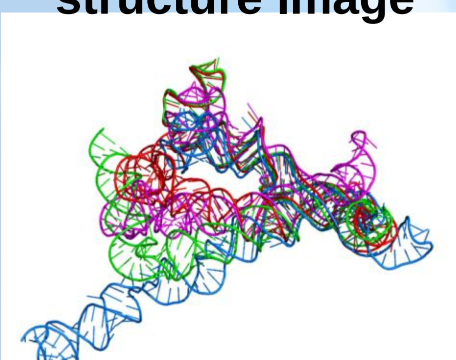
Mild patients



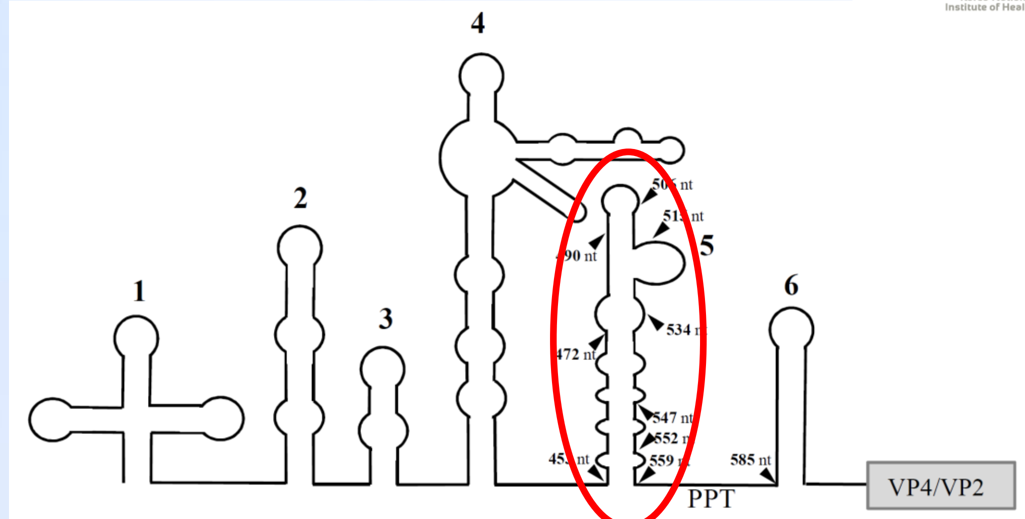
Severe patients

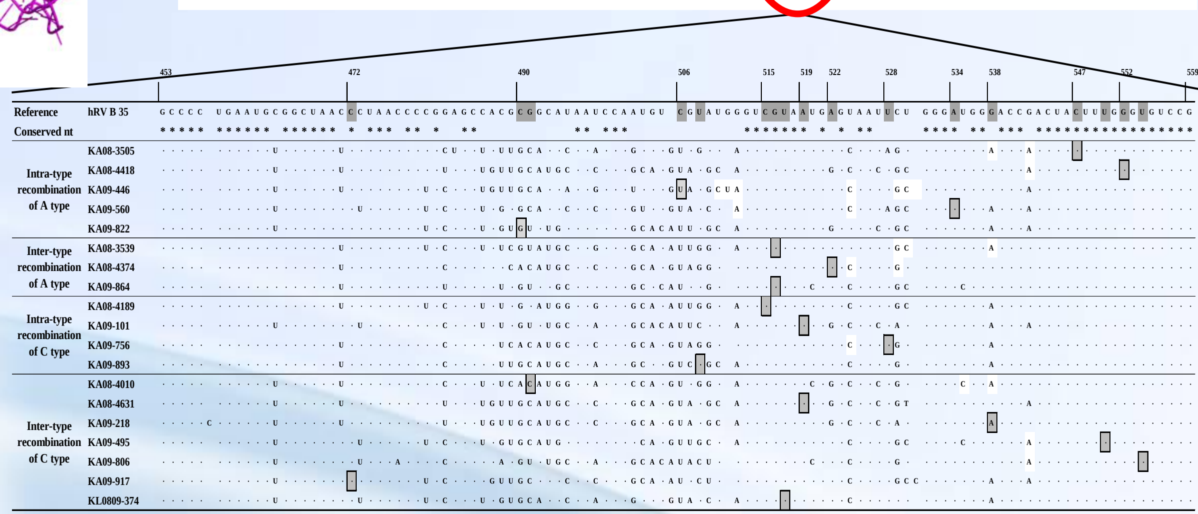


Merged RNA 3rd structure Image



Stem loop 4





Split tree analysis

OPEN ACCESS Freely available online

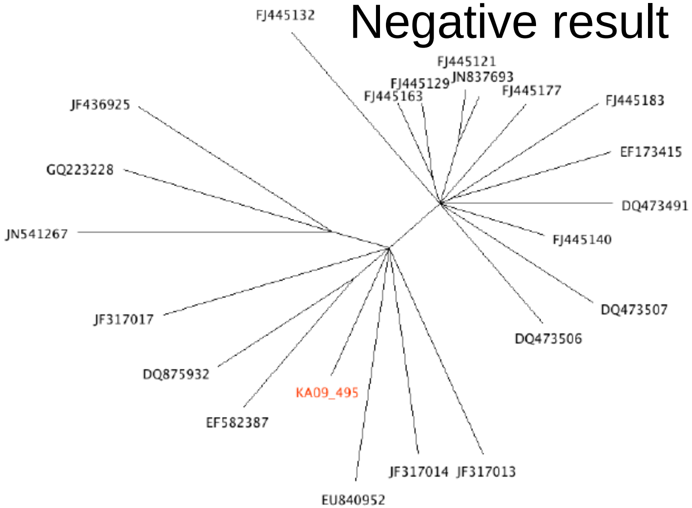
PLOS | ONE

Identification of Recombinant Human Rhinovirus A and C in Circulating Strains from Upper and Lower Respiratory Infections

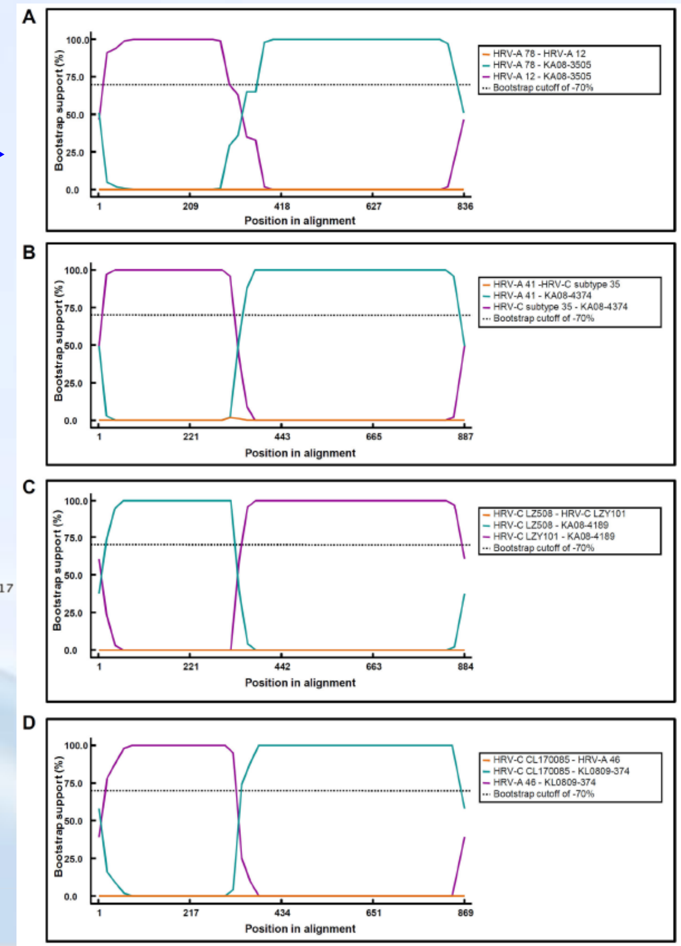
Hak Kim[1], Kisoon Kim[1], Dae-Won Kim[2], Hee-Dong Jung[1], Hyang Min Cheong[1], Ki Hwan Kim[3], Dong Soo Kim[3], You-Jin Kim[1*]

1 Division of Respiratory Viruses, Center for Infectious Diseases, Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongwon-gun, Chungbuk, Republic of Korea, 2 Systems Biology Team, Center for Immunity and Pathology, Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongwon-gun, Chungbuk, Republic of Korea, 3 Department of Pediatrics, Yonsei University College of Medicine, Severance Children's Hospital, Seodaemun-gu, Seoul, Republic of Korea

