Genetic Diversity of Influenza Virus & Related Groups

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Environment & Evolution

- Rapidly changing global and local environments:
  - Global climate changes
  - Changes in agriculture, industry, urbanization,
  - Changes in food production & practices
  - Changes in human-animal ecology
- Environmental changes affect the evolution, dynamics of biodiversity and genetic diversity

Biodiversity & Genetic Diversity

- What biodiversity: biodiversity found in genes, species, and ecosystem processes
  - Biodiversity in gene → genetic diversity
  - Genetic diversity: variety of genetic material within a species or a population
- *Why is it important*: Genetic diversity plays an important role in the survival and adaptation of species

Evolution – mutation - replication

- ❑Evolution requires mutation
- ❑Mutation ~ inacurate replication
- ❑Inaccurate replication ~ replication speed

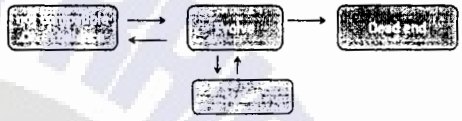
Human	100000	30 kb	100 bp/min
Mouse	25000	150 kb	2.200 bp/min
Toad	15000	200 kb	500 bp/min
Fruit fly	3500	40 kb	2.600 bp/min
Yeast	500	40 kb	3.600 bp/min
Bacterium	1	4200 kb	50.000 bp/min
Virus	?	?	?

Virus evolution

- What : The constant change of a viral population in the face of selective pressures
- What type:
  - Mutation (point, deletion, insertion),
  - recombination,
  - Reassortment (non reciprocal recombination)
  - selection : positive and negative pressure select for particular preexisting mutant
- Where: within host cells
- Virus evolution is fast:
- Fast generation time
- Produce large numbers of progeny
- High rates of mutation
- **virus evolution is inescapable**

Two general pathways for virus evolution

- **Co-evolution with host**
  - Advantage: prosperous host = prosperous virus
  - Disadvantage: virus shares same fate as host
- **Infection of multiple host species:**
  - Advantage: if one host species is compromised the virus can replicate in another
  - Disadvantage: cannot optimize for any one situation
- Risks: new-emerging & re-emerging diseases



Mutation in RNA Virus?

Genome replication relied on RNA polymerases

DNA polymerase	I	II	III		
5'-3' polymerase	+	+	+	+	+
3'-5' exonuclease	-	-	+	+	+
DNA polymerase	I	II	III		
5'-3' polymerase	+	+	+		
3'-5' exonuclease	+	+	+		
5'-3' exonuclease	+	-	-		

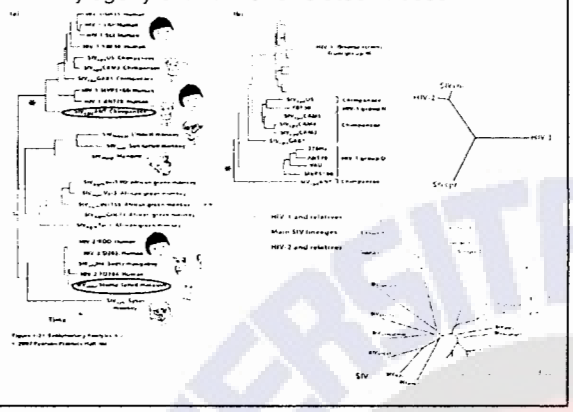
RNA polymerase	
Core polymerase	RNA elongation
Sigma factor	Transcription initiation

RNA polymerase has no capability to detect mismatch synthesis → mutation

Emerging of HIV

- Family: Retroviridae
  - Sub family: *Orthoretrovirinae*
    - Genus: Lentivirus
      - Species: HIV1 & HIV2
  - Extremely rapid RNA virus replication
  - Genome (+) ssRNA:
    - No proofreading mechanism leading to accumulation of mutations
    - The progeny of a single virus can differ greatly in antigenic configuration (gp120 and gp160) from the parent → viral evolution

