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Avian Influenza H5N1

Influenza A H5N1 Replication Sites in Humans

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- Despite severe and generalized clinical manifestations, the result of multiple organ dysfunction, previous limited autopsy data failed to show evidence of viral replication
- This study investigated a case of fatal H5N1 disease in a child for tissue tropism caused by the virus in the lungs and other organs

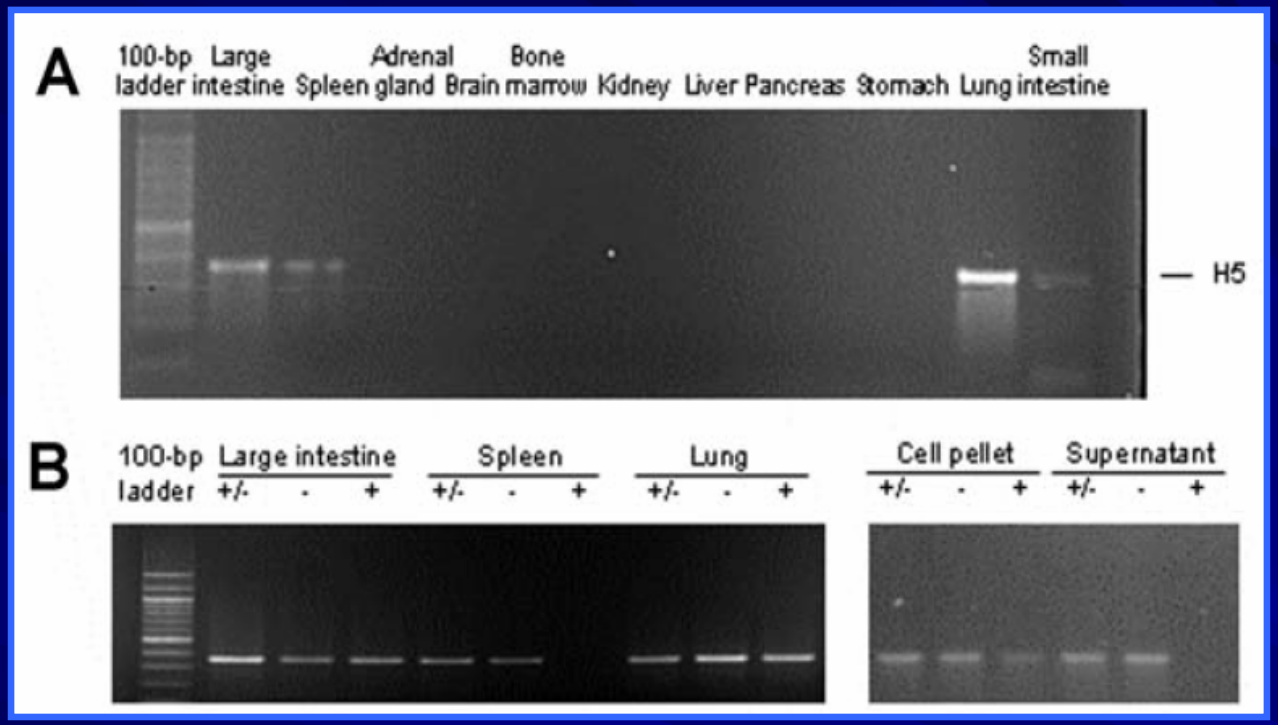


Figure H5 influenza viral RNA detection in selected tissue