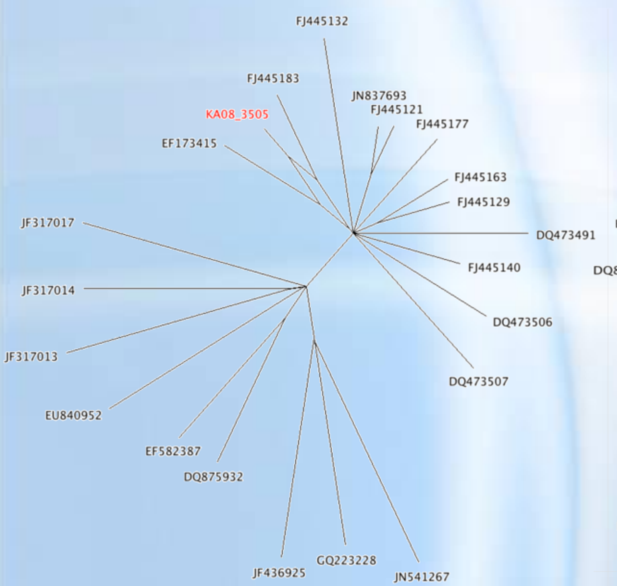
Negative result



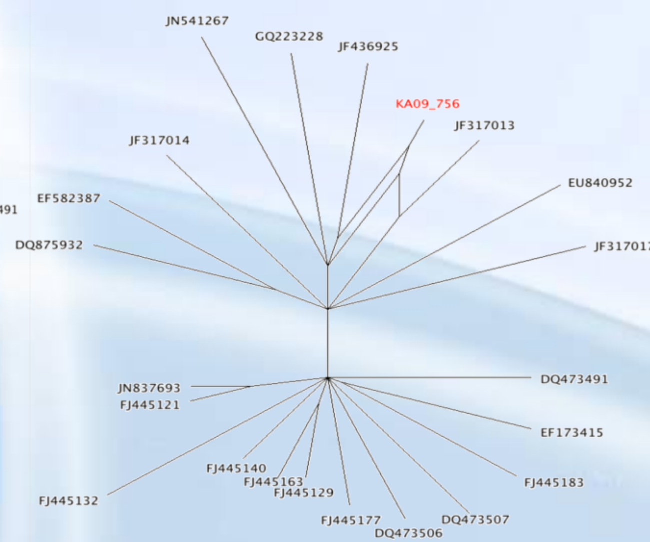
RDP analysis



Positive results



Positive results



Impacts on Disease severity of the HRV

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rationale

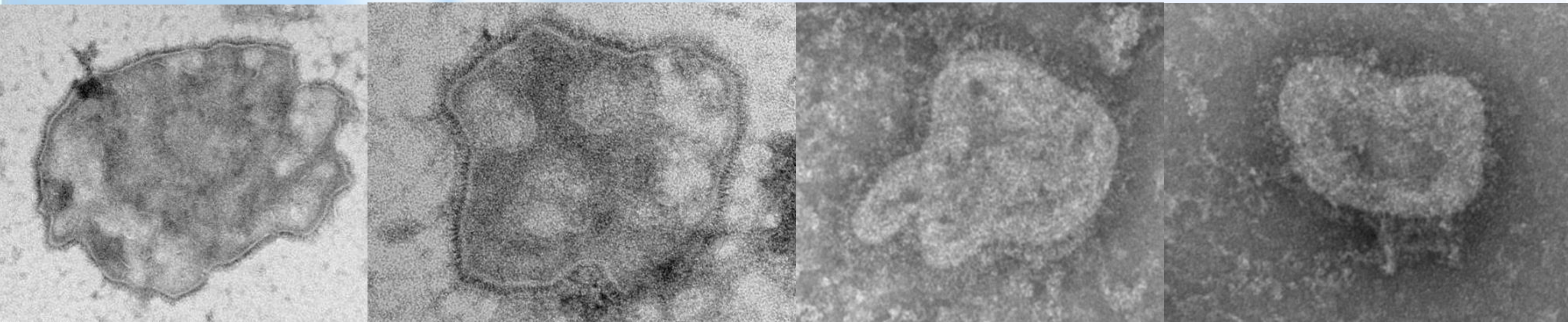
- 연중 발생 (ARI 환자의 약 10%이상)으로 높은 의료비용 발생
- 150개 이상의 혈청형 및 지속적인 변이
- 새로운 유전형 발견, 중복감염의 중요성, 중증도 변화가능성, 천식악화와의 연관성 부각
- 치료제 및 백신없음

Goal

- 질병예방 및 관리를 위한 명확한 factor는 알려지지 않아 보건학적으로 그 중요성이 점차 부각되는 hRV의 병인론 해석이 시급히 필요
- 국내 연구 활성화가 이루어지지 않은 hRV의 병인론 연구의 기초 확립
- Virulence determination을 통한 치료방향 정립
- 치료제 개발가능성 타진

국립보건연구원
Korea National Institute of Health

RSV
(호흡기세포융합바이러스)



호흡기세포융합바이러스(RSV)

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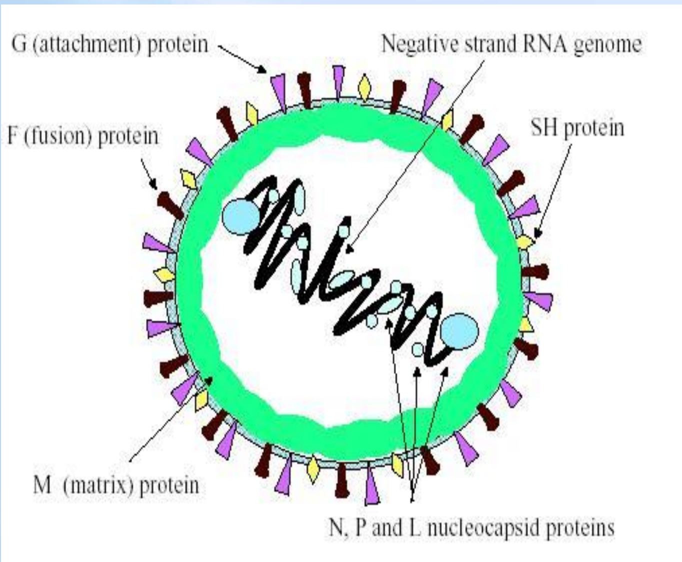
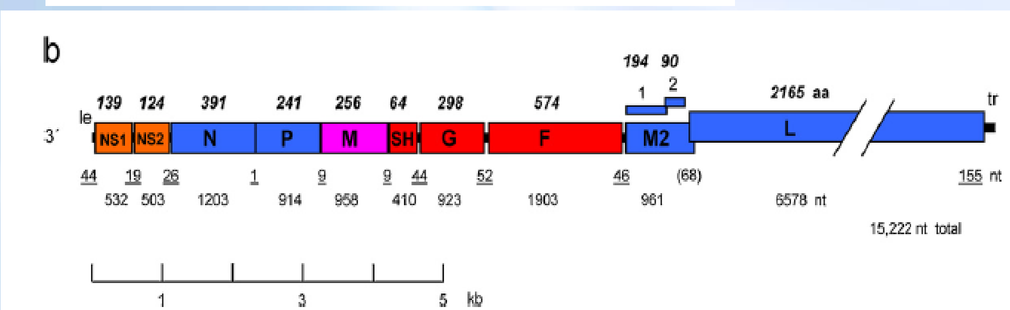
Korea National Institute of Health

Respiratory Syncytial Virus
Paramyxoviridae, Pnuemovirus





Envelope spikes
G attachment
F attachment, fusion
SH pentameric; ion channel?