Phylogeny of HIV-1 and related viruses



Corona Virus

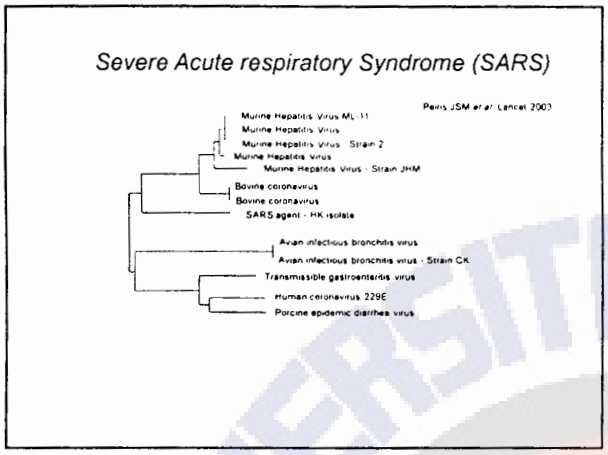
- Ordo: Nidovirales
- Family: Coronaviridae
- Genus: Coronavirus
- Characteristic:
  - Enveloped, (+) sense ssRNA viruses
- Coronaviruses infect and cause disease in many animal species: bats, mice, birds, bogs, pigs, cattle, human

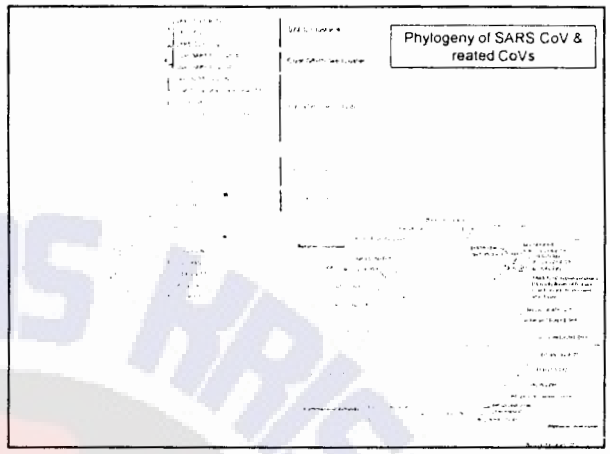
Coronavirus

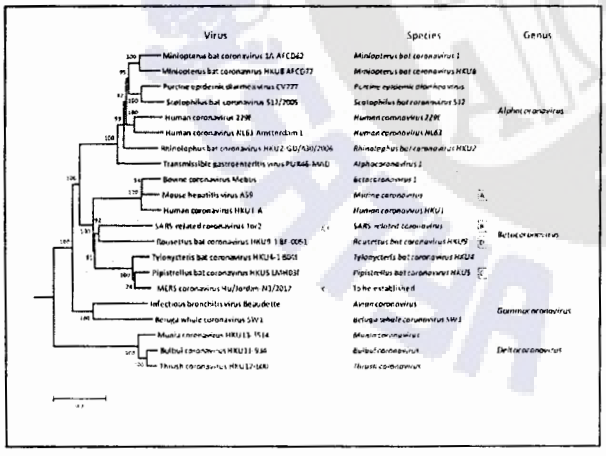
Group I		
TGEV, PRCoV	pig	porcine transmissible gastroenteritis virus
CCoV	dog	canine respiratory coronavirus
FECoV	cat	feline enteric coronavirus
FICoV	cat	feline infectious peritonitis virus
RbCoV	rabbit	rabbit respiratory coronavirus
Group II		
HCoV-OC43	human	human respiratory coronavirus
MHV	mouse	murine hepatitis virus
SDAV	rat	sialodacryoadenitis virus
HEV	pig	porcine hemagglutinating virus
BCoV	cow	bovine respiratory coronavirus
TCoV	turkey	turkey respiratory coronavirus
Group III		
IBV	chicken	avian bronchitis virus
TCoV	turkey	turkey respiratory coronavirus

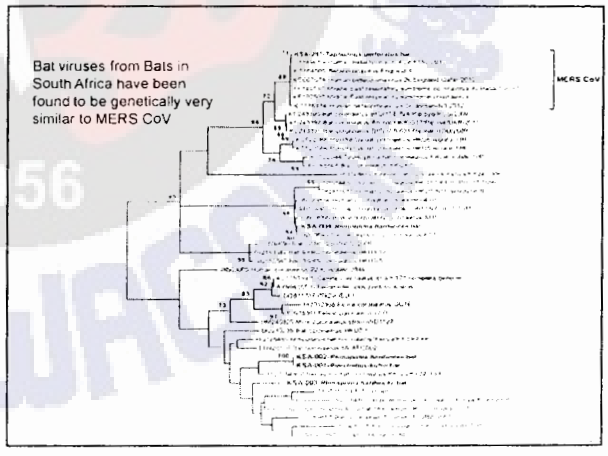
New corona viruses

1978	Porcine Epidemic diarrhea virus (PEDV) Probably from humans
1984	Porcine Respiratory Coronavirus
1987	Porcine Reproductive and Respiratory Syndrome (PRRS)
1993	Bovine corona virus
2003	SARS-CoV
2012	MERS-CoV


