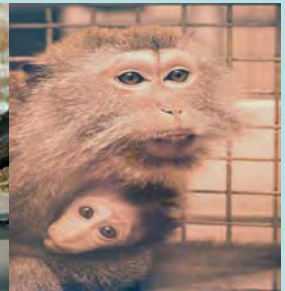
Albert Osterhaus
Department of Virology

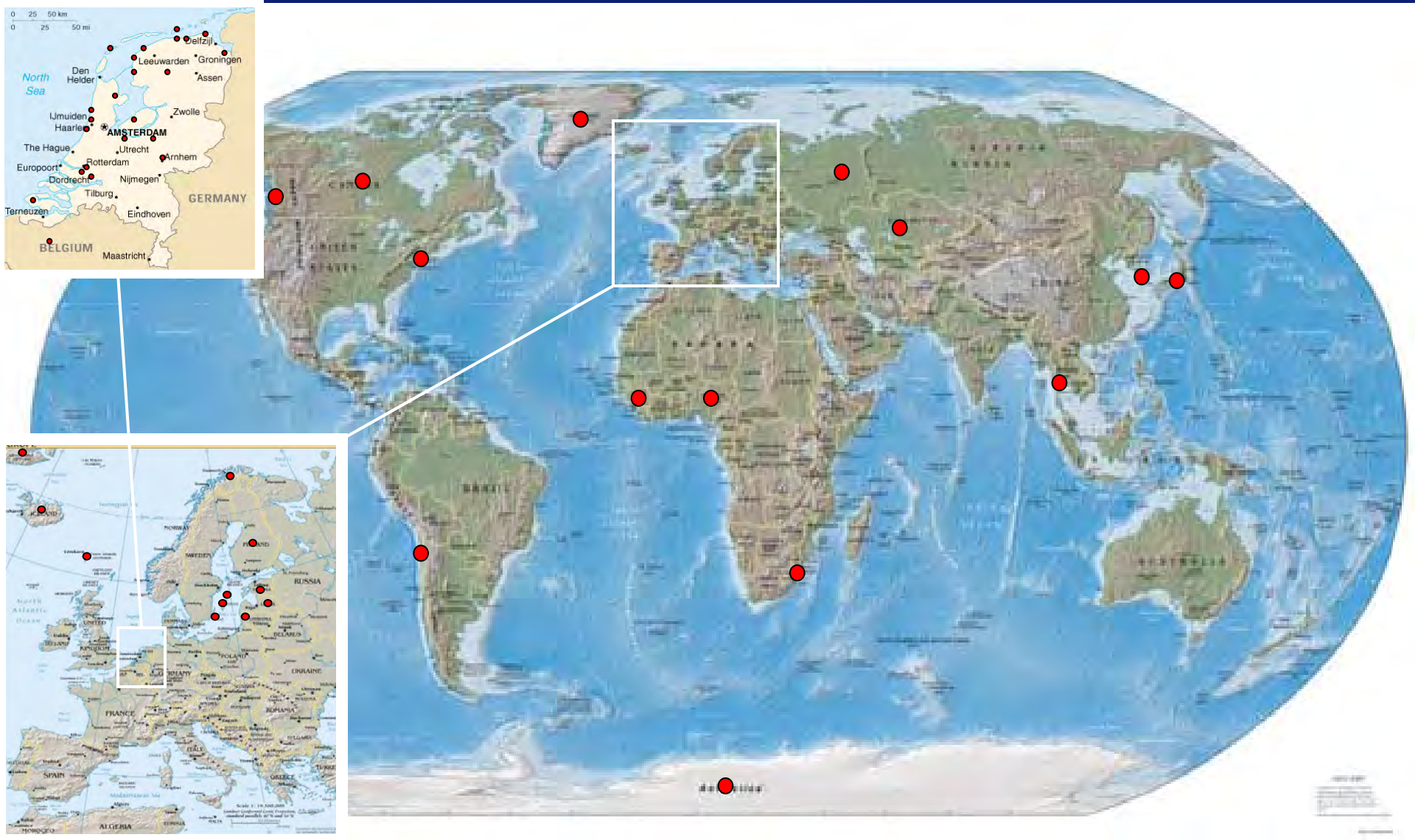
Interspecies and intraspecies transmission and re-assortment: the H7 influenza viruses



One Flu Strategic Retreat
Castel Brando, 02-01-2011

Avian influenza A virus surveillance

- Sampling sites 1998-present -



Avian influenza A virus surveillance
- Bird species, 1998-2005 -

Munster et al., 2005

Order	Family		# Species	# Samples
Anseriformes	*Anatidae*	Goose	8	6904
		Duck	17	13771
		Swan	3	226
Charadriiformes	*Charadriidae*	Plover, Lapwing	7	292
	Scolopacidae	Sandpiper, Dunlin, Snipe	31	2616
	Laridae	Gull, Tern	11	4099
	Alcidae	Guillemot	4	907
	Others	Others	7	103
Pelicaniformes	*Phalacrocoracidae*	Cormorants	2	899
Gruiformes	*Rallidae*	Rail, Coot	4	1029
10 others	*53 others*		221	5323
14 orders	*63 families*		315 species	36169 samples

Avian influenza A virus surveillance
- Positive bird species, 1998-2005 -

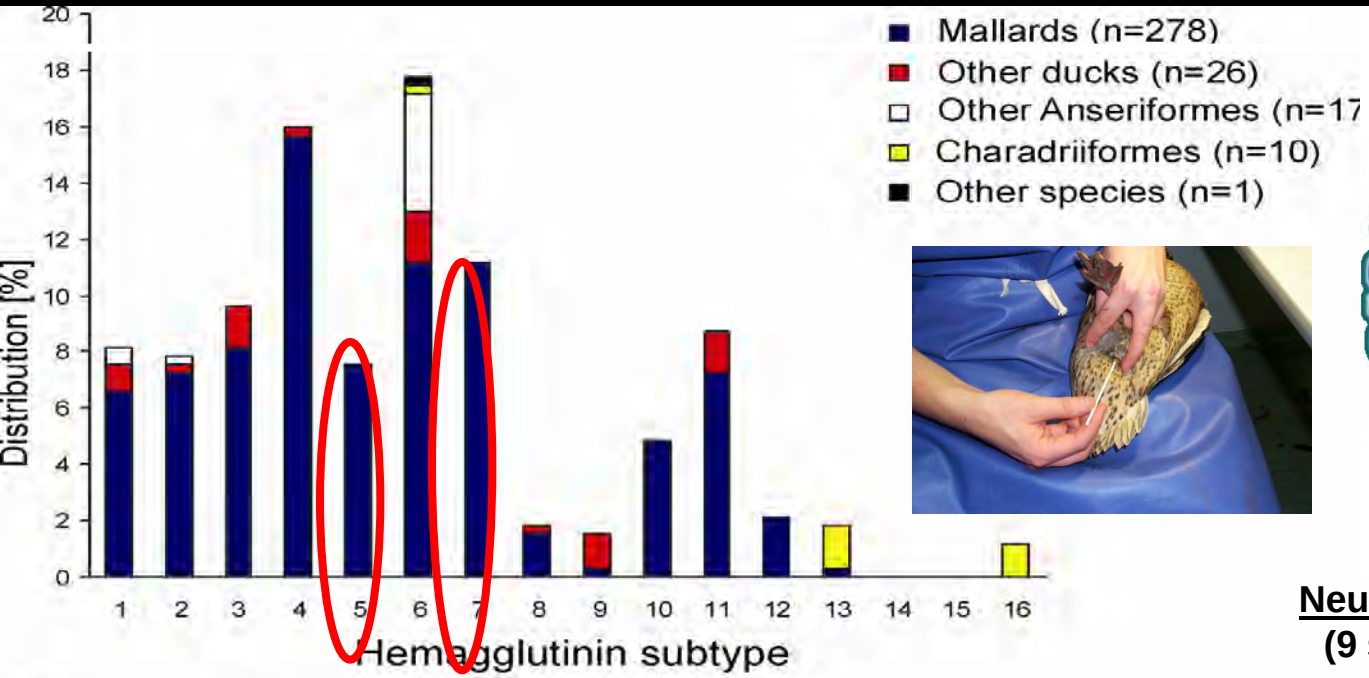
Order	Family	Species	Sampled	Positive	%
Anseriformes	Ducks	Mallard (Anas platyrhynchos)	8938	649	7.3
		Eurasian Wigeon (Anas penelope)	2538	76	3.0
		Common Teal (Anas crecca)	940	60	6.4
		Northern Pintail (Anas acuta)	448	13	2.9
		Gadwall (anas strepera)	298	8	2.7
		Northern Shoveler (Anas clypeata)	135	5	3.7
		Common Shelduck (Tadorna tadorna)	335	2	0.6
		Tufted Duck (Aythya fuligula)	62	2	3.2
		Common Eider (Somateria mollissima)	37	2	5.4
	Geese	White-fronted Goose (Anser albifrons)	3821	82	2.1
		Barnacle Goose (Branta leucopsis)	1139	8	0.7
		Greylag Goose (Anser anser)	455	11	2.4
		Brent Goose (Branta bernicla)	413	4	1.0
		Bean Goose (Anser fabalis)	315	2	0.6
		Pink-footed Goose (Anser brachyrhynchus)	285	6	2.1
	Swans	Tundra Swan (Cygnus colombianus)	153	3	2.0
		Mute Swan (Cygnus olor)	47	1	2.1
Charadriiformes	Gulls	Black-headed Gull (Larus ridibundus)	1583	14	0.9
		Common Gull (Larus canus)	226	1	0.4
		Herring Gull (Larus argentatus)	753	5	0.7
		Greater Black-backed Gull (Larus marinus)	28	2	7.1
	Waders	Red Knot (Calidris canutus)	229	1	0.4
		Red-necked Stint ()	5	1	20
Alcidae	Auks	Guillemot (Uria aalge)	817	3	0.4
Rallidae	Rails	Common Coot (Fulica atra)	237	1	0.4
Muscicapidae	Flycatchers	Pied Flycatcher (Ficedula hypoleuca)	19	1	5.3