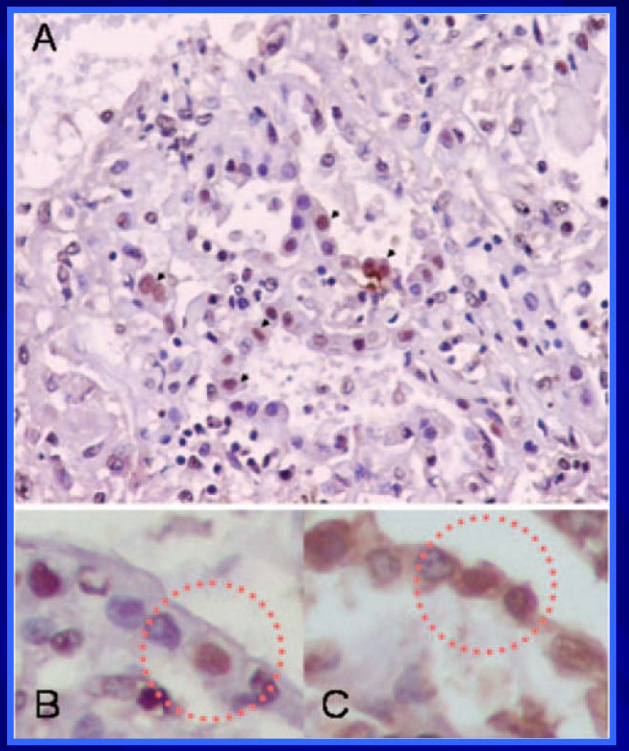
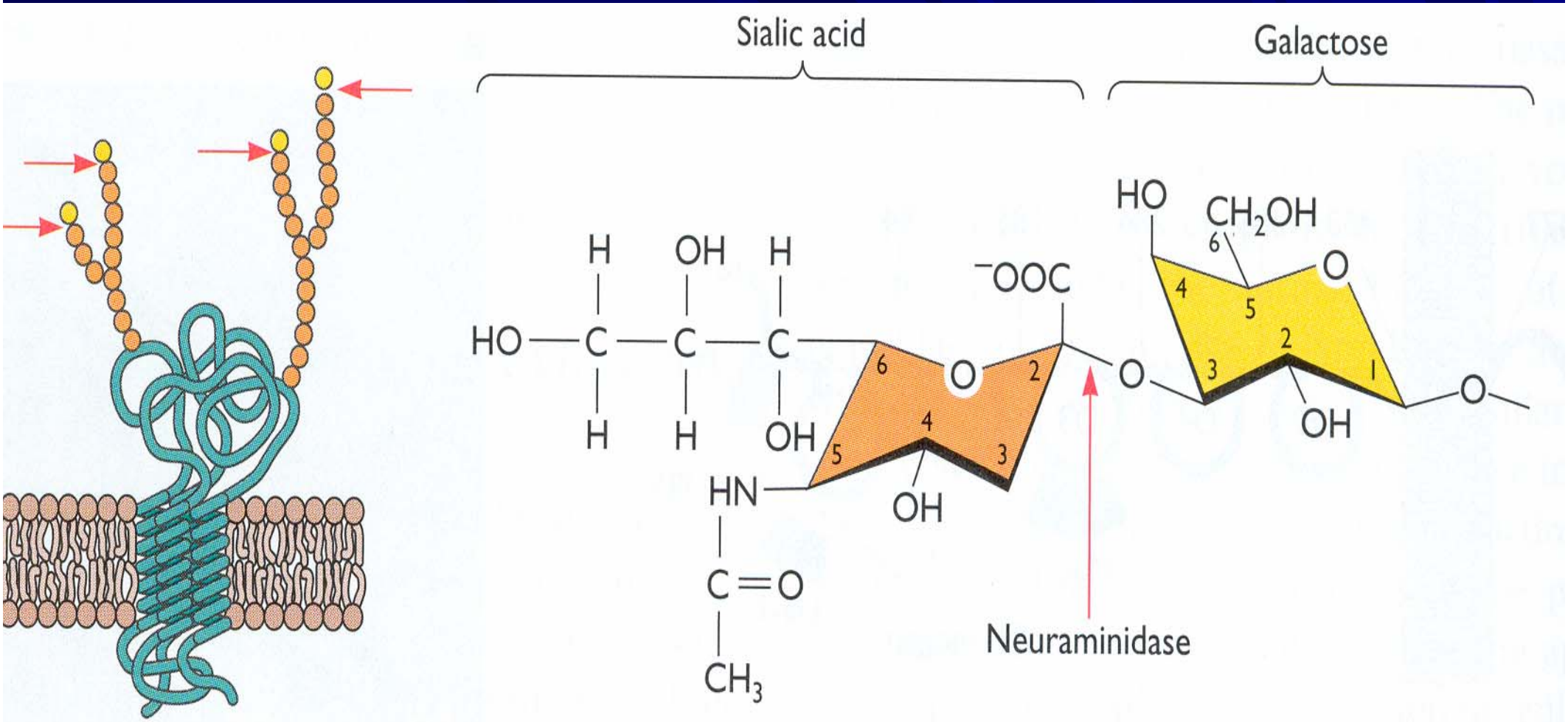


Figure Immunohistochemistry in lung tissue

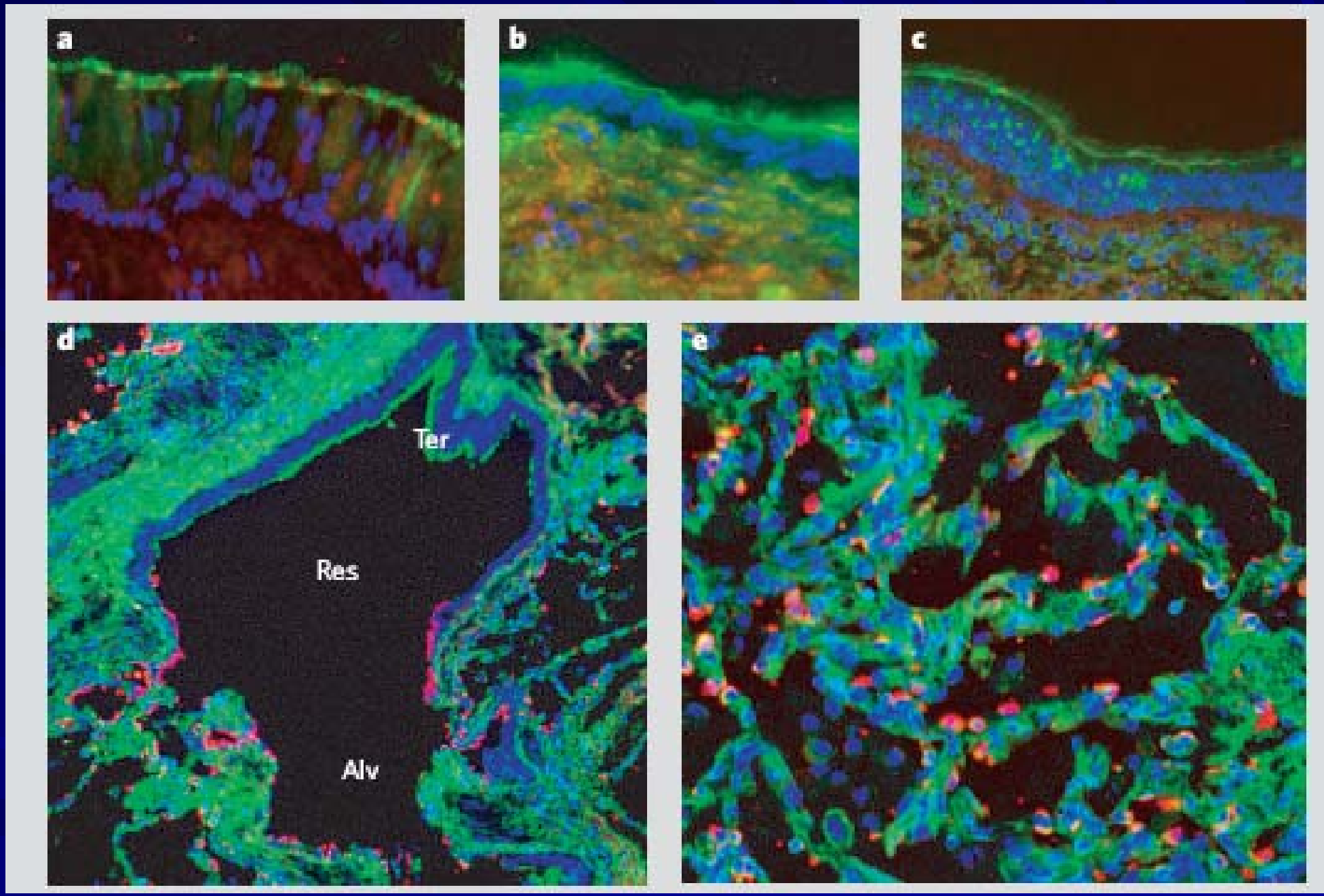
- H5-specific RNA was detected in the lung, spleen, and small and large intestines
- (+) and (-) stranded viral RNA was found in the lung, small and large intestines, but only (-) stranded RNA was detected in the spleen
- Immunohistochemical analysis detected influenza A virus antigen-positive cells in lung tissue.
- Antigen-positive cells were type II pneumocytes