Severe Acute respiratory Syndrome (SARS)
Peiris JSM et al. Lancet 2003
Murine Hepatitis Virus ML-11
Murine Hepatitis Virus
Murine Hepatitis Virus - Strain 2
Murine Hepatitis Virus
Murine Hepatitis Virus - Strain JHM
Bovine coronavirus
Bovine coronavirus
SARS agent - HK isolate
Avian infectious bronchitis virus
Avian infectious bronchitis virus - Strain GK
Transmissible gastroenteritis virus
Human coronavirus 229E
Porcine epidemic diarrhea virus

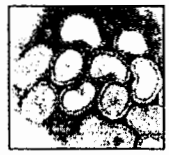

Phylogeny of SARS CoV & reated CoVs


Virus
Species
Genus


Bat viruses from Bats in South Africa have been found to be genetically very similar to MERS CoV
MERS CoV

Influenza Virus

- Ordo Mononegavirales
- Family Orthomyxoviridae
- Genus Orthomyxovirus
- Species: Influenza virus
  - Type A
    - infects human & animals
  - Type B
    - infects human
  - Type C
    - infect human & pigs



- Subtype : H1-H16 & N1-N9
- Pathogenicity:
  - Highly Pathogenic
  - Low Pathogenic

	Hemmaglutinin subtype					Neuraminidase subtype			
	Human	swine	Horses	Birds		Human	swine	Horses	Birds
H1	●	●		●	N1	●	●		●
H2	●			●	N2	●	●		●
H3	●	●	●	●	N3				●
H4				●	N4				●
H5				●	N5				●
H6				●	N6				●
H7			●	●	N7			●	●
H8				●	N8				●
H9				●	N9				●
H10				●					
H11				●					
H12				●					
H13				●					
H14				●					
H15				●					
H16				●					

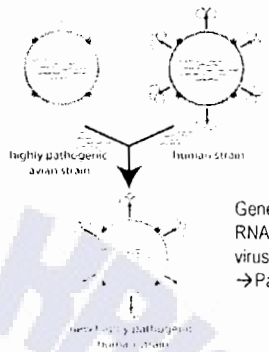
H17 N10 → Bats

Public Health Threat by Influenza

- Seasonal Flu
  - always lurking
  - Influenza virus A H_3N_2, H_1N_1 (classical), endemic human population
- Bird Flu, recently by AIV H_5N_1 & *H_7N_9 (China)*
  - Direct / indirect zoonotic infection from infected bird
  - Very limited human to human transmission (no clear evidence)
- Swine Flu, influenza virus A H_1N_1 (classical) endemic swine population; potentially infect human *vice-versa*
- Influenza virus easily mutation
- 2009 flu pandemy?

Mutation of Influenza Virus

- **Recombination:**
  - genom VAI : (-)ssRNA , segmented ~> genetic reassortment (non-reciprocal recombination) → antigenic shift → pandemi potential
- **Mutation:**
  - RNA-pol has no proof reading activity → mutation 1000-10000x more often than DNA-pol. → point mutaion → antigenic drift → epidemi potential
  - *secondary structure of* RNA genome → insertion/deletion during replication



How antigenic shift, or reassortment, can result in novel and highly pathogenic strains of human influenza

Genetic reassortment → exchange of RNA genome segment between 2/more virus strains→ antigenic shift →Pandemic potential?

Pandemics and Pandemic Potentials of Influenza

• 1918-19 "Spanish flu" √	H1N1
• 1957 "Asian flu" √	H2N2
• 1968 "Hong Kong flu"√	H3N2
• 1976 "Swine flu" episode	H1N1
• 1977 "Russian flu"	H1N1
• 1997 "Bird flu" in HK	H5N1
• 1999 "Bird flu" in HK	H9N2
• 2003 "Bird flu" in Netherlands	H7N7
• 2004 "Bird flu" in SE Asia	H5N1
• 2009 Swine Flu/Mexican Flu √	H1N1
• 2013 "Bird Flu" China	H7N9

Virulence factors of AIV

- ⦿Ability to infect lower respiratory tract
- ⦿A strong induction of pro-inflammatory cytokines and chemokines (cytokine storm)
- ⦿Apoptosis induction
- ⦿Systemic infection/viremia (spreading beyond the respiratory tract)
- ⦿Evasion of innate immune response (IFN)

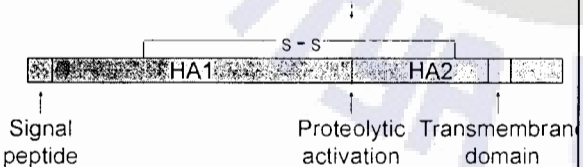
→ ***These can be identified based on sequence analysis***