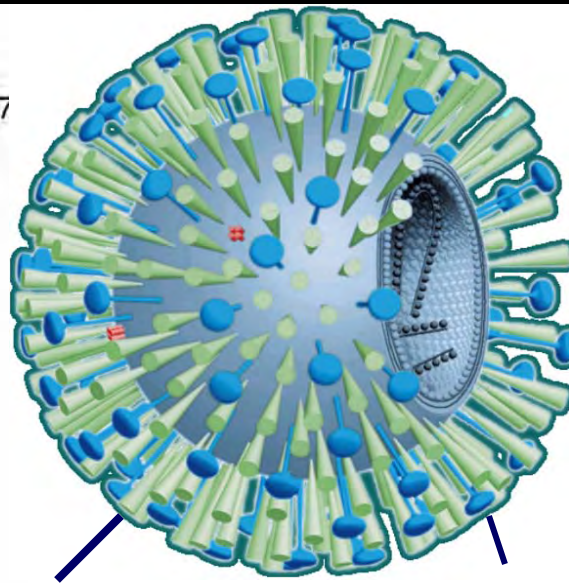
Munster et al., 2005

Influenza (LPAI/HPAI) surveillance in wild birds

- Hemagglutinin and neuraminidase subtypes -

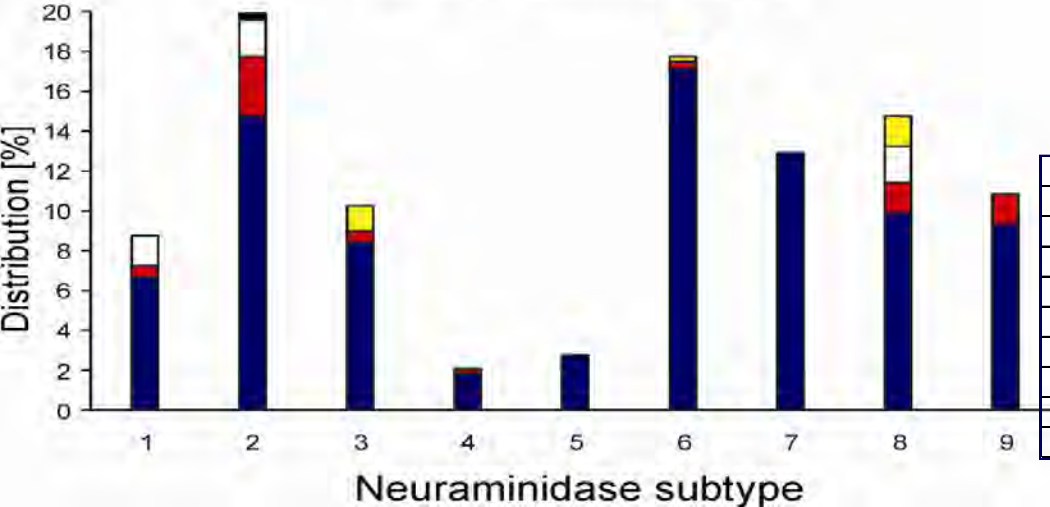
Erasmus MC
University Medical Center Rotterdam





Neuraminidase (9 subtypes)

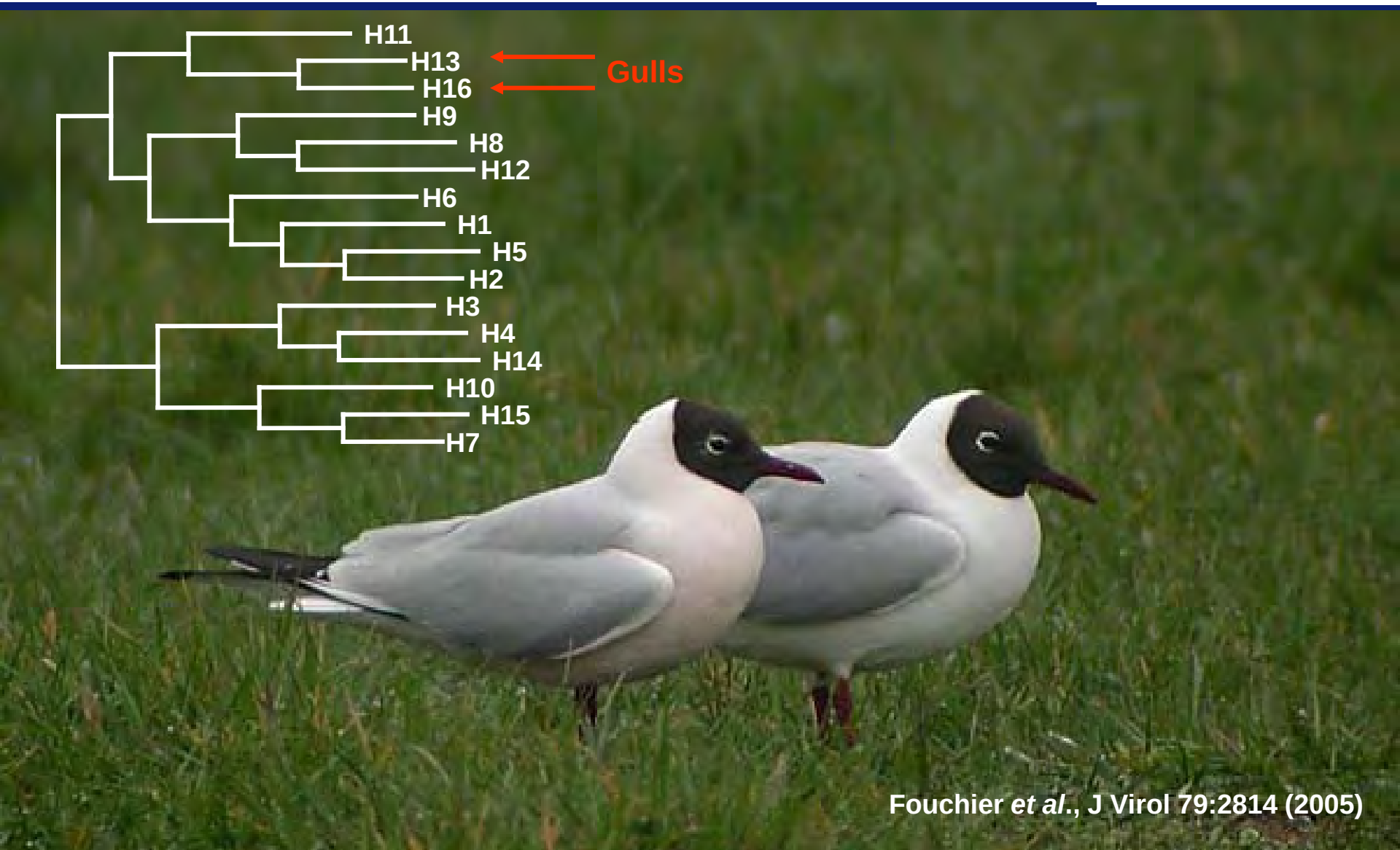
Haemagglutinin (16 subtypes)