Uiprasertkul, M. *et al. Emerg. Infect. Dis.* 2005



Human influenza strains bind preferentially to α-(2, 6)-linked sialic acid, whereas avian strains prefer α-(2, 3)- linked sialic acid

Flint et al, 2004



Shinya, 2006

In situ hybridization

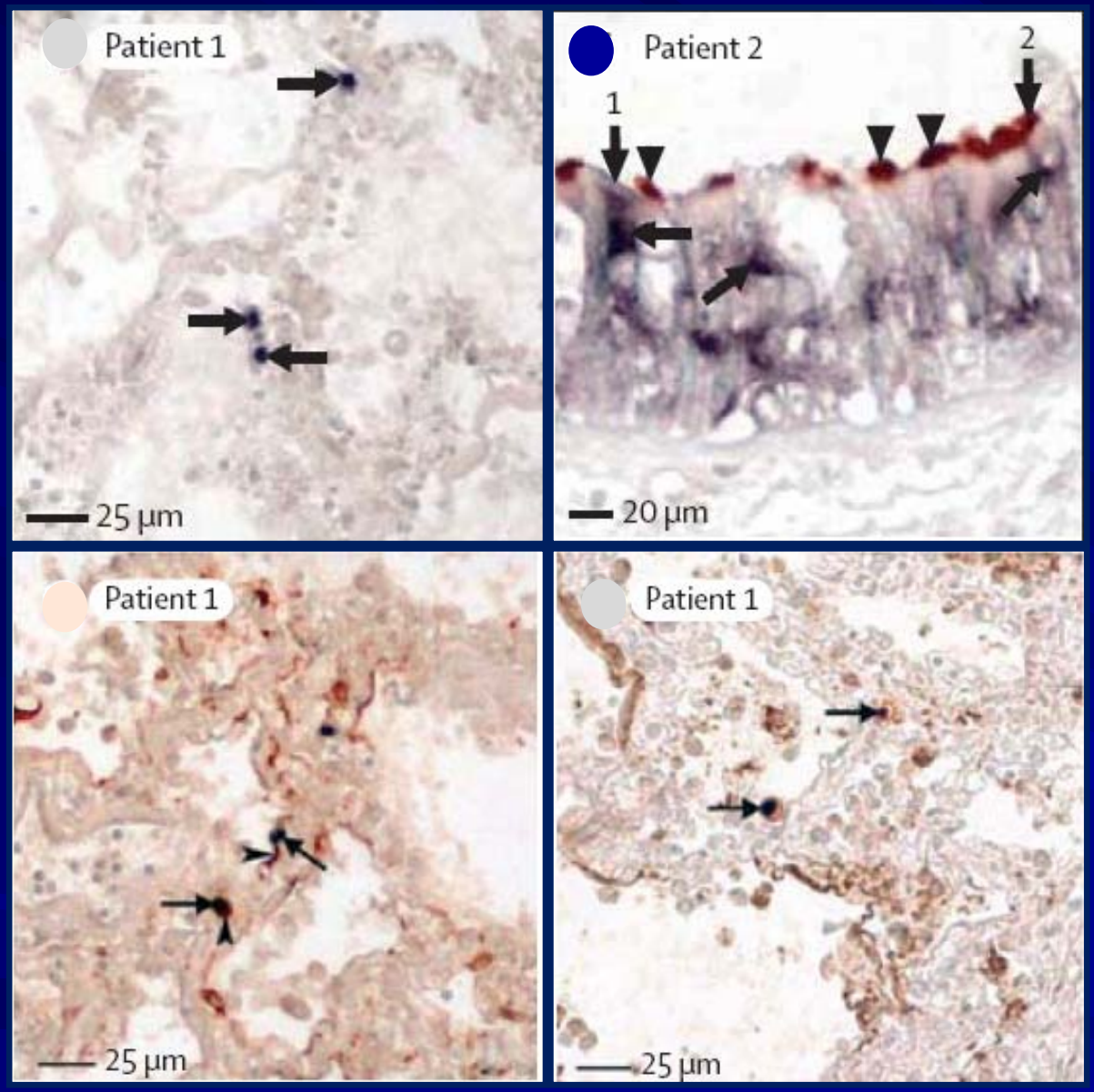
Table1 Result of in situ and immunohistochemistry in selected organ and cell type