Neutralization and protective antigens

Inner envelope face
M assembly

Ribonucleocapsid
N nucleoprotein
P phosphoprotein
L polymerase
M2-1 transcription processivity factor

Regulatory
M2-2 transcription↓ RNA replication

Nonstructural
NS1
NS2

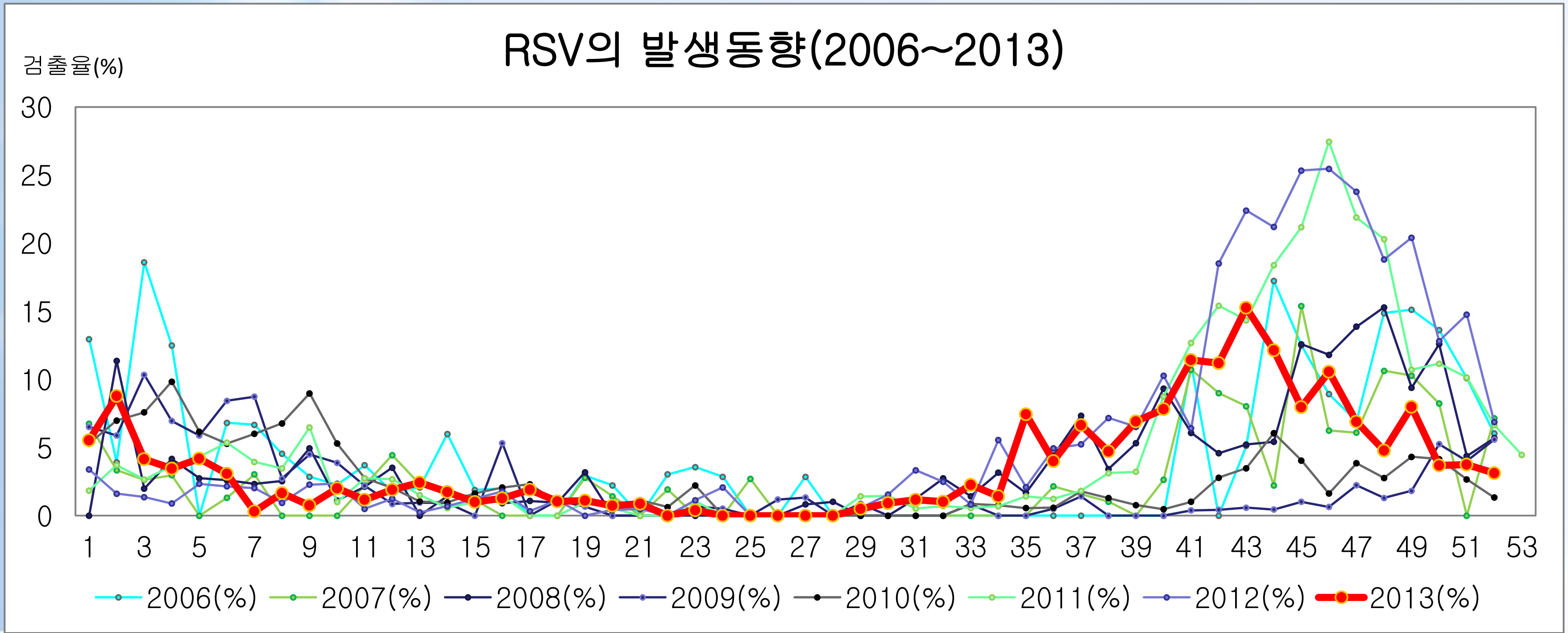
Inhibit IFN induction, signaling and apoptosis

RSV의 공중보건학적 중요성

- 전 연령에서 감염
- 특히 2세가 되기 전에 한번 이상씩 감염
- 폐렴의 약 50%를 차지
- 소아의 세기관지염 원인중 90% 이상 차지
- 고위험 그룹에 특히 문제가 심각
  - 조산아(1.2% <37weeks gestation)
  - 선천성 심장 또는 폐질환을 가진 영아
    (5.2%, 4.1% fatality, Shelagh et al., 2012)
  - 면역저하환자(성인의 경우)
- 예방을 위한 백신은 아직 없으며, 수동면역법으로 쓰이는 humanized mAb, Palivizumab(Synagis™) 의 접근성 문제
- RSV 특이 치료제 없음

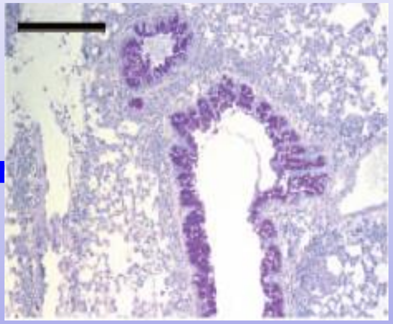
우리나라의 RSV 감염현황

- 가을-겨울 급성 상기도 감염증 환자의 약 10%-20% 가 RSV 감염(KINRESS).
- RSV감염자의 약 90%는 5세 이하 영유아.
- 특히 중증 하기도 질환자의 ~50%에서 RSV가 확인됨.
  - **2세 미만의** 연령군에 심각



백신개발 현황 (I)

Vaccine Failure



Formalin inactivated vaccine (FI-RSV, Lot 100)
Clinical trials 1965-6 in the USA
No protection against RSV and caused more sever
disease in vaccinees
Two fatalities
Alveolitis, Th2 "skew"

Prevention (Vaccine)

No vaccine approved although intensive effort during several decades

Basic : F/G/M protein
Ph-I : Attnuated recombinant virus with G/F
Ph-II : Live attenuated, F subunit

제한적 예방 및 치료 : 인간항체 Palivizumab (Synagis)

글로벌 백신개발 현황 (II)

국립보건연구원 Korea National Institute of Health

RSV Vaccine Snapshot

PATH

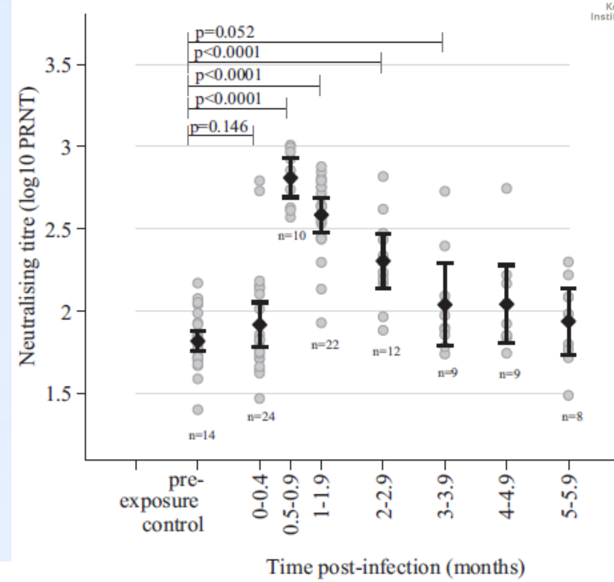
	Preclinical	Phase 1	Phase 2	Phase 3	Market Approved
Live-Attenuated	Codagenix: RSV; intravacc: Delta-G RSV; St. Jude Children's Research Hospital: SeV/RSV; Emory University: RSV; Pontificia Universidad Católica de Chile: BCG	MedImmune, NIH/NIAID/LID: Medi-RSV ΔM2-2; NIH/NIAID/LID: RSV ΔNS2 Δ1313; MedImmune, NIH/NIAID/LID: RSV cps2	MedImmune, NIH/NIAID/LID: Medi-559, RSV; MedImmune: Medi-534, RSV/PIV3		
Whole-Inactivated	NanoBio: RSV				
Particle-based	Agilvax: VLP; Fraunhofer: VLP; Georgia State University: VLP; TechnoVax: VLP; Artificial Cell Technologies, Inc.: Peptide microparticle; Mucosis: VLP; Ruhr-Universität Bochum RUB: VLP; University of Massachusetts: VLP; Emory University: VLP; Mymetics: Virosome; Takeda Vaccines: VLP; VLP Biotech: VLP		Novavax: RSV F Nanoparticle		
Subunit	IMV: DPX-RSV; NIH/NIAID/VRC: RSV pre-F protein; PeptiVir: RSV peptides; VIB: SH protein; University of Illinois: RSV F protein; Instituto de Salud Carlos III: RSV F protein; Novartis Vaccines: RSV F protein; Renaptys: RSV peptides; University of Georgia: RSV G protein; University of Saskatchewan: RSV F protein	GSK GlaxoSmithKline: RSV F protein			
Nucleic Acid	CureVac: RNA; INOVIO: DNA; Novartis Vaccines: RNA; Ruhr-Universität Bochum RUB: DNA				
Gene-based Vectors	AlphaVax: Alphavirus; Bavarian Nordic: MVA; RuenHuei Biopharma: Adenovirus; University of Pittsburg: Adenovirus; AmVac: Sendai virus; Janssen: Adenovirus; Ruhr-Universität Bochum RUB: Adenovirus; Vanderbilt University Medical Center: Alphavirus	Okairos: Adenovirus/MVA (Acquired by GSK)			
Combination	BRM: DNA prime, Protein boost; Fudan University: DNA+protein combo				