	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16
N1	X	X				X					X					
N2	X	X	X	X	X	X			X	X	X					
N3		X	X	X	X		X				X					X
N4								X		X						
N5		X	X			X				X		X				
N6	X		X	X	X	X				X	X		X			
N7	X	X					X			X	X					
N8	X		X			X				X	X	X	X			
N9		X		X	X		X			X	X					

Identification of a novel HA subtype (H16)

- Black-headed Gull (*Larus ridibundus*) -



Fouchier *et al.*, J Virol 79:2814 (2005)

Avian influenza A virus surveillance

- HA and NA subtypes -

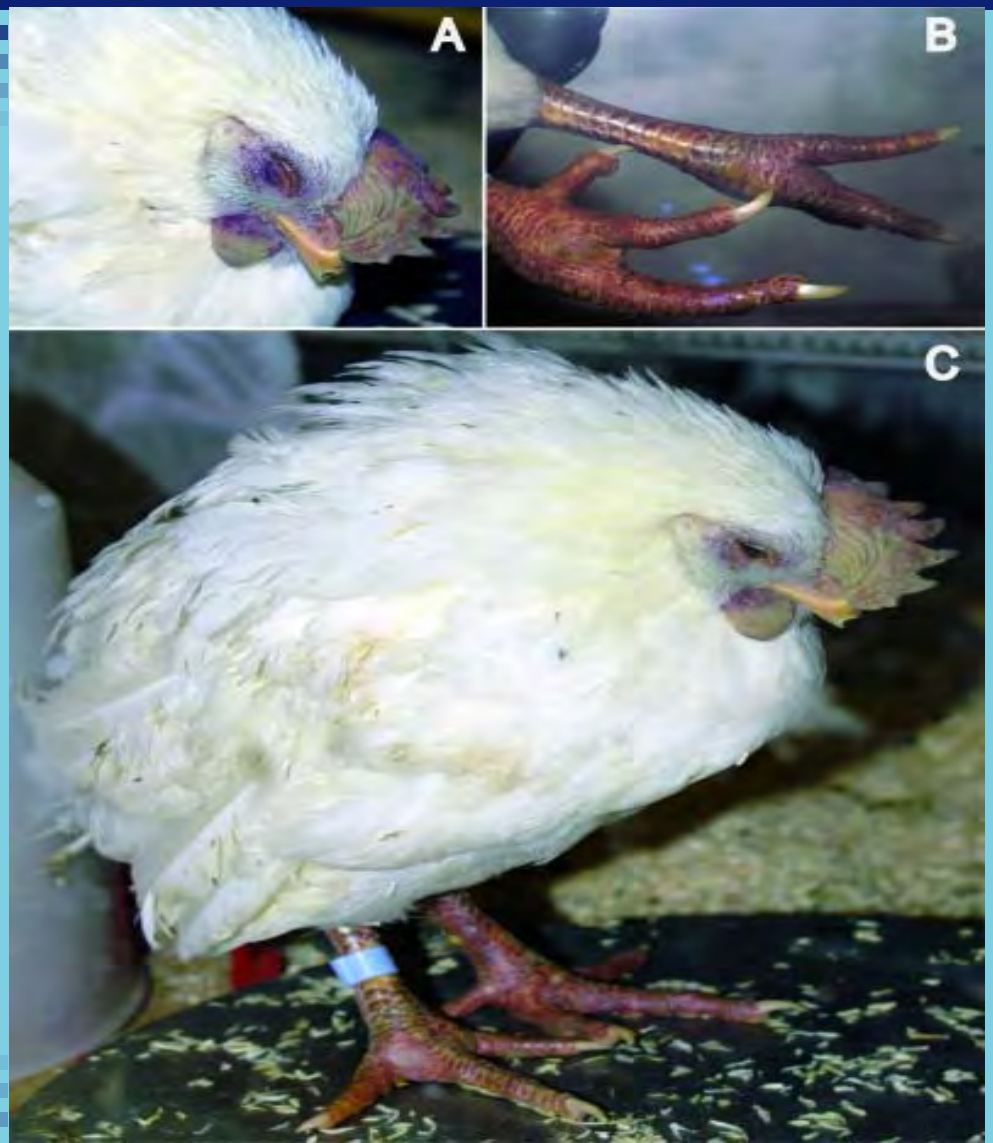
Fouchier *et al.*, J Virol., 2005
Munster *et al.*, Emerg Inf Dis., 2005
Olsen *et al.*, Science., 2006

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16
N1	X	X				X					X					
N2	X	X	X	X	X	X			X	X	X					
N3		X	X	X	X		X				X					X
N4								X		X						
N5		X	X			X				X		X				
N6	X		X	X	X	X				X	X		X			
N7	X	X					X			X	X					
N8	X		X			X				X	X	X	X			
N9		X		X	X		X			X	X					