	Patient 1		Patient 2		Fetus	
	ISH*	IHC†	ISH	IHC	ISH	IHC
Trachea	+/+	+/+	+/+	+/+	..	..
Bronchi	–/–	–/–	–/–	–/–	+/+	+/+
Alveolar pneumocytes	+/+	+/+	–/–	–/–	+/+	+/+
Lymph nodes	+/+‡	..	+/+‡	+/+‡	..	..
Spleen	–/–	–/–	–/–	–/–	..	..
Heart	–/–	–/–	–/–	–/–	–/–	–/–
Endothelial cells	–/–	–/–	–/–	–/–	–/–	–/–
Hepatocytes	–/–	–/–	–/–	–/–	–/–	..
Kupffer cells	–/–	–/–	–/–	–/–	+/+	..
Kidneys	–/–	–/–	–/–	–/–	–/–	..
Small intestine	+/+	–/–	+/+	–/–	–/–	..
Brain	..	..	+/+	+/+	..	..
Syncytiotrophoblasts	–/–	–/–	n/a	n/a	n/a	n/a
Cytotrophoblasts	+/+	+/+	n/a	n/a	n/a	n/a
Hofbauer cells	+/+	+/+	n/a	n/a	n/a	n/a
Circulating mononuclear cells	–/–	–/–	–/–	+/+	+/+	+/+

- Positive signal in lungs, trachea, lymph node, brain, small intestine and Hofbauer cells and cytotrophoblasts of the placenta.

GU, J. *et al. Lancet* 2007

Respiratory tract

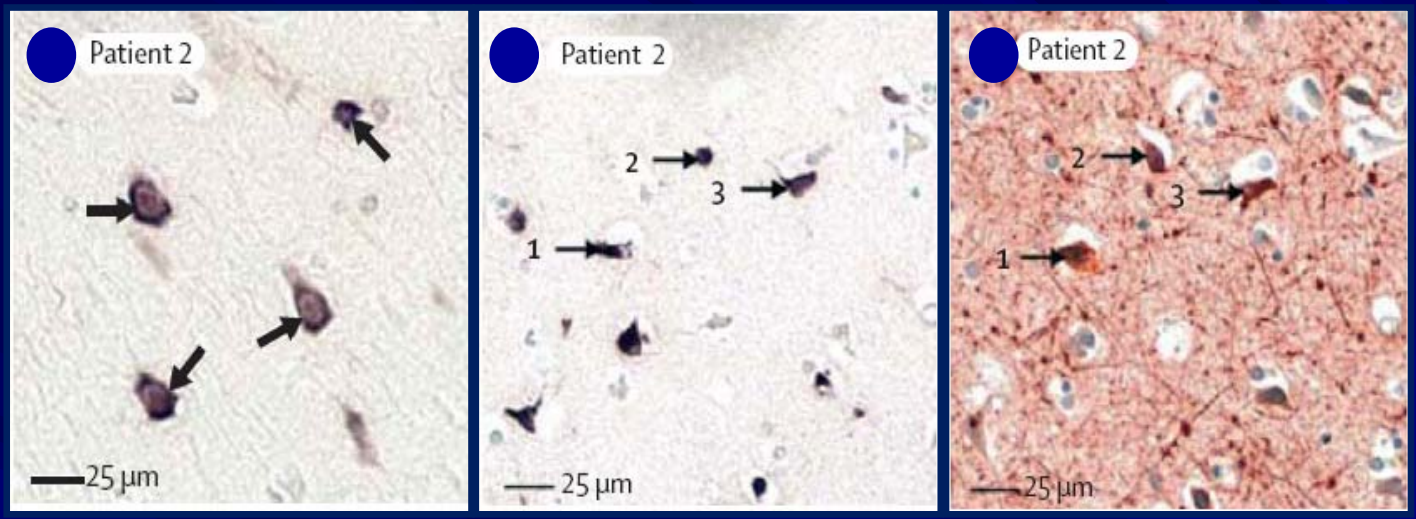


- Positive signal in tracheal epithelial cells and alveolar epithelial cell
- Negative signal in bronchi and bronchioles
- No positive signal in endothelial cell, macrophage, lymphocyte, or any other cell type in lung or blood.