Avian Influenza
Factors Influencing Pathogenicity

- Polygenic
- Associated with H5 and H7 subtypes
  - HA glycoprotein plays a dominate role
  - Presence of multiple basic amino acids at the cleavage site of the HA glycoprotein
  - RBS specificity
- Associated with N, PB2, NS1
- Pathogenic strains evolve from nonpathogenic lineages

Roles of H

- Binds to host cell receptor → host specificity
- Fusion with host cell endosome membrane → uncoating
- Sensitivity to proteases at cleavage site → pathogenicity (HPAIV /LPAIV)



Molecular detection of pathogenicity

Virus isolates	Cleavage sites sequence
A/Goose/Guangdong/1/1996	TPQRERRRKKR/GLFGAIAGFIE
A/Chicken/West-Java/2003	SPQRERRRKKR/GLFGAIAGFIE
A/Chicken/Jembrana/2004	SPQRERRRKKR/GLFGAIAGFIE
A/Layer/MGL/MHW-CS-2/2005	SPQRERRRKKR/GLFGAIAGFIE
A/Chicken/GK/2006	SPQRE\RRKKR/GLFGAIAGFIE
A/Chicken/JK1/2006	SPQRE\RRKKR/GLFGAIAGFIE
A/Chicken/MGL/2007	SPQRE\RRKKR/GLFGAIAGFIE
A/Layer/Solo/MHW-CS-25/2007	SPQRE\RRKKR/GLFGAIAGFIE
A/Layer/Jabar/MHW-CS-02/2008	SPQRE\RRKKR/GLFGAIAGFIE
A/Broiler/SMG/MHW-CS-03/2008	SPQRE\RRKKR/GLFGAIAGFIE
A/Broiler/Klungkung/2009	SPQRE\RRKKR/GLFGAIAGFIE

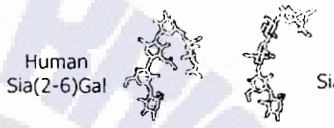
All Indonesian isolates of AIV are still HPAIV

Virus Pandemi Influenza

- 3 pandemy in 20th century :
  - 1918 (H1N1)
  - 1957 (H2N2)
  - 1968 (H3N2)
- None of them belongs to HPAIV based on HA cleavage site
  - Sepertinya phenotipe HPAI tidak dibutuhkan virus influenza untuk menjadi virus pandemi

Does receptor binding specificity of influenza viruses influence transmission of H5N1 viruses in mammals?

- Human/mammals influenza viruses prefer binds to alpha 2,6 linkages
- Avian influenza viruses prefer binds to alpha 2,3 linkages



Presence of α2,3-sialosides-linked virus receptor in tissues

	human	cat	dog	chicken	pigs
Trachea	-	-	+	++	++
Bronchi	+/-	+/-	+	++	++
Alveoli	+	+	+	++	++

Presence of α2,6-sialosides-linked virus receptor in tissues

	human	cat	dog	chicken	pigs
Trachea	++	++	++	-	++
Bronchi	++	++	++	-	++
Alveoli	++	++	++	-	++
Intestine	-	-	-	+	-

Detection of mutation on *receptor binding sites*

Virus isolates	Receptor binding sites													
	91	129	130	131	132	133	134	149	151	182	186	190	191	192
A/Goose/Guangdong/1/1996	Y	S	G	V	S	S	A	W	I	N	E	L	Y	Q
A/Layer/PWT/MHW-RBS-12/2003	Y	S	G	V	S	S	A	W	I	N	E	L	Y	Q
A/Chicken/Jembrana/2004	Y	S	G	V	S	S	A	W	I	N	E	L	Y	Q
A/Chicken/GK/2006	Y	S	G	V	S	S	A	W	I	N	E	L	Y	Q
A/Quail/Solo/MHW-RBS-11/2007	Y	S	G	V	S	S	A	W	I	N	E	L	Y	Q
A/Layer/Jabar/MHW-RBS-02/2008	Y	S	G	V	S	S	A	W	I	N	E	L	Y	Q

Sampai dengan isolat 2008 mutasi tidak melibatkan asam-amino pada receptor binding sites
Perubahan pada receptor binding site berpotensi perubahan spesifitas hospes
(still avian specific)