Subtypes detected in wild birds globally

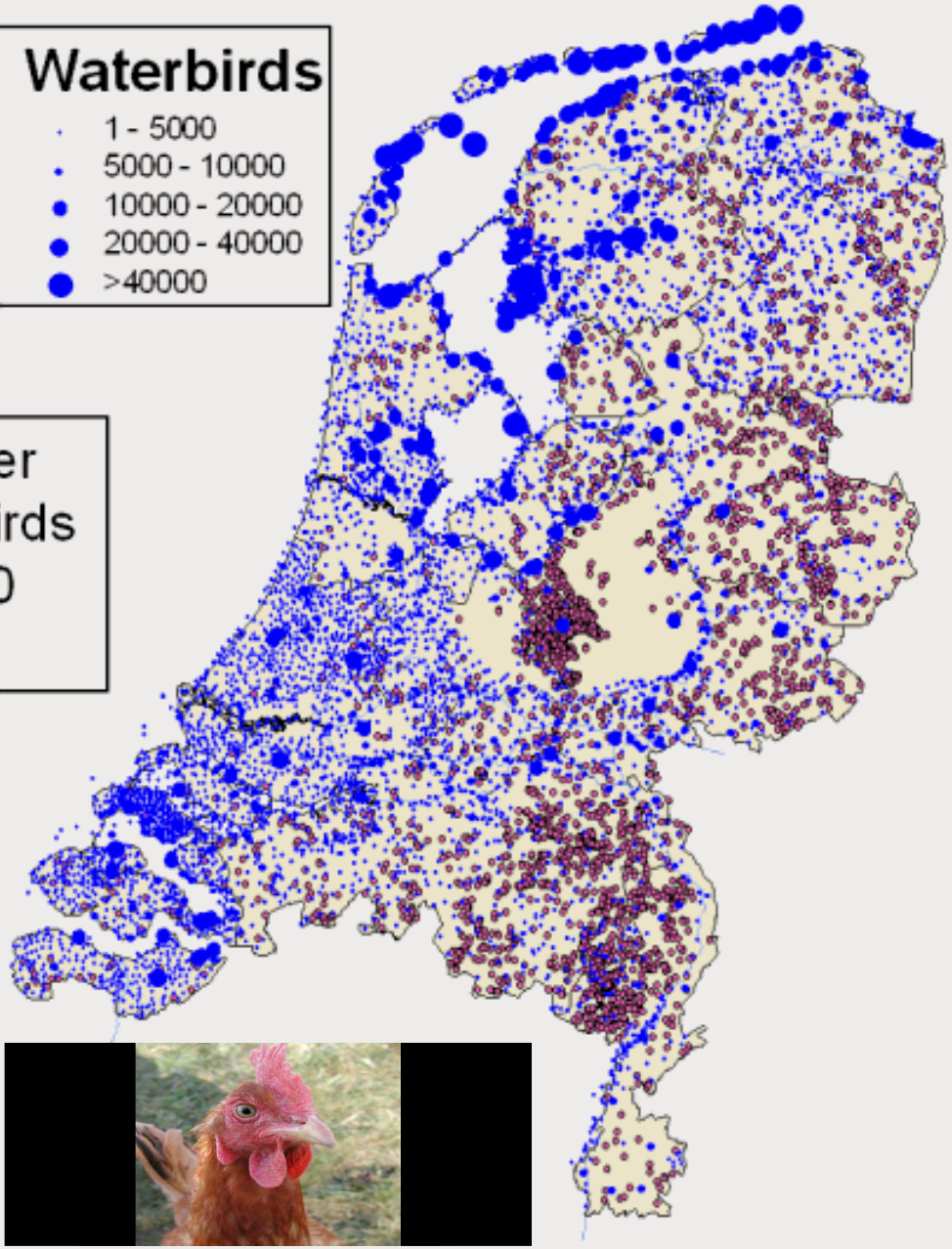
X Subtypes detected in wild birds in our own studies (Northern Europe)

Insertion of a multibasic cleavage motif into the HA of a low-pathogenic avian influenza H6N1 virus induces a highly pathogenic phenotype.