Figure In situ hybridization in respiratory tract

GU, J. *et al. Lancet* 2007

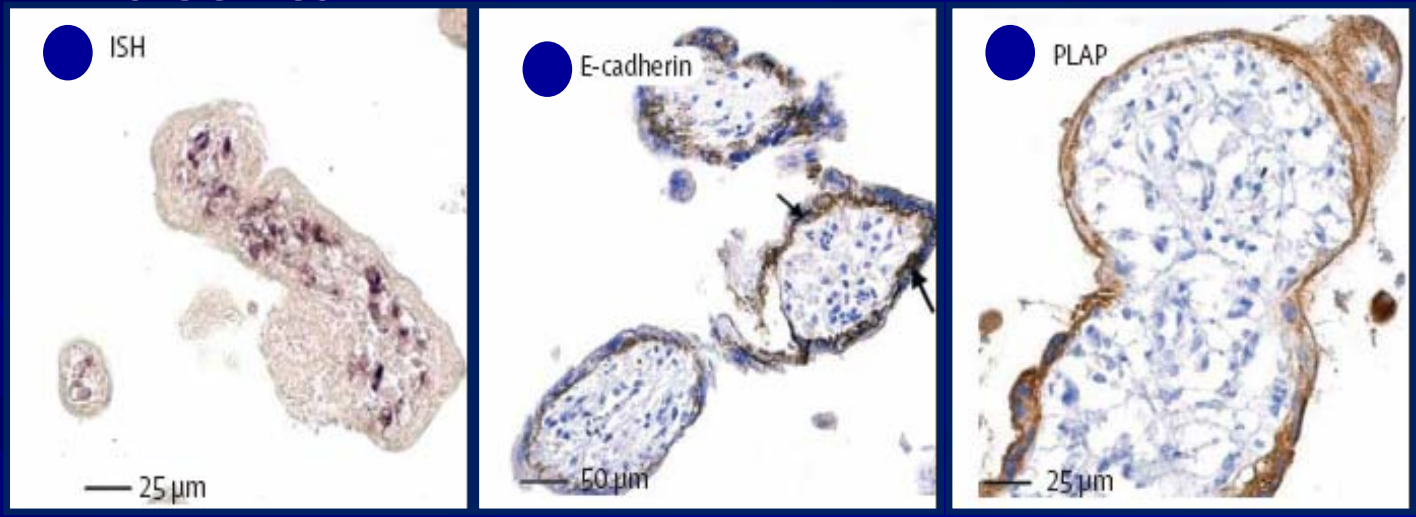
Brain



- Positive signal in cytoplasm of brain
- Double labeling showed positive cells is neurons cells

Figure In situ hybridization in brain

Placenta



- Positive signal in chorionic villi
- Double labeling showed positive cell is Hofbauer cells and cytotrophoblastic cells

Figure In situ hybridization in placenta

GU, J. *et al. Lancet* 2007

Lymph node

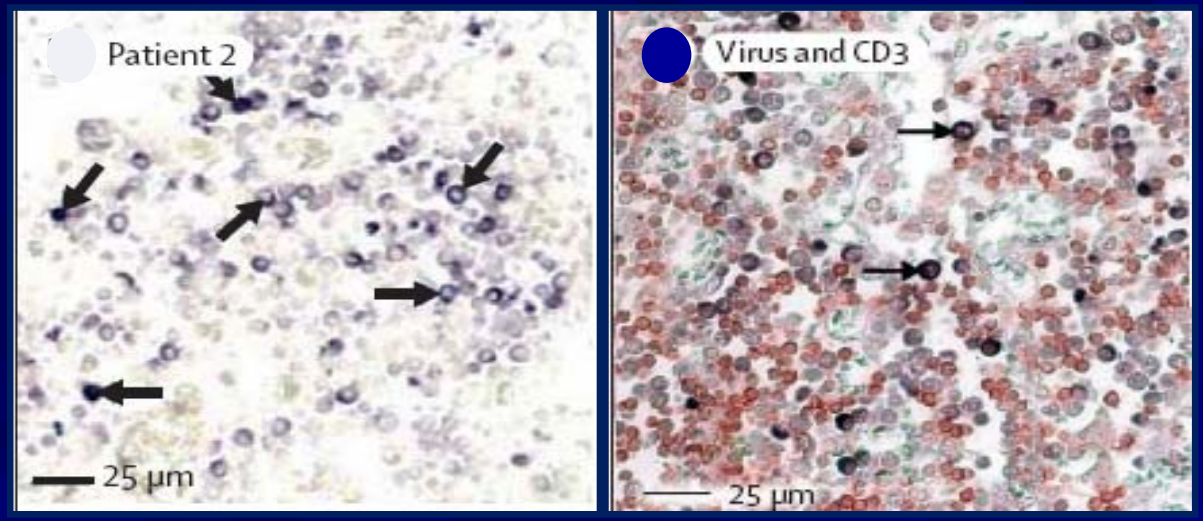


Figure In situ hybridization in lymph node

- Positive signal in cytoplasm of mononuclear cell in lymph node
- Double labeling showed positive cell is T lymphocytes

Small intestine

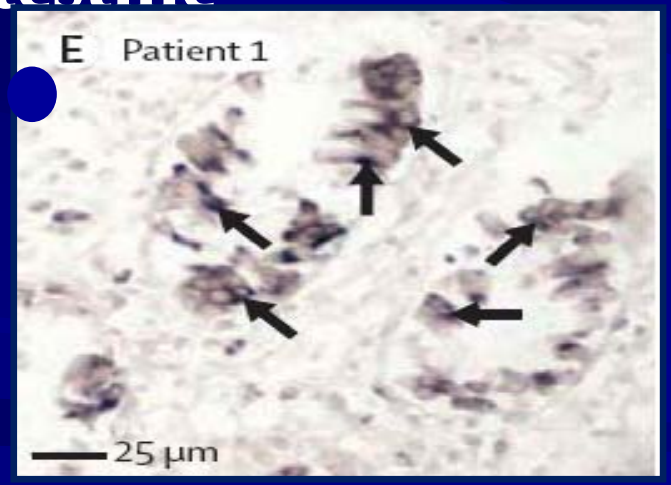


Figure In situ hybridization small intestine

- Positive signal in cytoplasm of mucosal epithelial cells of small intestine

GU, J. *et al. Lancet* 2007

Fetus

Table1 Result of in situ and immunohistochemistry in selected organ and cell type