Updated: February 25, 2014

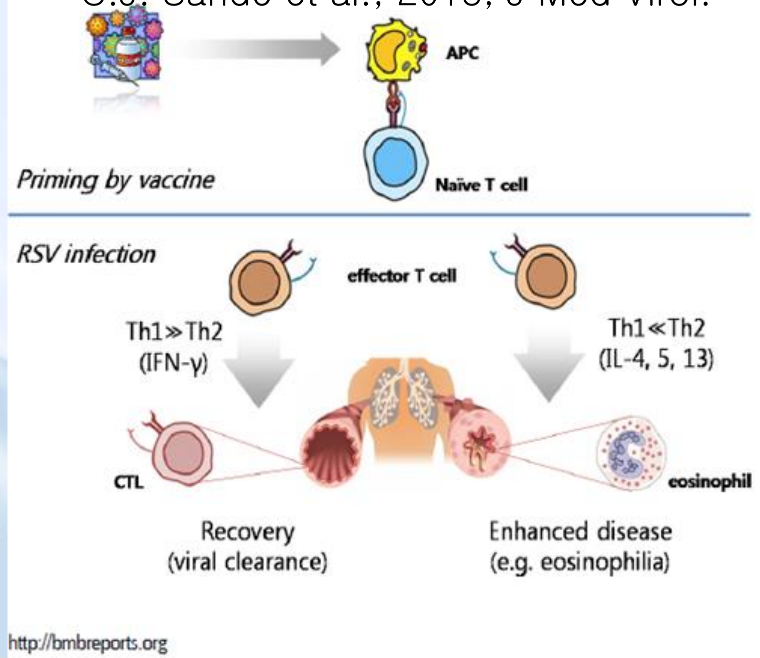
Source http://sites.path.org/vaccinedevelopment/respiratory-syncytial-virus-rsv/

RSV 백신개발의 어려움

- 지속적인 반복감염(2-3회)
- Absent of long lasting immune response
- Maternal antibody
- 고전적인 백신 trial에 의한 severity 증가
  - FI-RSV vaccine의 실패 (부작용)
- 감염에 의한 lung eosinophilia 발생
  - Th 1반응 억제 및 Th2 반응 증가
- Immune escape
  - Fractalkine (CX3C)
  - Attachment 단백질의 고도 당화:N 및 O 고도 당화 (*N*- or *O*-glycosylation)
  - NS1, NS2
  - Genetic diversity, periodic circulation



C.J. Sande et al., 2013, J Med Virol.

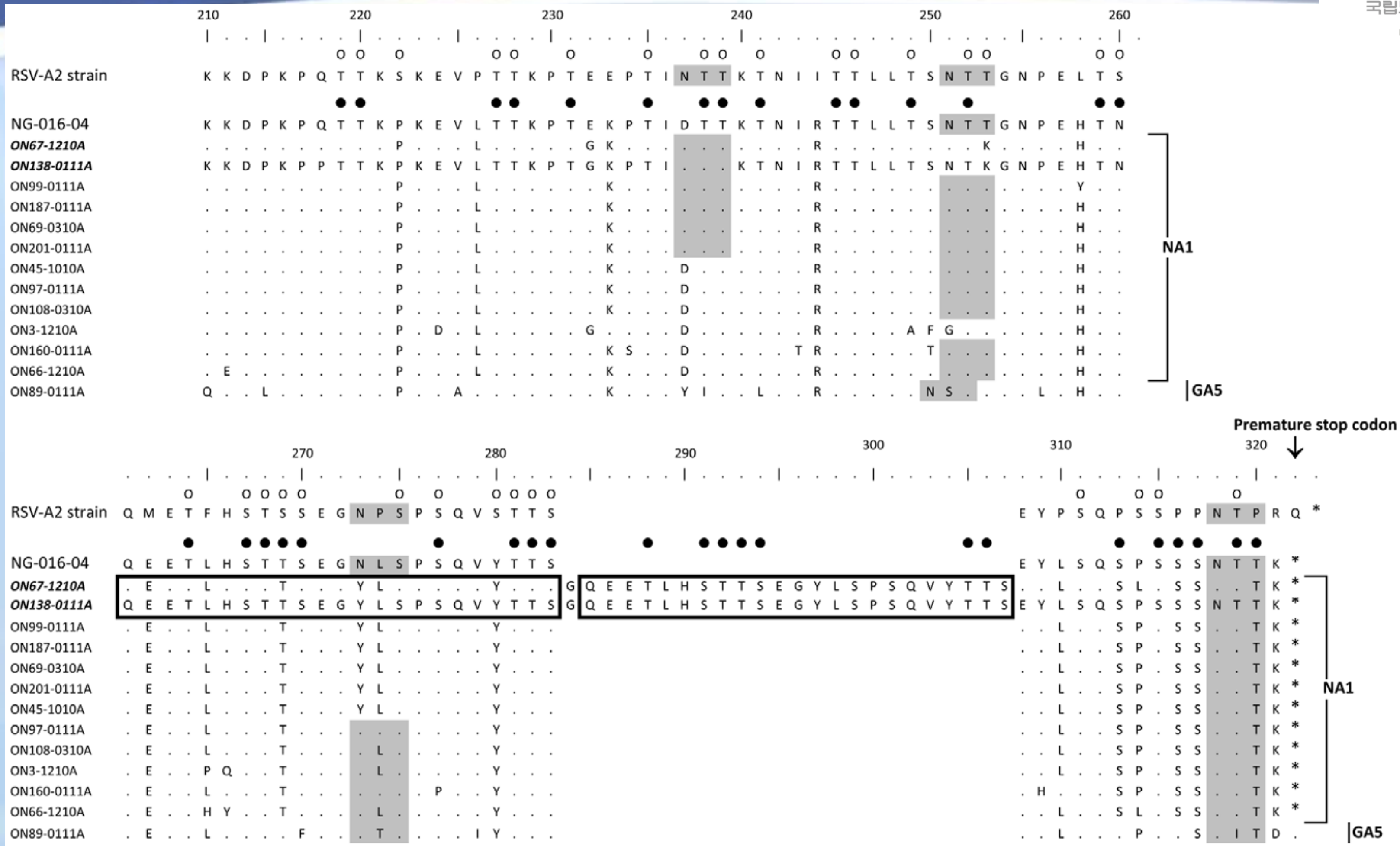


RSV A-72 new subtype 발견

- 2010-2011 season 지속적인 분자역학 분석중에 발견
- G 유전자 carboxy-terminal에 72 bases (23 a.a.)가 중복되어있음을 확인
- 기존의 RSV/B 60 bases 중복과는 위치가 다름
- Potential antigenic site로서 항원 구조에 영향을 미칠 가능성 있음
- Cell mediated growth pattern이 상이
- Replace speed가 매우 빠름

G gene Duplication in RSV-A72 strain

국립보건연구원
Korea National Institute of Health



2010-2011 winter season in Ontario:

a novel genotype with a 72 nt duplication near NA1 genotype

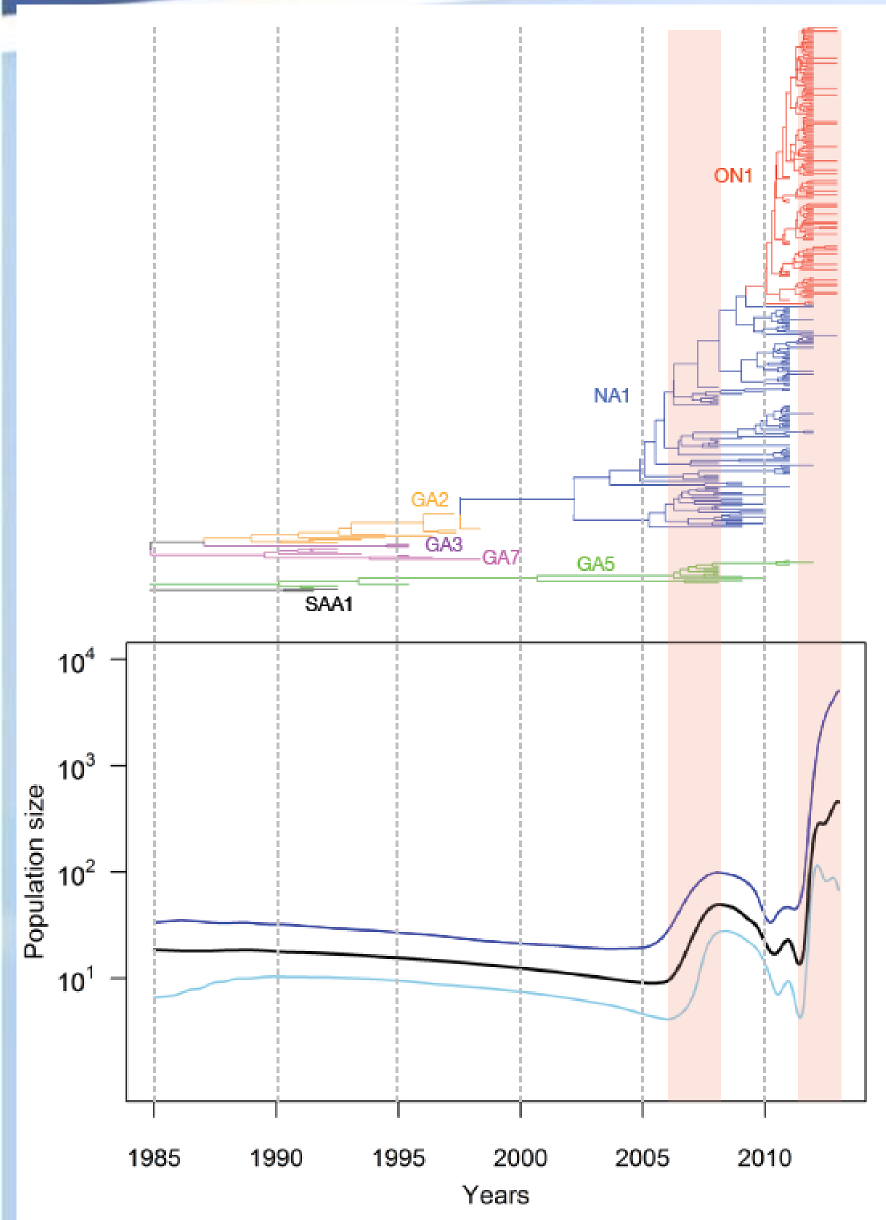
Predicted N-glycosylation sites are shaded in gray.