Munster et al., J.Virol 2010

Birds in the Netherlands



Total number wild waterbirds *c.* 5,000,000

Source: Sovon

Domestic birds

Each red dot represent a location of various kinds of bird farming

Total number domestic birds *c.* 90,000,000 of which *c.* 5,000,000 roam outside

Source: PVE

WETLANDS INTERNATIONAL

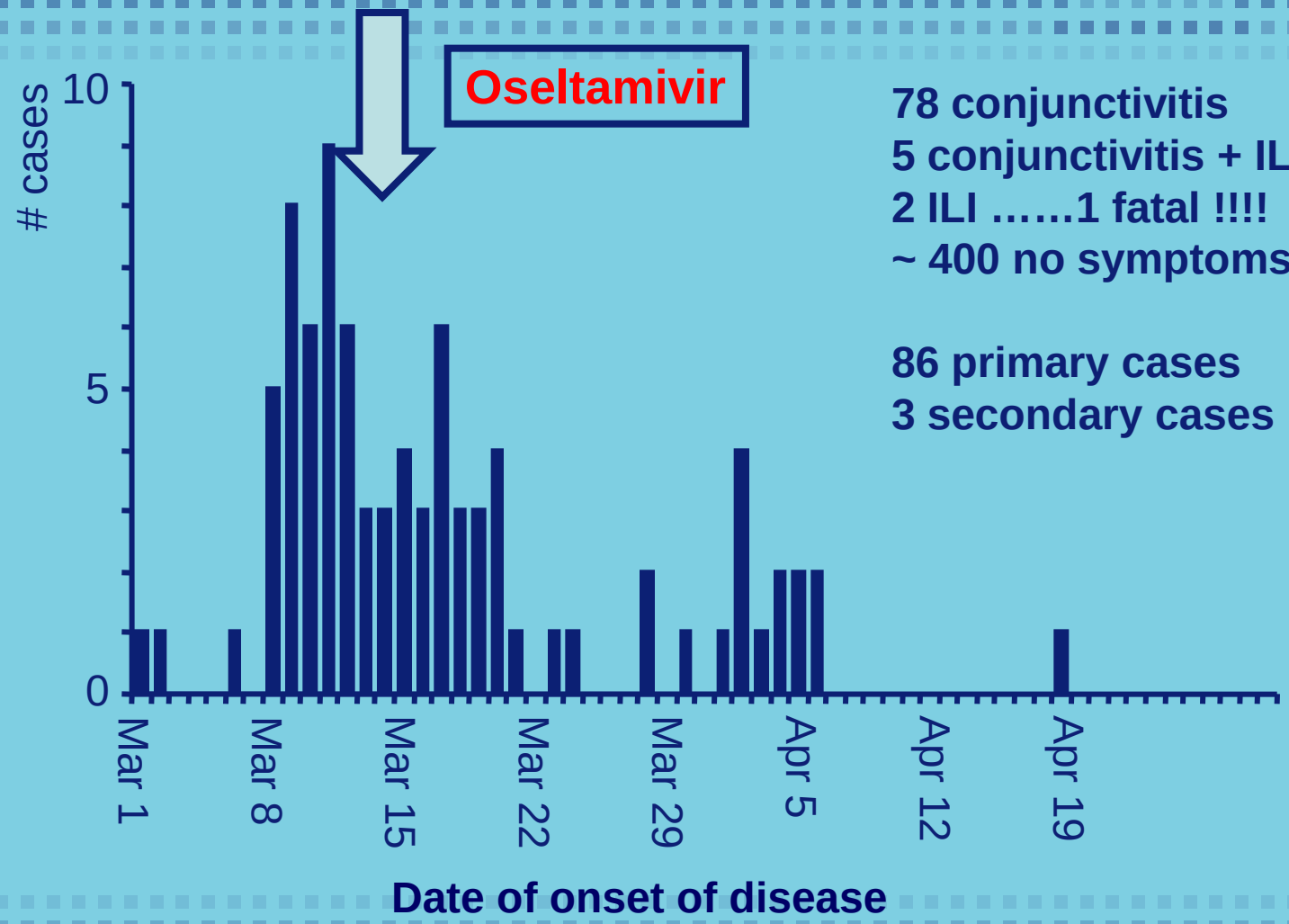
Sovon

Fowl plague (H7N7), NL 2003

H7N7 in The Netherlands 2003

- Human cases -

R.Fouchier *et al.*, PNAS 2004
M.Koopmans et al., Lancet 2004

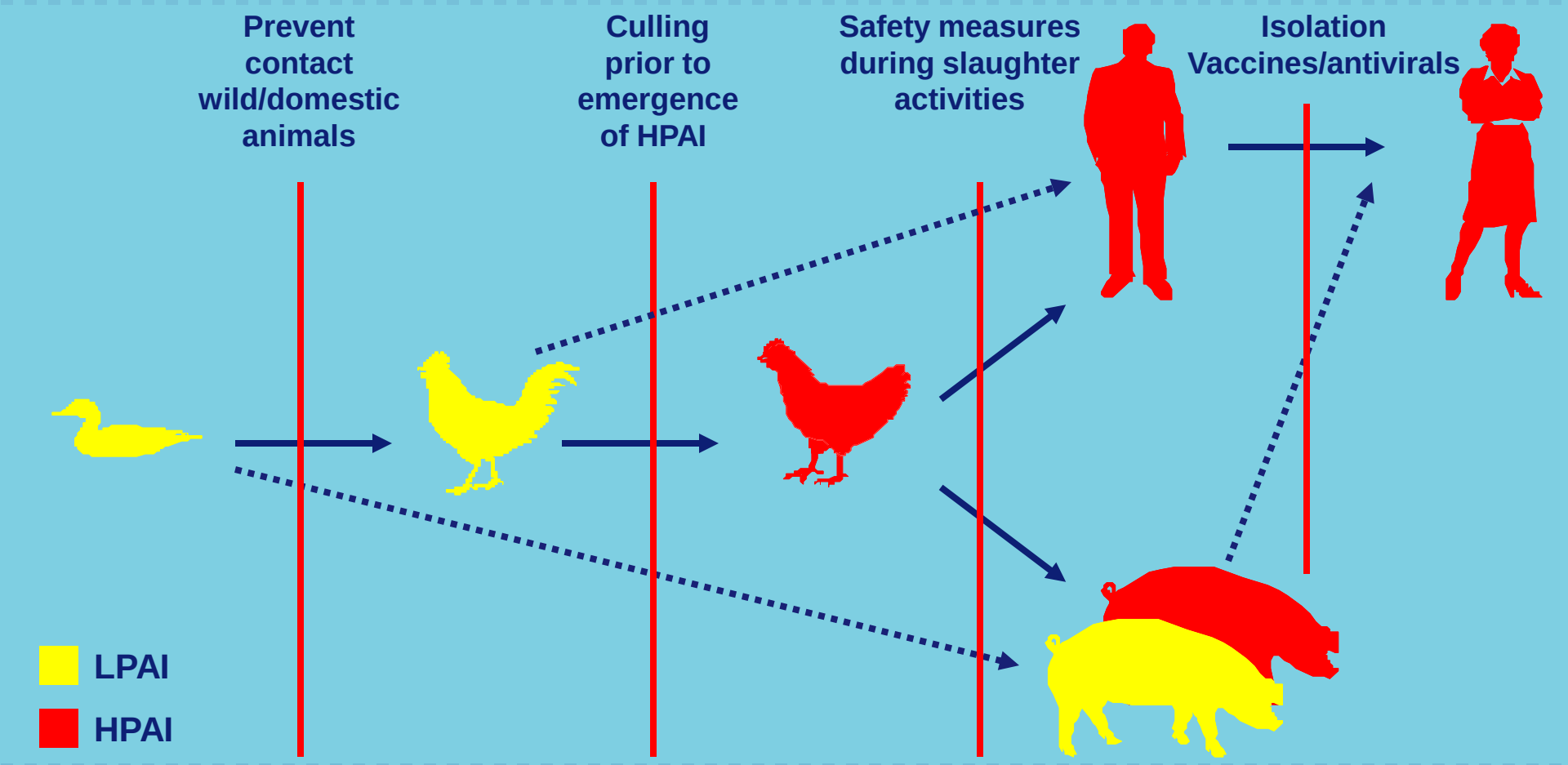


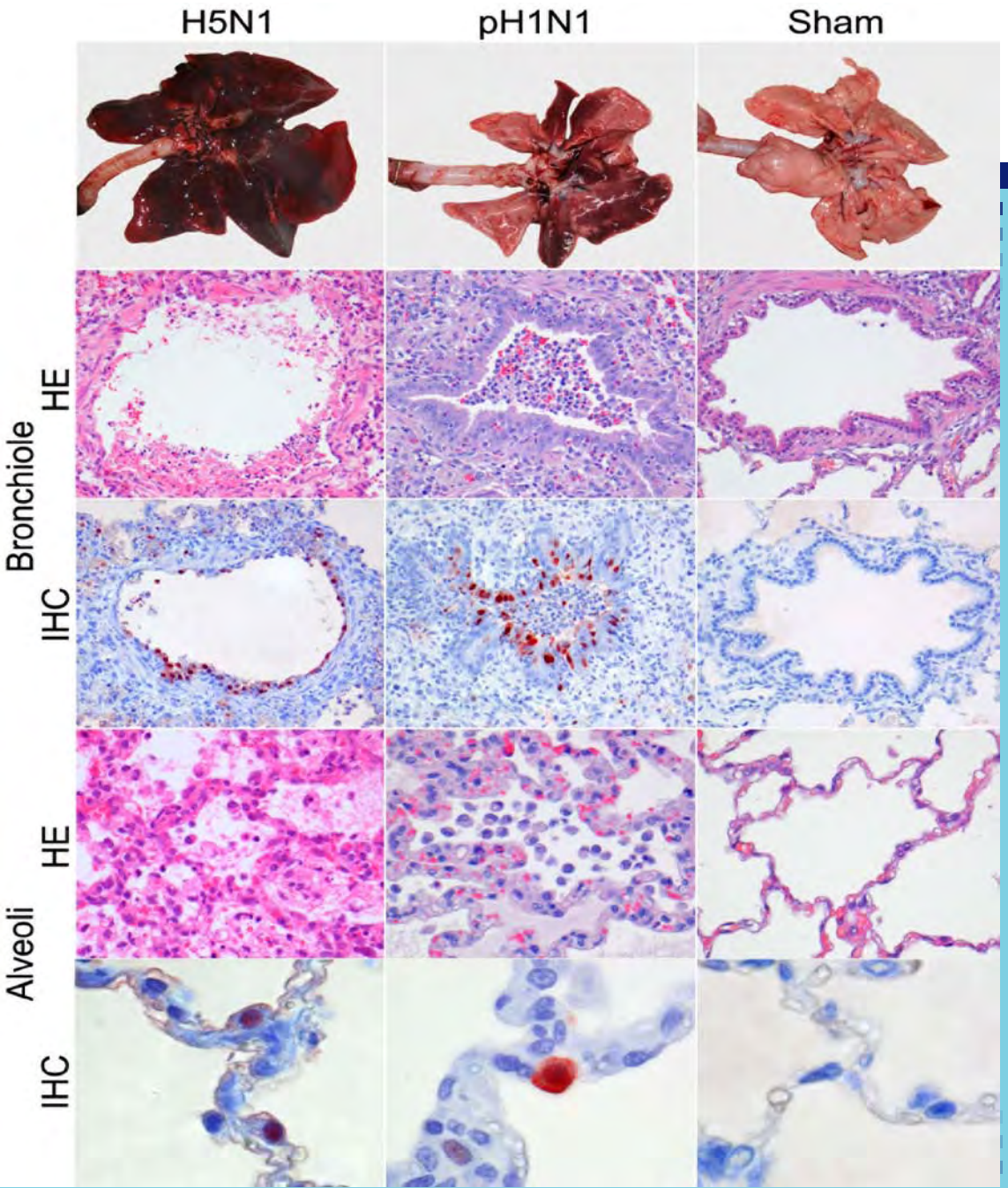
78 conjunctivitis
5 conjunctivitis + ILI
2 ILI ……1 fatal !!!!
~ 400 no symptoms