Roles of NA

- Hydrolyze *galactose ~ N-acetylneuraminic*
  - important for cleaving respiratory mucus
  - important for releasing virus from infected cell
  - → balance activity of HA & NA → efficiency of infection
- NA of AIV of chicken isolates can not cleave 4-O-acetyl SA (human respiratory mucus) → inhibitor analog receptor → AIV of chicken isolates not readily infect human
- genetic reassortment AIV of chicken and human isolates →potential threat

Roles of NS

- Confront *host innate immune respons* (IFNα / β & TNF-α) *antagonist IFN α / β activity*
- ✓AIV – H5N1/97 which infect chicken & human Hong Kong : Protein NS with 92(Glu) → resistant to IFNα / β & TNF-α ; protein NS with 92(Asp) → sensitive to IFNα / β & TNF-α (Seo *et al*, 2002)
- ✓AIV of chicken isolates Indonesia : NS with 92(Glu)→ risks ?

Roles of PB2

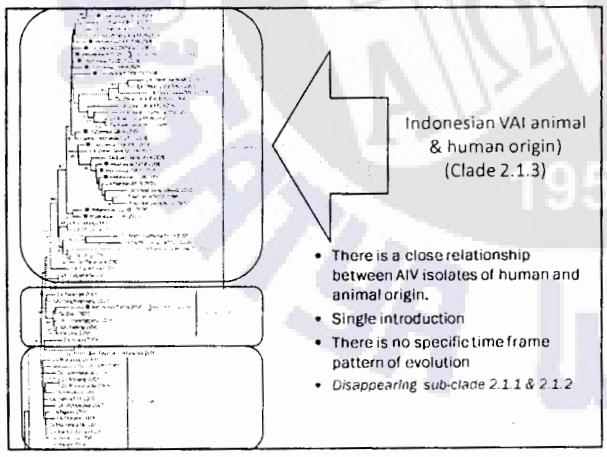
- The appearance of the PB2 E667K mutation has provided the virus with the ability to replicate efficiently at human body temperatures
- Some of AIV of Indonesian human isolates shows this character → pay attention to

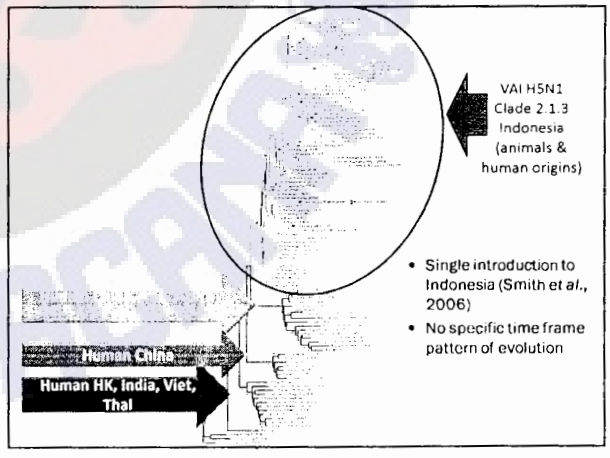
Influenza virus in birds

- All subtypes can be found in wild aquatic birds
- Potential as reservoir for influenza viruses in domestic birds & mammals
- There are different in pathogenicity (HPAIV & LPAIV)
- HPAIV mostly restricted to H5 & H7
- Recent outbreak in poultry H5N1
- Some of this virus have infected human (bird flu), no human to human transmission → this virus still avian specific

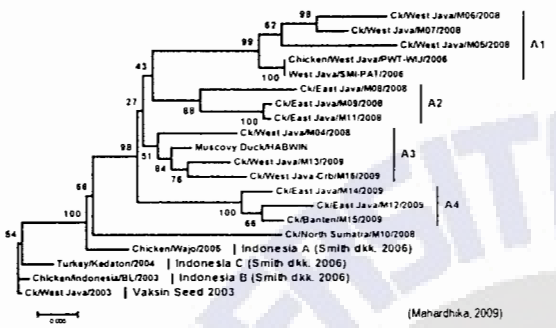
Avian influenza in Indonesia

- Sub type : H_5N_1
- First identified in 2003
- Chickens, quails and ducks affected
- Both commercial and village poultry
- Human cases since July 2005
- Incidence varies across the country
- Consider as a zoonotic disease.
- *Has Indonesian AIV mutated far ?*
- *Pandemic potentials ?*