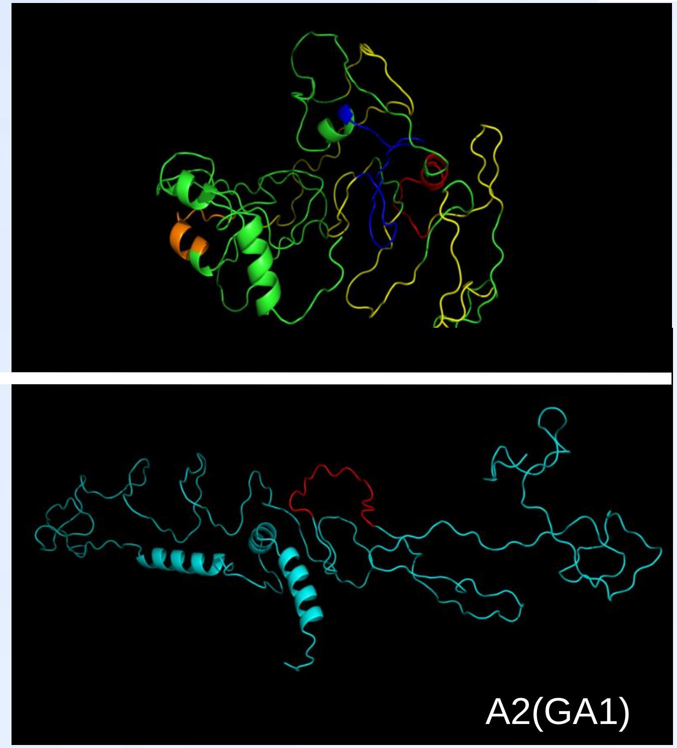
Predicted O-glycosylation sites in RSV-A strain A2 are indicated by small unfilled circles.

When compared to the NA1 reference strain, conserved O-glycosylation sites are indicated by black circles.

PLoSONE March2012, Vol7 issue3

Evolution dynamics using Bayesian skyline plot





I-TASSER server

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Infection, Genetics and Evolution
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ELSEVIER

Rapid replacement of human respiratory syncytial virus A with the ON1 genotype having 72 nucleotide duplication in G gene

You-Jin Kim [a], Dae-Won Kim [b], Wan-Ji Lee [a], Mi-Ran Yun [b], Ho Yeon Lee [a], Han Saem Lee [a], Hee-Dong Jung [a], Kisoon Kim [a,*]

[a] Division of Respiratory Viruses, Center for Infectious Diseases, Korea National Institute of Health, Cheongwon-gun, Chungbuk-do 363-951, Republic of Korea
[b] Systems Biology Team, Center for Immunity and Pathology, Korea National Institute of Health, Cheongwon-gun, Chungbuk-do 363-951, Republic of Korea

중증과 관련된 호흡기 바이러스 특성

	Human Respiratory Syncytial Virus (RSV)	Human Rhinovirus (HRV)
특성	- 100~300nm, ssRNA enveloped virus - Paramyxovirus과 Pneumovirus속의 한 종류 - subgroup A와 B로 분류됨 - A와 B subgroup 모두 일반 자연상태에서 공기 중에 순환 - Nucleolin을 receptor로 사용함	- 30nm, ssRNA virus - Picornavirus과 enterovirus속에 속함 - 최소 100종 이상이 알려져 있음 - A,B와 **C type으로 분류됨** - 여러가지 serotype이 한 시즌에 유행 - ICAM-I과 LDLR을 receptor로 사용함
임상 특징	- 소아의 중증하기도 호흡기 질환의 대표적인 원인 - 급성후두기관지염(croup)도 유발시킴 (10% of all cases) - 청소년 이상이나 성인에서는 증상이 매우 완화되어 나타남	- 2-4일 안으로 증상이 최대로 나타나며 최대 7일까지 지속됨 - 코의 상피세포에 가장 먼저 감염됨 - 재채기, 코막힘, 인후염 등이 발생 - 알레르기 / 천식 발병 및 활성에 영향 - 중증도의 변화

NIH
국립보건연구원
Korea National Institute of Health

KINRESS

Korea Influenza and Respiratory Viruses Surveillance System

인플루엔자 및 호흡기바이러스 실험실감시(KINRESS)

NIH 국립보건연구원 Korea National Institute of Health

➢ 인플루엔자실험실표본감시 (Korea Influenza Surveillance Scheme : KISS)

: 인플루엔자의사환자 대상 (2000년 부터)

➢ 급성호흡기 감염증 감시 (Acute Respiratory Infection Surveillance Network : ARI-Net) : 급성상기도감염증 환자 대상 (2005년 말부터)

➢ KISS 사업

- 인플루엔자 음성 검체에 대한 원인 병원체 규명 요구가 증대
- 하절기 감시업무 취약: 신종 혹은 계절 인플루엔자 유행 조기 감지업무 강화 필요

➢ 급성호흡기감염증 감시사업 자문위원 및 연구결과 활용방안에서 KISS와 ARI-NET의 바이러스 분야를 통합 운영하는 것이 바람직하다는 점이 제기

☞호흡기 바이러스 유행 양상에 대한 종합 분석 및 신속 대응을 위해 2009년 5월 "호흡기바이러스 실험실통합감시사업 (KINRESS)" 운영

Contents of the KINRESS

구 분	KISS(00.9~09.4)	ARI-Net (05.12~09.4)	KINRESS(09.5~13.9)
대상병원체	Influenza virus (A/H1, A/H3, B)	Influenza virus (A/H1, A/H3, B) PIV, RSV ,ADV, hBoV hCoV, hRV, hEV	Influenza virus (A/H1, A/H3, B) PIV 1, 2, 3, RSV ,ADV, hBoV hCoV, hRV, hMPV
참여기관	127개 의료기관 (1,2차)	65개 의료기관 (1,2차)	100여개 의료기관(1,2차)
검체종류	Throat swab	Nasal aspirate	**Throat swab**
실험실진단법	바이러스 분리 및 유전자진단	유전자진단	유전자진단 (인플루엔자 바이러스분리)
환류방법	매주 (인플루엔자표본감시 소식지) 매절기 (감시결과보고서)	매주	매주 환류
대상 시도	16개	16개	16개
대상환자	인플루엔자 의사환자	급성 상기도감염증	인플루엔자 의사환자를 포함한 급성 호흡기 증상 환자
검체 수집	보건환경연구원 주체	검체수송관리 용역사업체	검체수송관리 용역사업체

➢(http://www.cdc.go.kr 질병관리본부 홈페이지)

작성일 : 2016년 1월 21일

www.cdc.go.kr

질병으로부터 자유로운 세상을 여는 질병관리본부

급성감염증 병원체 감시정보

Acute Infectious Agents Laboratory Surveillance Reports(Weekly)

목차

▌2016년 3주 인플루엔자 및 호흡기바이러스 주별 발생 양상 (2016. 01. 10 ~2016. 01. 16)

◎ 최근 4주 급성호흡기 감염증 원인바이러스 검출 현황 (2015. 12. 20 ~ 2016. 01. 16)