86 primary cases
3 secondary cases

Avian influenza H7N7 infections in The Netherlands 2003

- Chain of transmission and intervention strategies -





Cats experimentally infected with HPAI-H5N1 virus; pandemic H1N1 virus

Kuiken *et al*.,2008

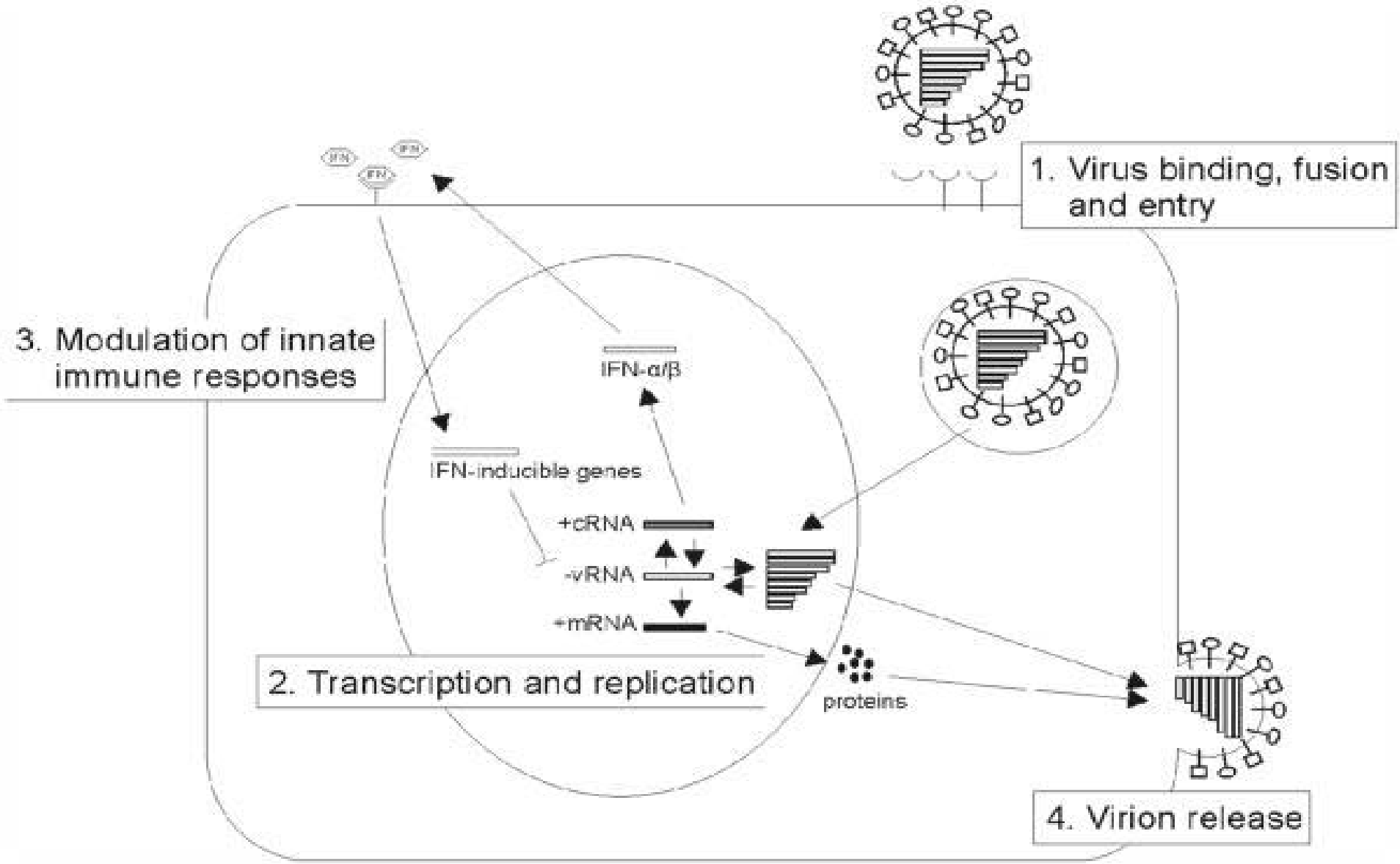
v.d.Brand *et al*., EID 2010

v.d. Brand et al., Am. J. Pathol. 2010

HPAI-H7N7 virus
isolated from a fatal human case causes
respiratory disease in cats but does not spread systemically.

J.Clin Virol., de Wit et al., 2009

Overview of the current knowledge of determinants of pathogenicity in mammals



Influenza A virus:
major determinants of pathogenicity for mammals

Spanish flu (H1) virus:

- **PB2 E627K (Taubenberger *et al*., 2005)**
- **HA (Kobassa *et al*., 2004)**
- **NA (Tumpey *et al*., 2005)** ***replication in absence trypsine***
- **NS1 (Geiss *et al*., 2002)** ***IFN pathway (not in mice lacking Mx1 gene)***

H7 viruses (LPAI/HPAI)

- **PB2 E627K (Munster et al., 2007; Shinya *et al*., 2007)**
- **HA (Munster et al., 2007)** ***attachment pattern***

H5 viruses (HPAI)

- **PB2 E627K (Hatta *et al*., 1997)**
- **PB2 D701N (Li *et al*., 2005)** ***duck virus in mice***
- **HA (van Riel *et al*., 2006; Shinya *et al* 2006)** ***attachment pattern***
- **NS1 (Seo *et al*., 2002)**

H7N7 in The Netherlands

- Sequence analysis of full-length genomes -

Fouchier et al., PNAS 2004
De Wit et al., 2009

Virus	# substitutions in			
Gene	A/Netherlands/33/03		A/Netherlands/219/03	
Segment	Nucleotide	Amino acid	Nucleotide	Amino acid
1 (PB2)	2	0	8	5 (S79I,V297I,R355K,Q563R,E627K)
2 (PB1)	0	0	2	0
3 (PA)	0	0	5	1(F666L)
4 (HA)	0	0	4	3(I13S,A143T,K416R)
5 (NP)	0	0	1	0
6 (NA)	0	0	5	4(N308S, A346V, T442A, P458S)
7 (MA)	0	0	0	0
8 (NS)	1	1 (K126R)[2]	1	1(V137I)[2]

1. Sequences were compared to those of A/Chicken/Netherlands/1/03
2. Both mutations in NS1

Molecular Determinants of Adaptation of HPAI-H7N7 Viruses to Efficient Replication in the Human Host

PubMed Central, : J Virol. 2010 February; 84(3): 1597–1606. Published online 2009 November 25. doi: 10.1128/JVI.01783-09.

De Wit et al ., J.Virol 2010

TABLE 1.

Amino acid differences in HPAI H7N7 viruses isolated in the Netherlands in 2003

Virus	Amino acid at the indicated position in[a]:														
	PB2					PA	HA			NA				NS	
	79	297	355	563	627	666	13	143	416	308	346	442	458	126	137
A/Ck/NL/1/03	S	V	R	Q	E	F	I	A	K	N	A	T	P	K	V
A/NL/33/03 (CC)	S	V	R	Q	E	F	I	A	K	N	A	T	P	R	V
A/NL/219/03 (FC)	I	I	K	R	**K**	L	S	T	**R**	S	V	A	S	K	I
A/Ck/NL/03010132/03	I	I	K	R	E	L	S	T	K	S	V	A	S	K	I

[a]Amino acid positions are relative to methionine in the open reading frame. Unique amino acid substitutions in the FC virus are indicated in bold.

From: J Virol. 2010 February; 84(3): 1597–1606.
Published online 2009 November 25. doi: 10.1128/JVI.01783-09.