	Patient 1		Patient 2		Fetus	
	ISH*	IHC†	ISH	IHC	ISH	IHC
Trachea	+/+	+/+	+/+	+/+	..	..
Bronchi	−/−	−/−	−/−	−/−	+/+	+/+
Alveolar pneumocytes	+/+	+/+	−/−	−/−	+/+	+/+
Lymph nodes	+/+‡	..	+/+‡	+/+‡	..	..
Spleen	−/−	−/−	−/−	−/−	..	..
Heart	−/−	−/−	−/−	−/−	−/−	−/−
Endothelial cells	−/−	−/−	−/−	−/−	−/−	−/−
Hepatocytes	−/−	−/−	−/−	−/−	−/−	..
Kupffer cells	−/−	−/−	−/−	−/−	+/+	..
Kidneys	−/−	−/−	−/−	−/−	−/−	..
Small intestine	+/+	−/−	+/+	−/−	−/−	..
Brain	..	..	+/+	+/+	..	..
Syncytiotrophoblasts	−/−	−/−	n/a	n/a	n/a	n/a
Cytotrophoblasts	+/+	+/+	n/a	n/a	n/a	n/a
Hofbauer cells	+/+	+/+	n/a	n/a	n/a	n/a
Circulating mononuclear cells	−/−	−/−	−/−	+/+	+/+	+/+

- Positive signal in lungs, circulating mononuclear cells, and Kupffer cells in liver

GU, J. *et al. Lancet* 2007

Immunohistochemistry

Table1 Result of in situ and immunohistochemistry in selected organ and cell type

	Patient 1		Patient 2		Fetus	
	ISH*	IHC†	ISH	IHC	ISH	IHC
Trachea	+/+	+/+	+/+	+/+	..	..
Bronchi	−/−	−/−	−/−	−/−	+/+	+/+
Alveolar pneumocytes	+/+	+/+	−/−	−/−	+/+	+/+
Lymph nodes	+/+‡	..	+/+‡	+/+‡	..	..
Spleen	−/−	−/−	−/−	−/−	..	..
Heart	−/−	−/−	−/−	−/−	−/−	−/−
Endothelial cells	−/−	−/−	−/−	−/−	−/−	−/−
Hepatocytes	−/−	−/−	−/−	−/−	−/−	..
Kupffer cells	−/−	−/−	−/−	−/−	+/+	..
Kidneys	−/−	−/−	−/−	−/−	−/−	..
Small intestine	+/+	−/−	+/+	−/−	−/−	..
Brain	..	..	+/+	+/+	..	..
Syncytiotrophoblasts	−/−	−/−	n/a	n/a	n/a	n/a
Cytotrophoblasts	+/+	+/+	n/a	n/a	n/a	n/a
Hofbauer cells	+/+	+/+	n/a	n/a	n/a	n/a
Circulating mononuclear cells	−/−	−/−	−/−	+/+	+/+	+/+

- Distribution of immunohistochemical staining was consistent with that of In situ hybridization except the absence of viral antigen in small intestine

GU, J. *et al. Lancet* 2007

RT-PCR, real time RT-PCR, and NASBA

Table1 Result of RT-PCR, real time RT-PCR and NASBA for H5 detection in tissue

	RT-PCR and strand-specific RT-PCR						Real-time RT-PCR	NASBA-based H5 detection assay			
	Patient 1			Patient 2			Fetus	Patient 1		Patient 2	
	+/−	−	+	+/−	−	+		Optical density (405 nm)*	Result	Optical density (405 nm)*	Result
Lungs	Y	Y	N	Y	N	Y	Y	2·579	Y	0·961	Y
Trachea	..	..	..	Y	Y	Y	..	..	n/a	1·236	Y
Intestines	Y	Y	N	Y	Y	Y	N	2·277	Y	2·112	Y
Brain	..	..	..	Y	Y	Y	..	..	n/a	2·424	Y
Heart	Y	N	Y	Y	N	N	N	2·415	Y	2·555	Y
Spleen	Y	Y	N	Y	N	N	..	1·326	Y	1·442	Y
Liver	Y	N	N	Y	N	N	Y	2·176	Y	2·225	Y
Kidneys	Y	Y	N	Y	N	N	N	2·371	Y	1·587	Y
Lymph node	..	..	..	N	Y	N	..	..	n/a	1·107	Y
Placenta	Y	Y	Y	n/a	n/a	n/a	n/a	1·653	Y	n/a	n/a

+/−, −, and + represent total, negative-stranded, and positive-stranded RNA, respectively. Y=positive result. N=negative result. n/a=not applicable. *Samples regarded as H5-positive if absorbance higher than 0·45.