No specific geographical pattern of evolution

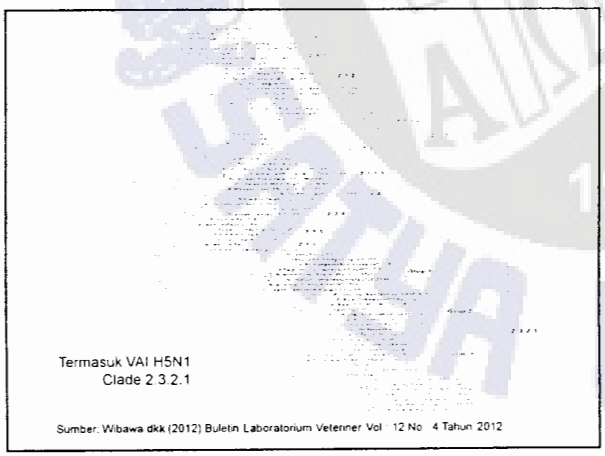


(Mahardhika, 2009)

Penyebab Wabah AI (pada itik)2012 / 2013

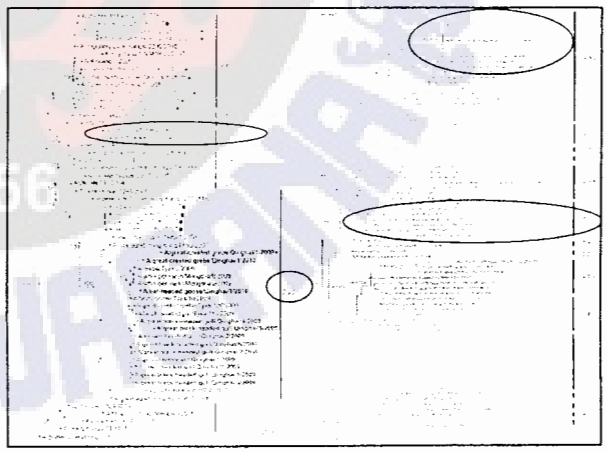
- Disebabkan oleh VAI H5N1
- *Analisis molekuler termasuk HPAIV clade 2.3.2*
- *Bukan hasil mutasi dari VAI penyebab wabah 2003 ~*

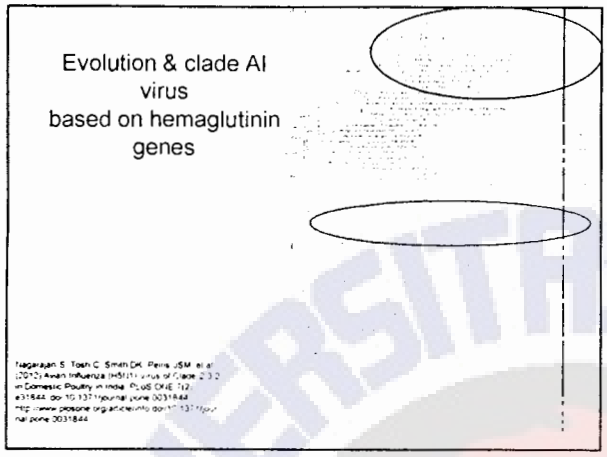
- Dari mana asal virus?

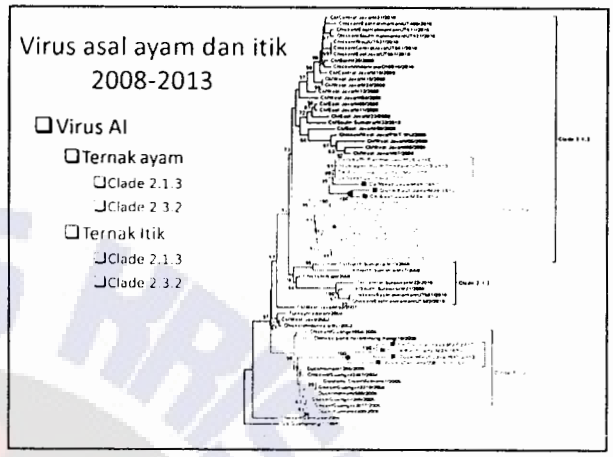


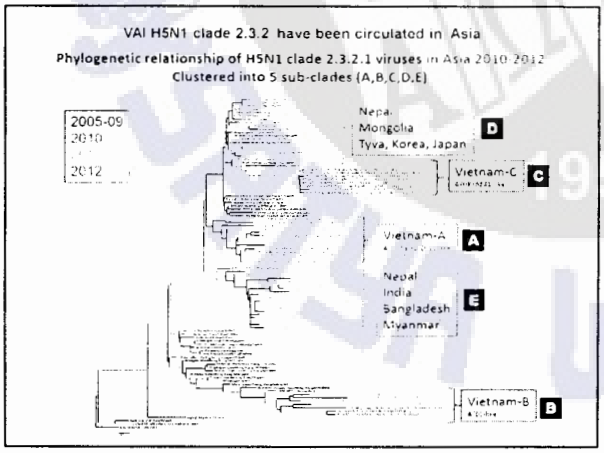
Termasuk VAI H5N1
Clade 2.3.2.1

Sumber: Wibawa dkk (2012) Buletin Laboratorium Veteriner Vol. 12 No. 4 Tahun 2012