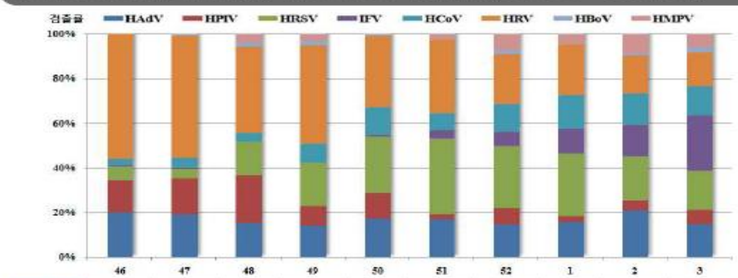
2015년~2016년	주별 검출률(%)	바이러스별 검출률(%)							
		HAdV	HPIV	HRSV	IFV	HCoV	HRV	HBoV	HMPV
52주	56.5	8.5	4.0	15.7	3.6	7.2	12.6	0.9	4.0
1주	49.5	7.9	1.4	13.9	5.6	7.4	11.1	0.0	2.3
2주	57.1	12.1	2.7	11.2	8.0	8.0	9.8	0.4	4.9
3주	55.0	8.2	3.6	9.5	13.6	7.3	8.2	1.4	3.2
누적*	52.7	5.2	6.0	3.5	14.1	2.4	17.4	2.3	1.9
2015†	52.6	4.9	6.2	3.0	14.4	2.1	17.9	2.3	1.8

- HAdV : 아데노바이러스, HPIV : 파라인플루엔자바이러스, HRSV : RS바이러스, IFV : 인플루엔자바이러스 [A(H1N1)pdm09바이러스 포함] HCoV : 코로나바이러스, HRV : 라이노바이러스, HBoV : 보카바이러스, HMPV : 메타뉴모바이러스
- 최근 4주 평균 221개의 호흡기검체에 대한 유전자 검사결과임.

◆ 인플루엔자바이러스(IFV)가 3주차에 13.6%[A(H1N1)pdm09: 10.4%, A(H3N2): 0.9%, B: 2.3%] 로 검출됨.

* 누적: 2015년 1주 – 2016년 3주 자료 (14. 12. 28 – 16. 01. 16)
† 2015: 2015년 1주 – 2015년 52주 자료 (14. 12. 28 – 15. 12. 26)
◦ 주별 통계는 잠정 통계이므로 변동 가능

◎ 최근 10주간 급성호흡기 감염증 원인바이러스별 발생 현황 (2015. 11. 08 ~ 2016. 01. 16)

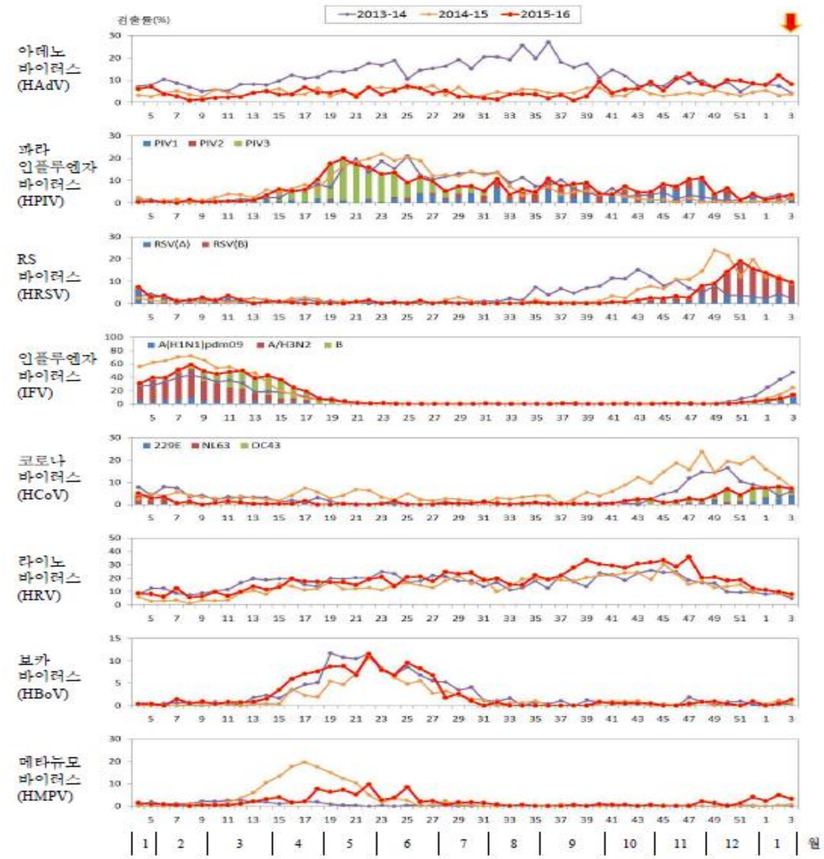


46	47	48	49	50	51	52	1	2	3
양성률* 47.5	56.0	47.1	43.7	51.3	49.8	50.7	46.3	51.6	50.5

* 양성률: 1종 이상의 바이러스가 검출된 환자의 비율(%)

www.cdc.go.kr
질병으로부터 자유로운 세상을 여는 질병관리본부
KOREA CENTERS FOR DISEASE CONTROL & PREVENTION

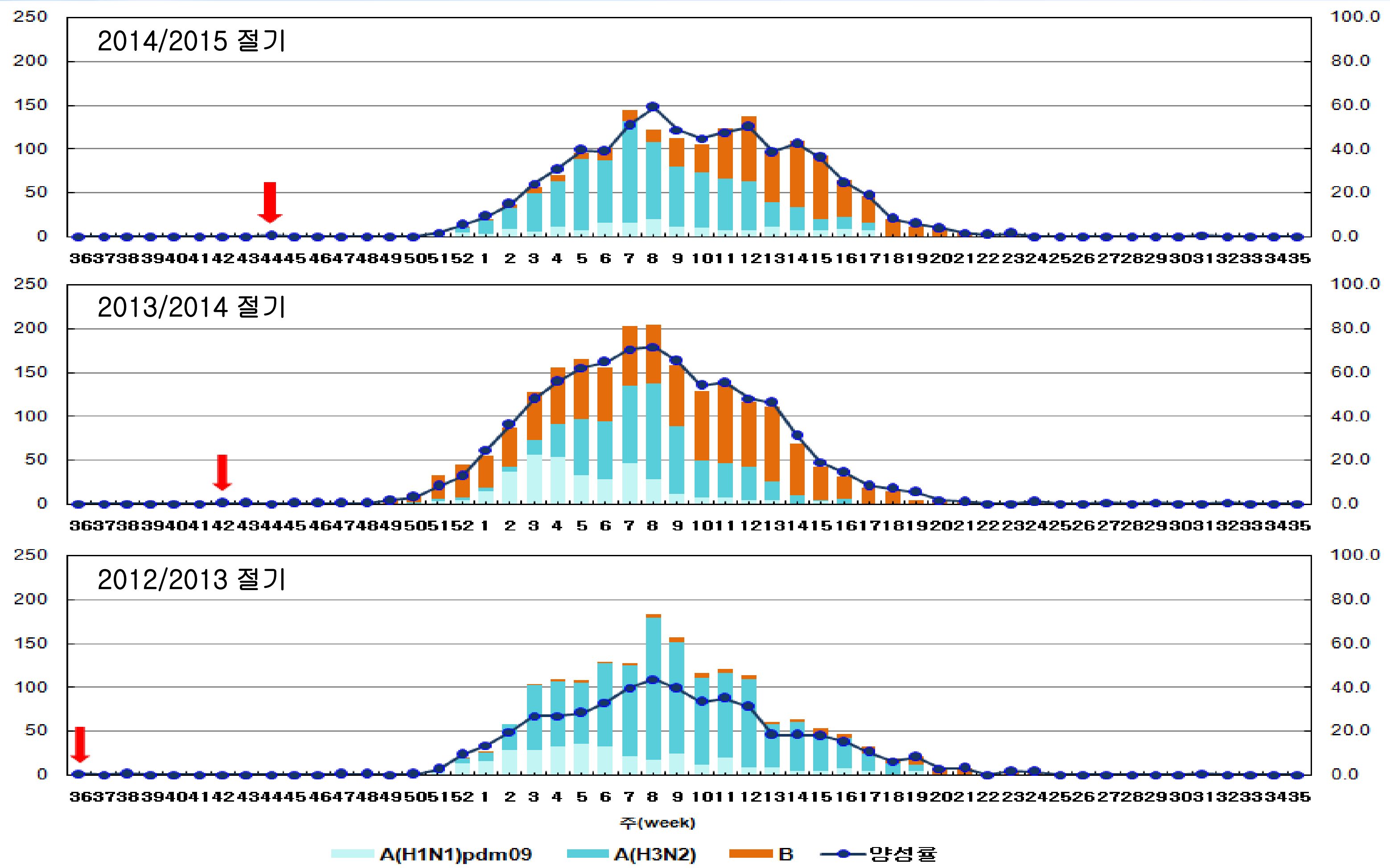
◎ 최근 3년 호흡기바이러스 주별 검출 현황 (2013. 01. 20 ~ 2016. 01. 16)



•2005년 12월 HAdV, HPIV, HRSV, IFV 감시로 시작하여, 2006년 11월 HCoV, HRV, HBoV를, 2011년 7월부터 HMPV 추가.