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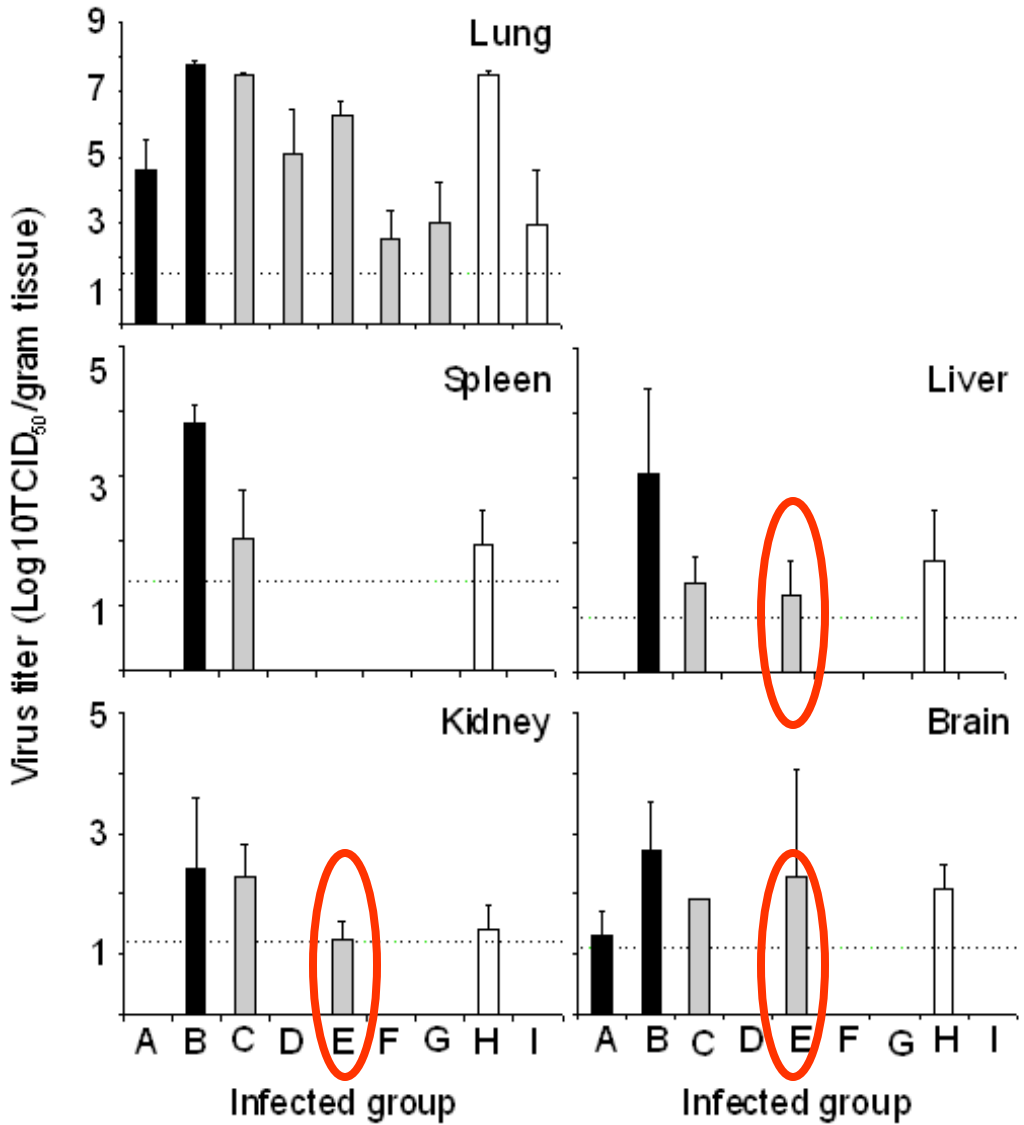
Human influenza A H7N7 virus pathogenicity

- Systemic replication in mice: PB2 and HA -

de Wit, et al. 2008



Black: wildtype viruses

- **A: Conjunctivitis case (CC)**
- **B: Fatal case (FC)**

Gray: re-assortants

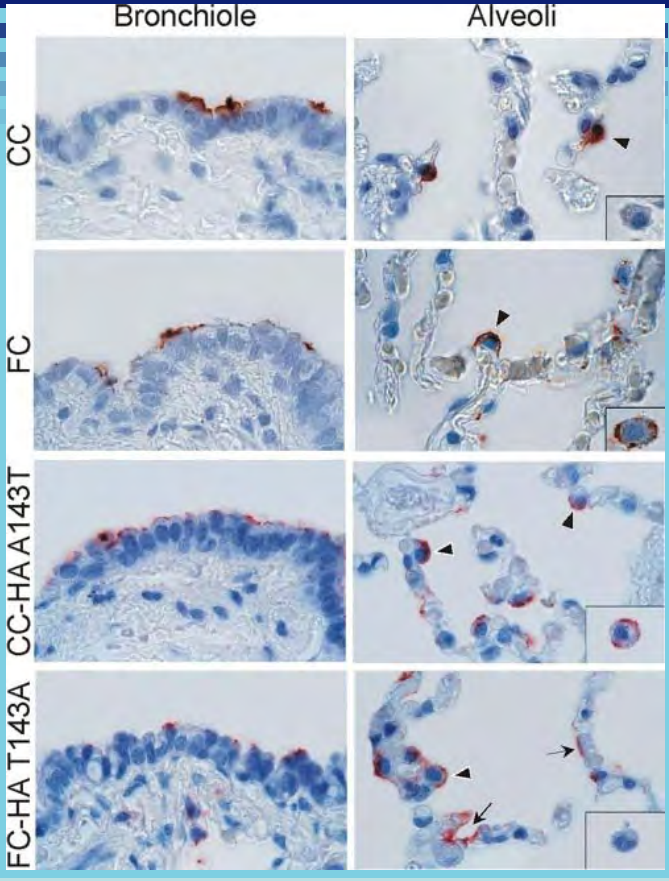
- **C: CC+PB2 of FC**
- **D: CC+PA of FC**
- **E: CC+HA of FC**
- **F: CC+NA of FC**
- **G: CC+NS of FC**

White; point mutants

- **H: CC + PB2 E627K**
- **I: FC + PB2 K627E**

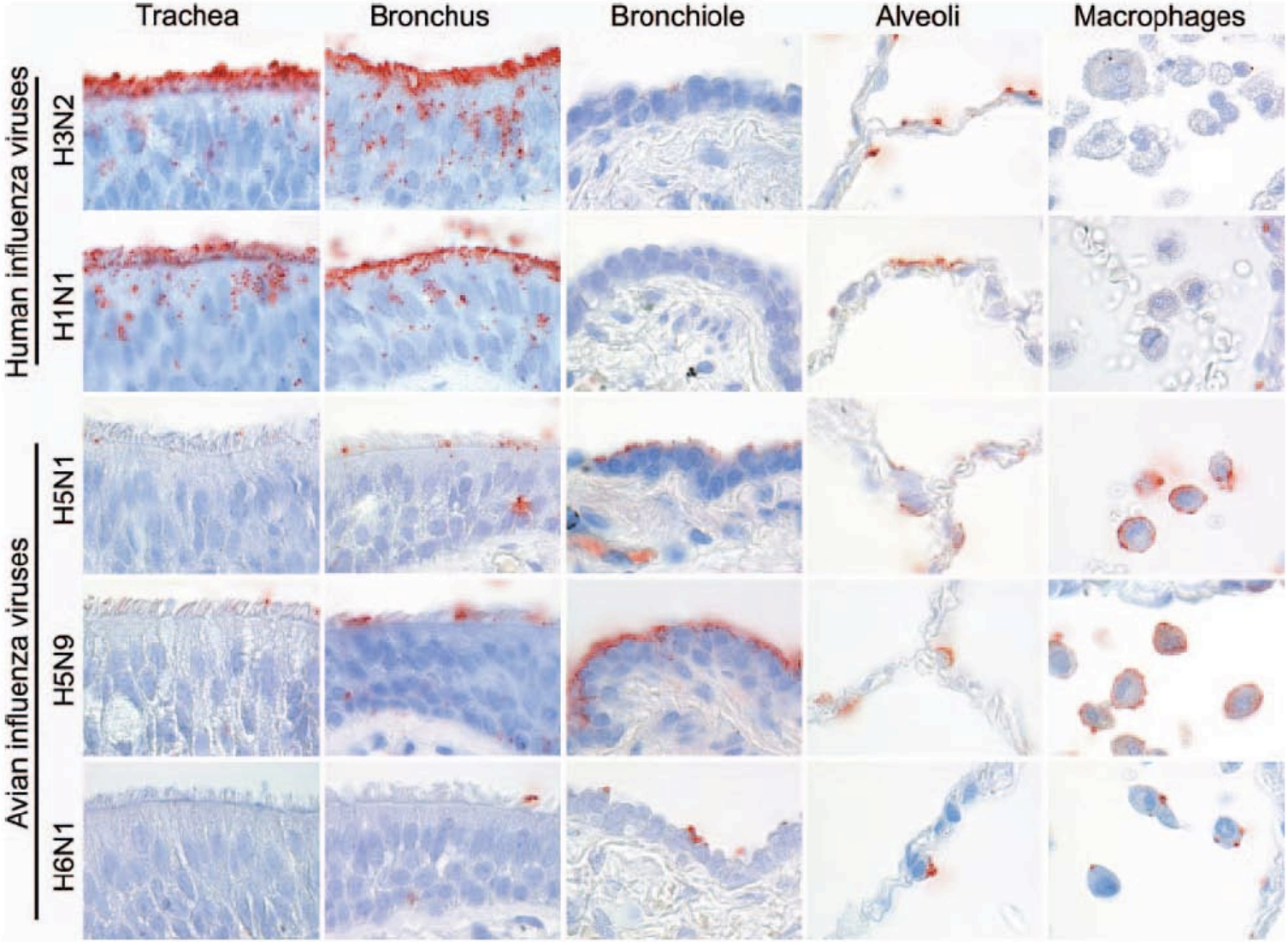
Molecular Determinants of Adaptation of Highly Pathogenic Avian Influenza H7N7 Viruses to Efficient Replication in the Human Host