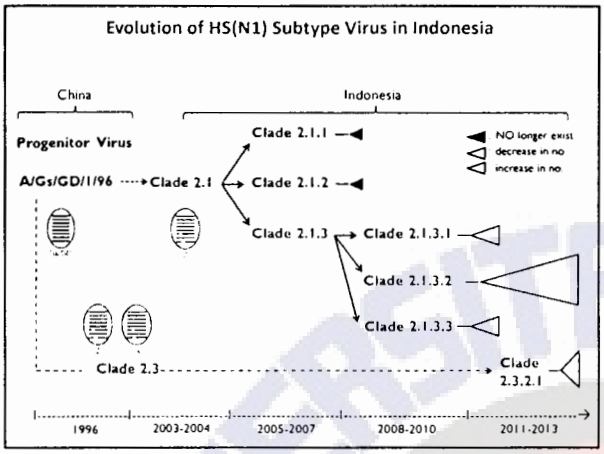


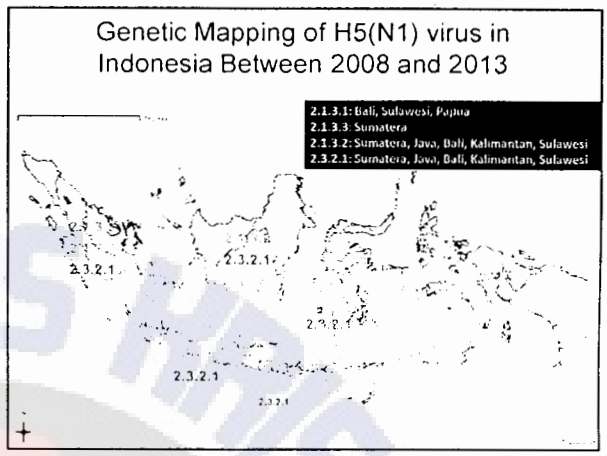




New Clade of VAI 2013

- Clade 2.3.2 mempunyai ciri molekuler virus influenza unggas
- Clade 2.3.2 mempunyai struktur antigenik yang agak berbeda dengan clade 2.1.3
- Bukan merupakan hasil mutasi VAI clade 2.1.3
- Merupakan pemasukan baru virus AI Clade 2.3.2., mungkin dari Asia daratan





Virus Influenza pada Orang & Babi

- Yang sering endemik pada populasi (orang)
  - Umumnya: H_1N_1, H_1N_2, H_3N_2, virus yg secara klasik endemik pada populasi (orang) agak berbeda dengan dengan yang ada di babi.
  - Kadang-kadang : H_2N_8, H_3N_8
- Yang endemik di babi:
  - Umumnya H_1N_1; beberapa H_1N_2 , H_3N_2
  - Asal dan karakter SIV ini dapat berbeda pada benua yang berbeda, misal Amerika, Eropa-Asia,

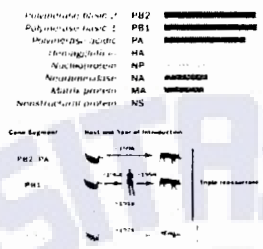
Flu Outbreak 2009

- Wabah influenza yang disebabkan oleh virus Influenza A (H_1N_1)
- Virus tsb pertama kali dideteksi pada orang pada bulan April 2009 di Meksiko, Amerika Serikat dan Canada
- Strain Virus yang diisolasi dari Meksiko dan AS identik
- Virus ini menyebar dari orang ke orang
- Virus influenza A (H_1N_1) baru / 2009 ini akhirnya menjadi strain pandemi

Influenza pandemic 2009

- Merupakan virus influenza A (H_1N_1) baru, yang sebelumnya belum pernah ditemukan pada babi atau orang
- Mengandung unsur genetik virus influenza yang secara normal bersirkulasi pada babi di Eropa & Asia, avian virus genes & human virus genes. (H_1 similar to H_1N_1classical swine influenza; N_1 from avian; PB_1 from H_3N_2 human; PB_2 from avian; NP from H_1N_1 classical swine; M from avian; NS from H_1N_1 classical swine).
- Scientists call this a "quadruple reassortant" virus