2014/2015 절기 IFV 실험실 감시 결과

- 주별 인플루엔자 바이러스 검출 현황 (과거)

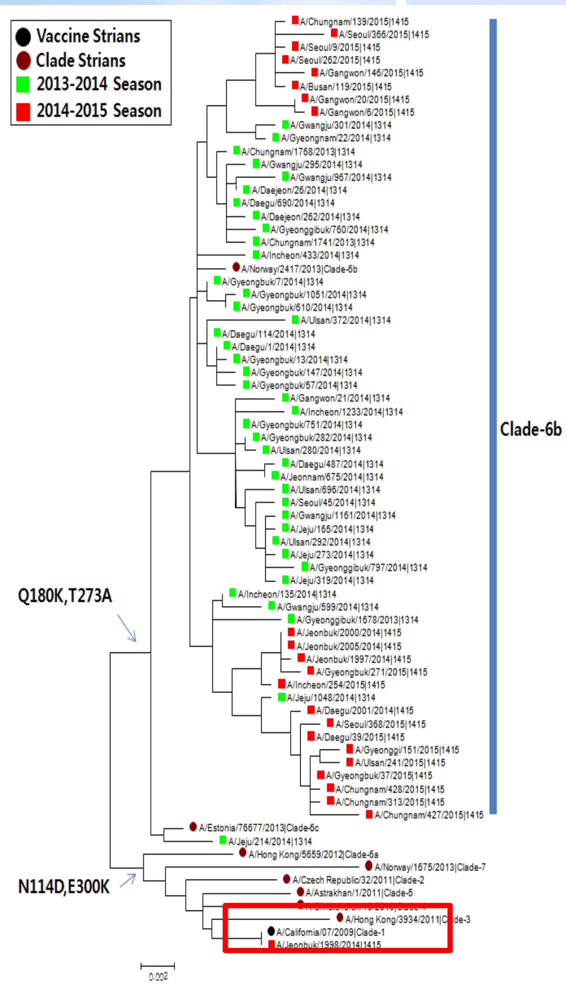


↓: 절기별 인플루엔자 바이러스 첫 검출시기

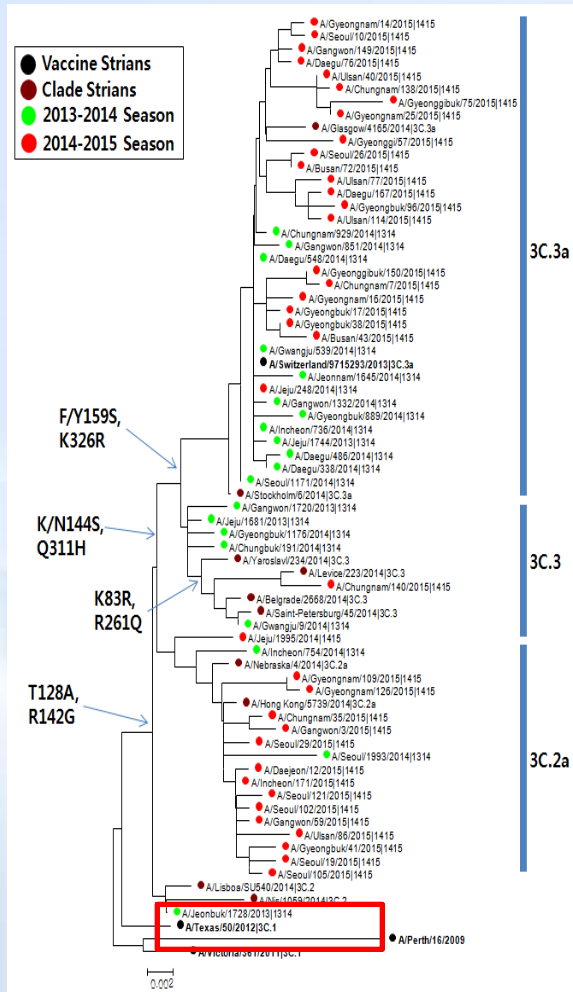
2014/2015 절기 IFV 유전자 분석

- 국내분리주의 유전자 분석 (HA)

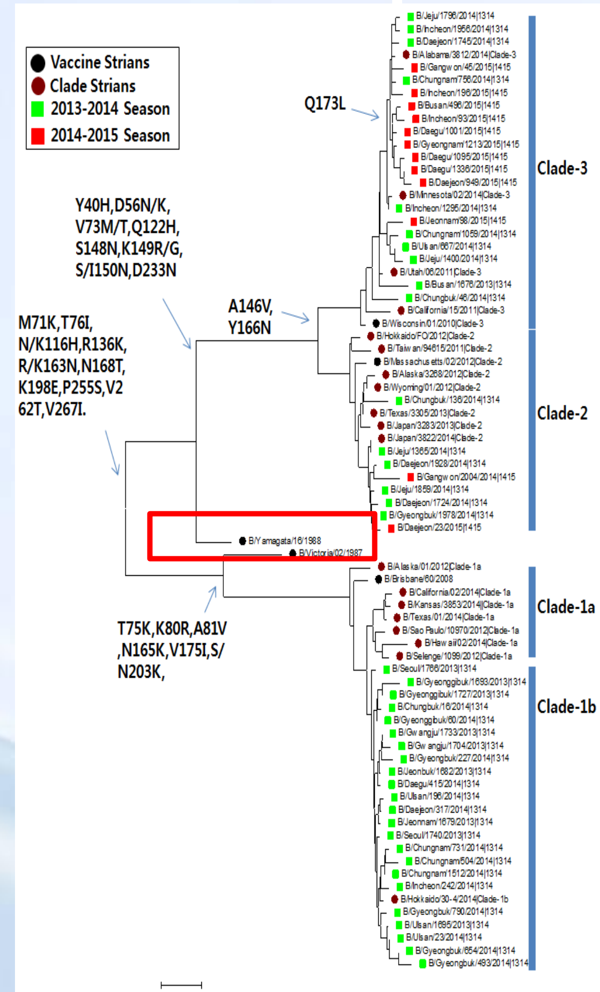
A(H1N1)pdm09



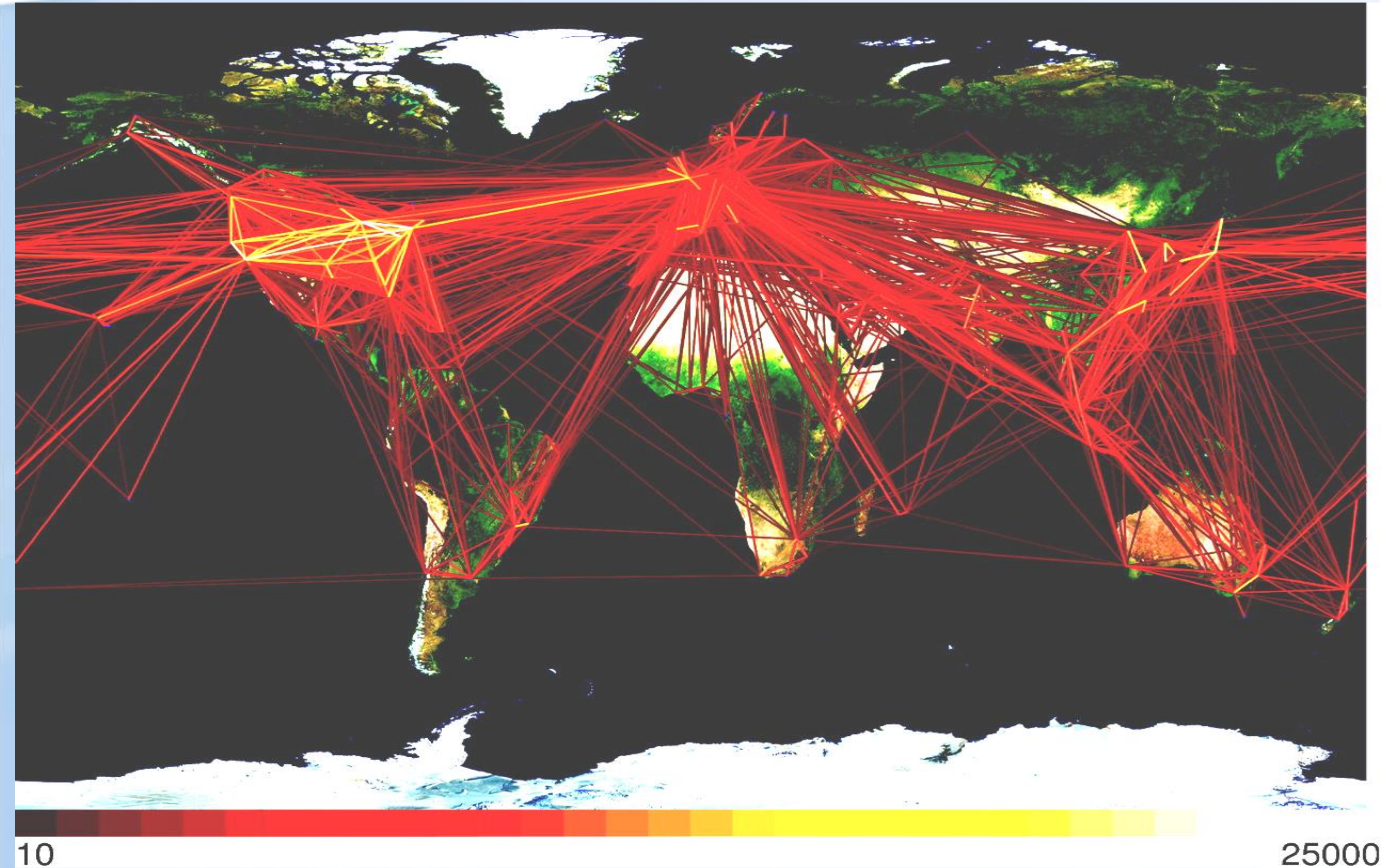
A(H3N2)