- De Wit et al ., J.Virol 2010



Patterns of attachment of the CC-HA143T and FC-HA143A viruses to tissues of the human lower respiratory tract. Attachment to bronchioles (left panels) and alveoli (right panels) is shown.
For comparison, attachment of the CC and FC viruses to these tissues is displayed,
Virus attachment is shown in red. The insets in the right panels show the pattern of virus attachment to alveolar macrophages.

Avian influenza A H5N1 virus
- HA: Receptor specificity -



Shinya et al., Nature 440, 2006

Van Riel et al., Science 2006

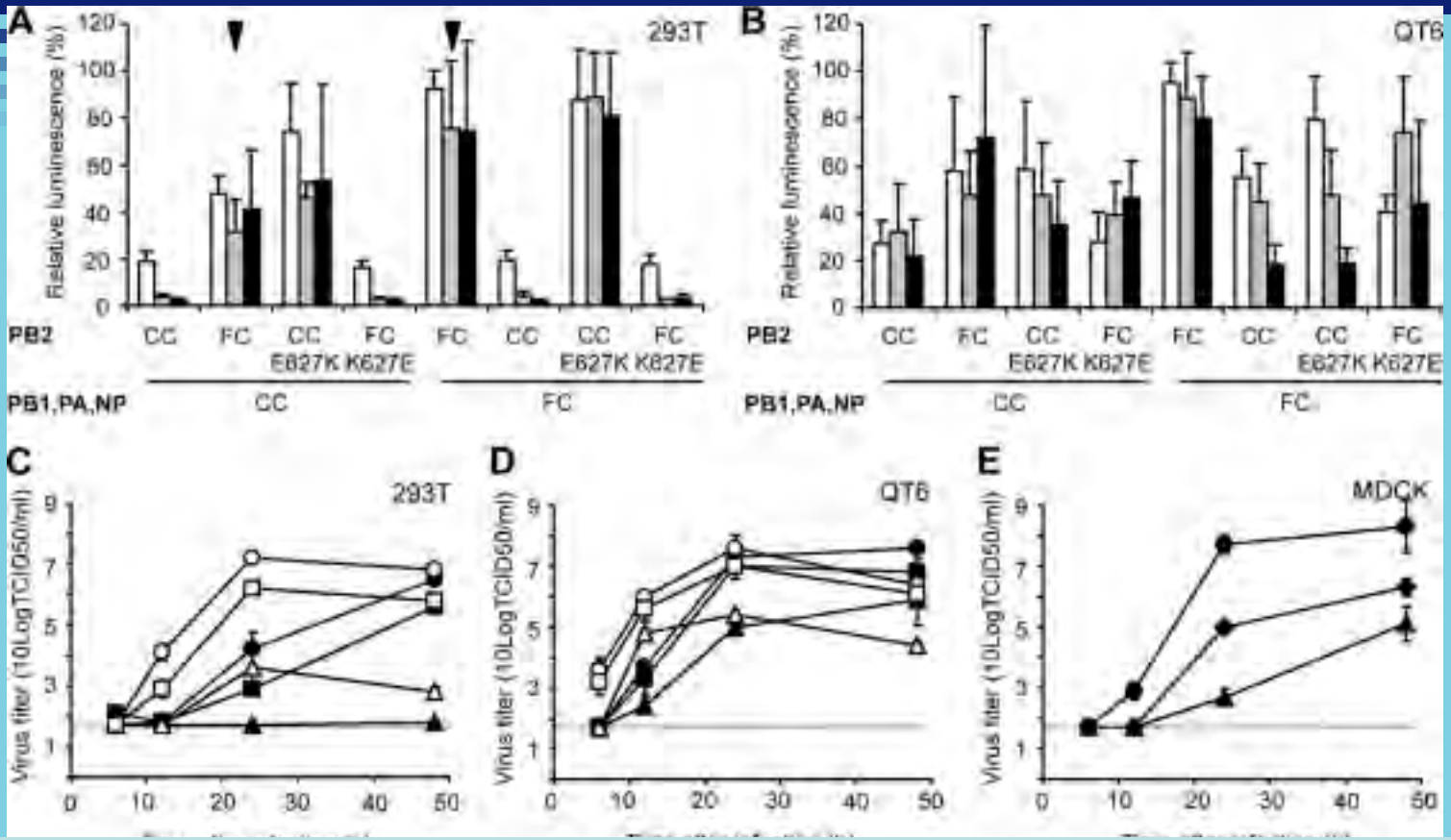
Van Riel et al., Am J Pathol 2007

Van Riel et al., Am J Pathol 2009

Van Riel et al., Am J Pathol. ***in press***

Molecular Determinants of Adaptation of Highly Pathogenic Avian Influenza H7N7 Viruses to Efficient Replication in the Human Host

De Wit et al ., J.Virol 2010



- Polymerase activities and replication kinetics of H7N7 viruses. (A and B)
- Minigenome assays were performed by transfecting 293T (A) or QT6 (B) cells with plasmids encoding the polymerase proteins and NP of the CC and FC viruses or mutants thereof.

Influenza A H7N7 virus
- Determinants of infectivity in human cells -

Virus Gene Segment	# amino acid substitutions in A/Netherlands/219/03 (FC)	Phenotype
1 (PB2)	5 (S79I,V297I,R355K,Q563R,E627K)	Replication
2 (PB1)	0	
3 (PA)	1(F666L)	Replication
4 (HA)	3(I13S,A143T,K416R)	Receptor binding
5 (NP)	0	
6 (NA)	4(N308S, A346V, T442A, P458S)	Particle release
7 (MA)	0	
8 (NS)	1(V137I)	

Sequence as compared to A/Chicken/Netherlands/1/03

Influenza virus adaptation to mammals
- Conclusions & discussion -

- Studies on zoonotic and pandemic influenza start revealing determinants of pathogenicity:

  - HA/NA; host cell receptors

  - Polymerase genes; body temp & cellular factors (PB2 E627K)

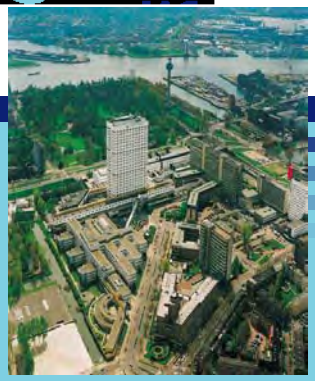
  - NS; innate immunity

- No final clues to determinants of transmissibiliy
  Animal models available: experiments ongoing

-Mutations and/or reassortment key in host adaptation

pathogenesis versus transmissibility

Influenza research at Erasmus MC
- The team leaders -



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Prof. Dr. T. Kuiken	Pathology
Prof. Dr. M. Koopmans	Public Health
Prof. Dr. C.A.B. Boucher	Antiviral research
Dr.A.van der Eijk / Dr.P.Fraaij	Clinical research
Prof.Dr. G.F. Rimmelzwaan	Immunology
Dr. M. Schutten	Diagnostics
Dr. A. Andeweg	Genomics

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