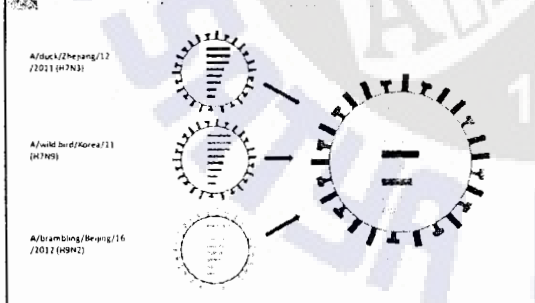
Novel H1N1



Bird flu virus H7N9 China

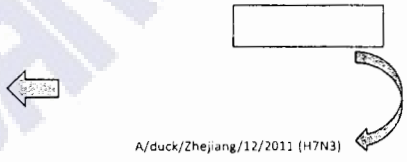
- A novel reassortant avian-origin influenza A (H7N9) virus isoloted from respiratory tract of 3 patiens and identified as influenza virus A (H7N9).
- Sequence analysis revealed that the genes of those 3 virus are avian origin, all six internal genes from avian influenza virus A (H9N2).
- Some Detected Mutation :
  - Substitution Q226L (H3 numbering) pada 210-loop pada gen hemagglutinin (HA) ditemukan pada virus A/Anhui/1/2013 dan A/Shanghai/2/2013, tetapi tidak pada virus A/Shanghai/1/2013
  - Mutasi T160A diidentifikasi pada 150-loop dalam gen HA
  - Delesi lima asam amino dalam neuraminidase (NA) stalk region

Hipothesis the origin of the genes of novel virus H7N9



Source : Kageyama et al., 2013

Pohon Filogenetika gen HA novel H7N9



Source : Kageyama et al., 2013

Pohon filogenetika
gen NA virus H7N9

A/wild bird/Korea/11
(H7N9)

Source : Kageyama et al., 2013

Pohon filogenetika gen
PB1 virus H7N9

A/Jiangsu/Beijing/16/2011 (H9N2)

Source : Kageyama et al., 2013

Pohon filogenetika
gen PB2 virus H7N9

Source : Kageyama et al., 2013


Thank You

LEMBAR PERTANYAAN
SEMINAR NASIONAL MIKROBIOLOGI FB-UKSW
SALATIGA, 24 JUNI 2014

Nama : Jacqueline H. S. L
Ditujukan kepada : Prof. drh. Widya Asmara

Pertanyaan :

1. Ada wacana bahwa apabila ibu yang sedang hamil mengalami cacar air, janin dalam kandungannya akan mendapat kekebalan terhadap cacar air, apakah benar ?

LEMBAR PERTANYAAN
SEMINAR NASIONAL MIKROBIOLOGI FB-UKSW
SALATIGA, 24 JUNI 2014

Nama : Richard d. Anggada
Ditujukan kepada : Prof. drh. Widya Asmara

Pertanyaan :

1. Apa manfaat bagi manusia mempelajari mutasi virus ? kan virus gampang bermutasi jadi akan sulit untuk mendapat manfaat mempelajari virus karena sulit membuat vaksin yang ditujukan untuk mencegah penyakit karena virus

LEMBAR PERTANYAAN
SEMINAR NASIONAL MIKROBIOLOGI FB-UKSW
SALATIGA, 24 JUNI 2014

Nama : Catherine Tiara (cath.kath@gmail.com)
Ditujukan kepada : Prof. drh. Widya Asmara, SU, Ph.D

Pertanyaan :

1. Bagaimana penggolongan clade dilakukan ? Apa yang membuat calde 2.1 dapat tersub-cladekan menjadi clade 2.1.1. maupun calde 2.1.2. dst.

2. Bagaimana identifikasi clade melalui sequencing dilakukan ? termasuk metode sequencingnya ?

3. Latar belakang: saya sangat tertarik dengan materi Bapak, sekaligus keingintahuan saya karena topic seperti ini masih awam bagi sebagian besar mahasiswa biologi

LEMBAR PERTANYAAN
SEMINAR NASIONAL MIKROBIOLOGI FB-UKSW
SALATIGA, 24 JUNI 2014

Nama : Rizky Dewi Darma Kusuma (ikydarmakusuma@gmail.com)
Ditujukan kepada : Prof. drh. Widya Asmara, SU, Ph.D

Pertanyaan :

1. Virus yang menyerang tumbuhan apakah bisa menyerang manusia/hewan ? seperti yang diketahui belum ada kasus seperti itu, apakah itu disebabkan oleh virus yang berbeda atau lingkungan yang tidak mendukung bagi virus untuk menulari manusia atau hewan ?