B



Potential transmission pathways



Past Pandemic CANNOT tell about the next one

Credit: US National Museum of Health and Medicine

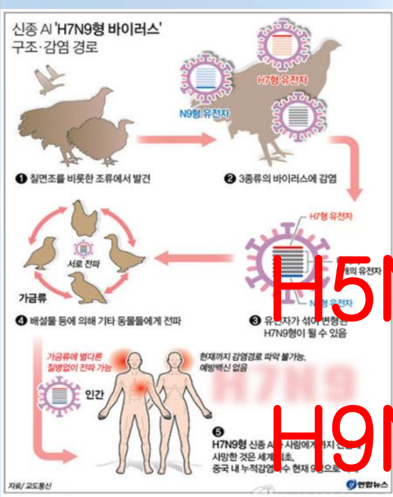
1918: "Spanish Flu"
A(H1N1)
50 –100 M deaths

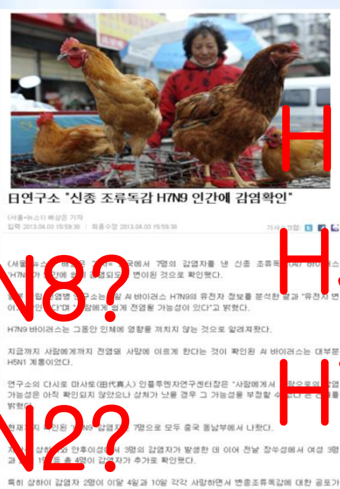
1957: "Asian Flu"
A(H2N2)
1–4 M deaths

1968: "Hong Kong Flu"
A(H3N2)
1–4 M deaths

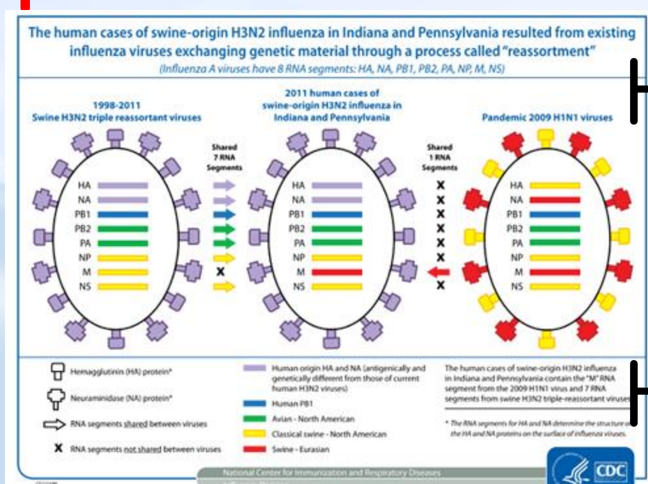
2009: "Pandemic flu"
A(H1N1)
0.1–0.4M deaths
(Lucky case)

What is Next pandemics?





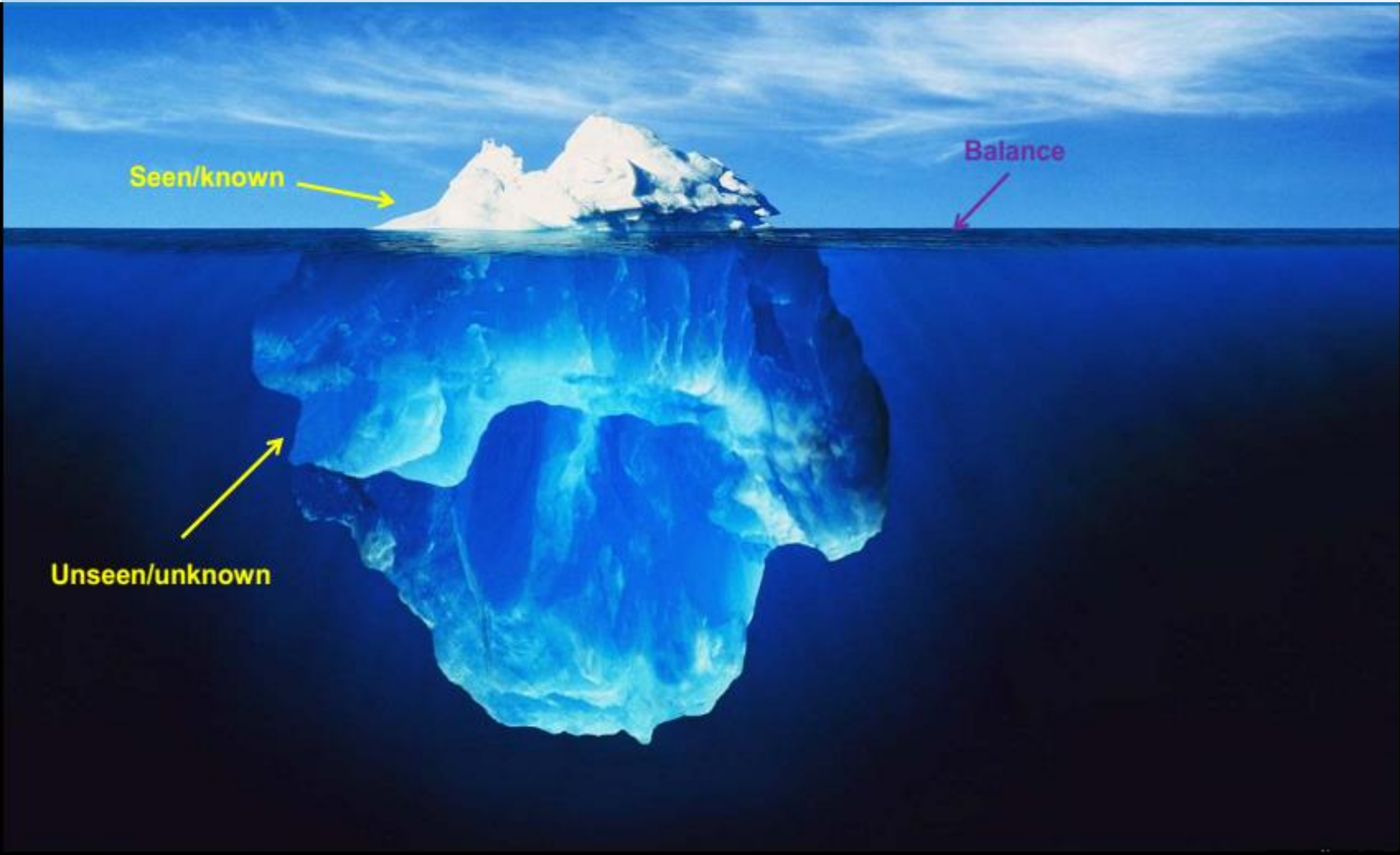
H5N6?
H5N1?
H5N8?
H7N9?
H9N2?



H1N2v?
H3N2v?

Isaac B. Weisfuse, NYC Department of Health & Mental Hygiene

Still we do NOT know lots of things under the Sea



감사합니다.

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국립보건연구원
Korea National
Institute of Health