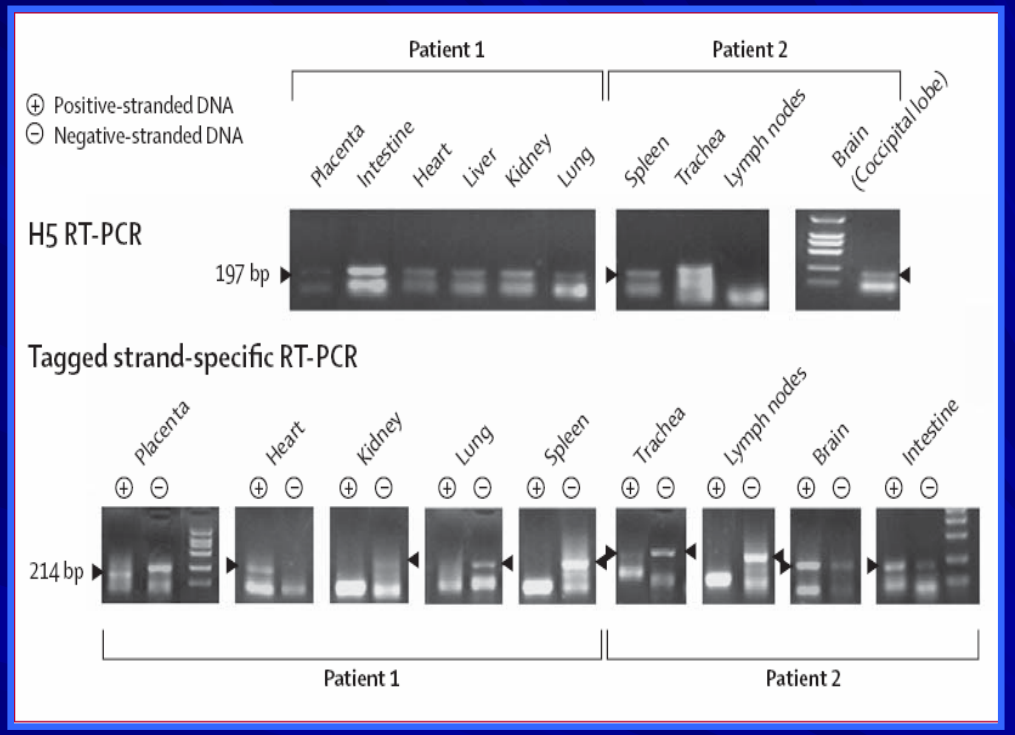


Figure H5 RT-PCR and tagged strand-specific RT-PCR on selected organ

- All organs tested showed positive RT-PCR result except lymph node

GU, J. *et al. Lancet* 2007

Apoptosis

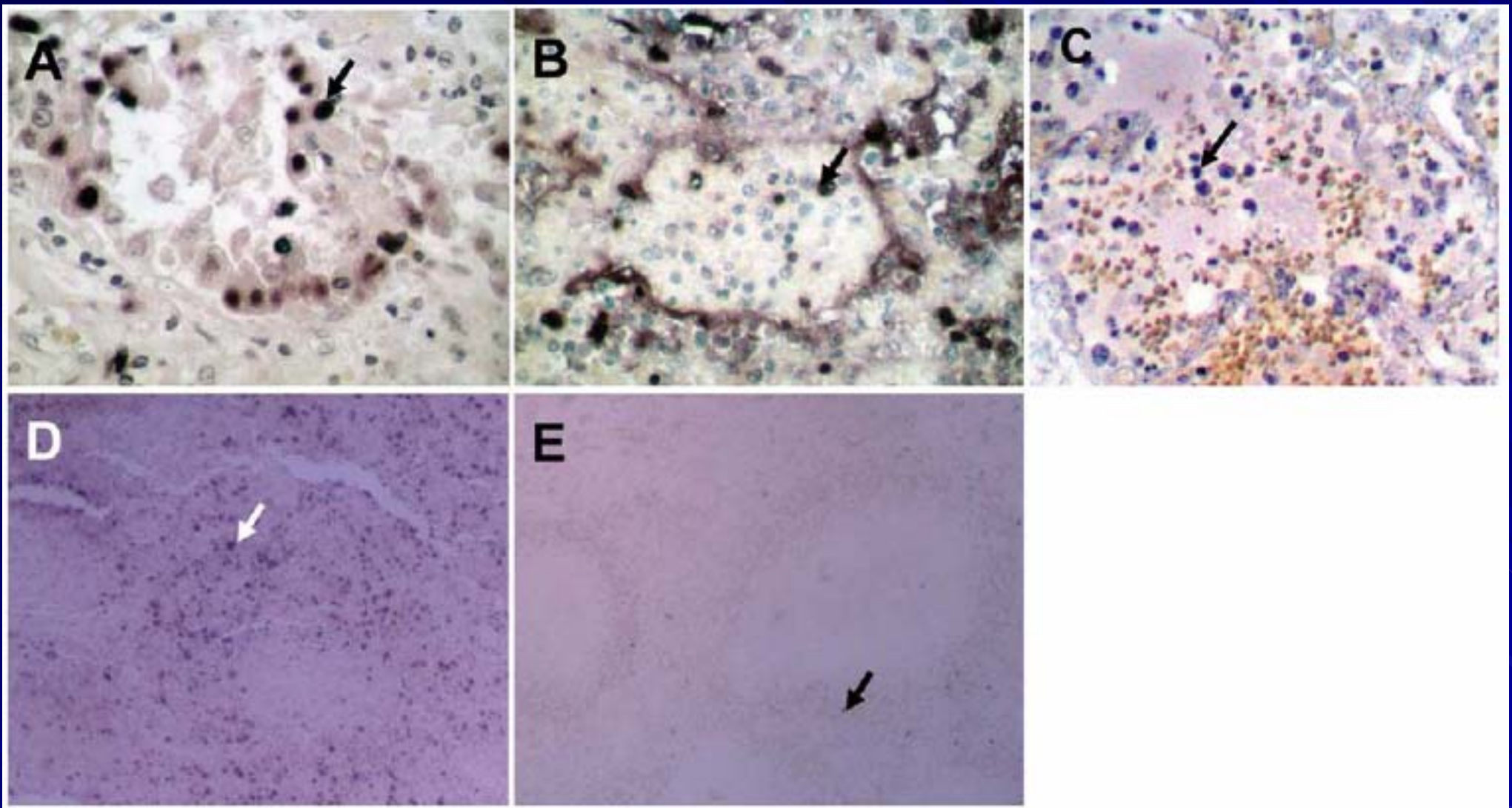
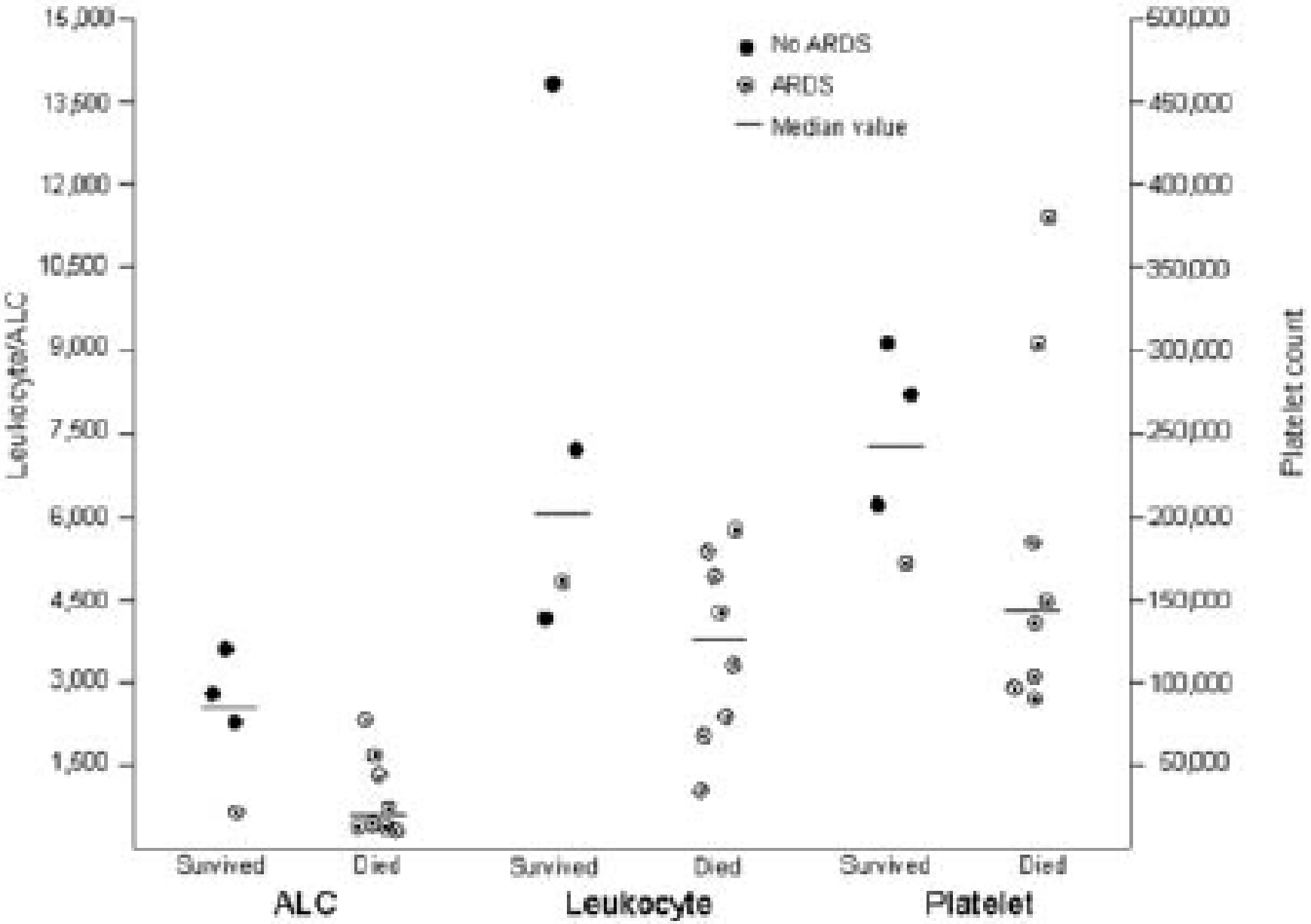


Figure 3. Terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick end-labeling staining showing numerous apoptotic alveolar epithelial cells in lung of patient B (A) and leukocytes in lung of patient A (B). C) Lung tissue from a patient with pneumonia caused by human influenza A (H5N1) virus showing apoptosis only in leukocytes. D) Spleen of patient B showing numerous apoptotic cells. E) Normal spleen tissue showing only a minimal level of apoptosis. Apoptotic cells are stained dark blue, and an apoptotic cell in each panel is indicated by an arrow. Magnification ×400 in A, B, and C; ×100 in D and E.

Uiprasertkul, 2007



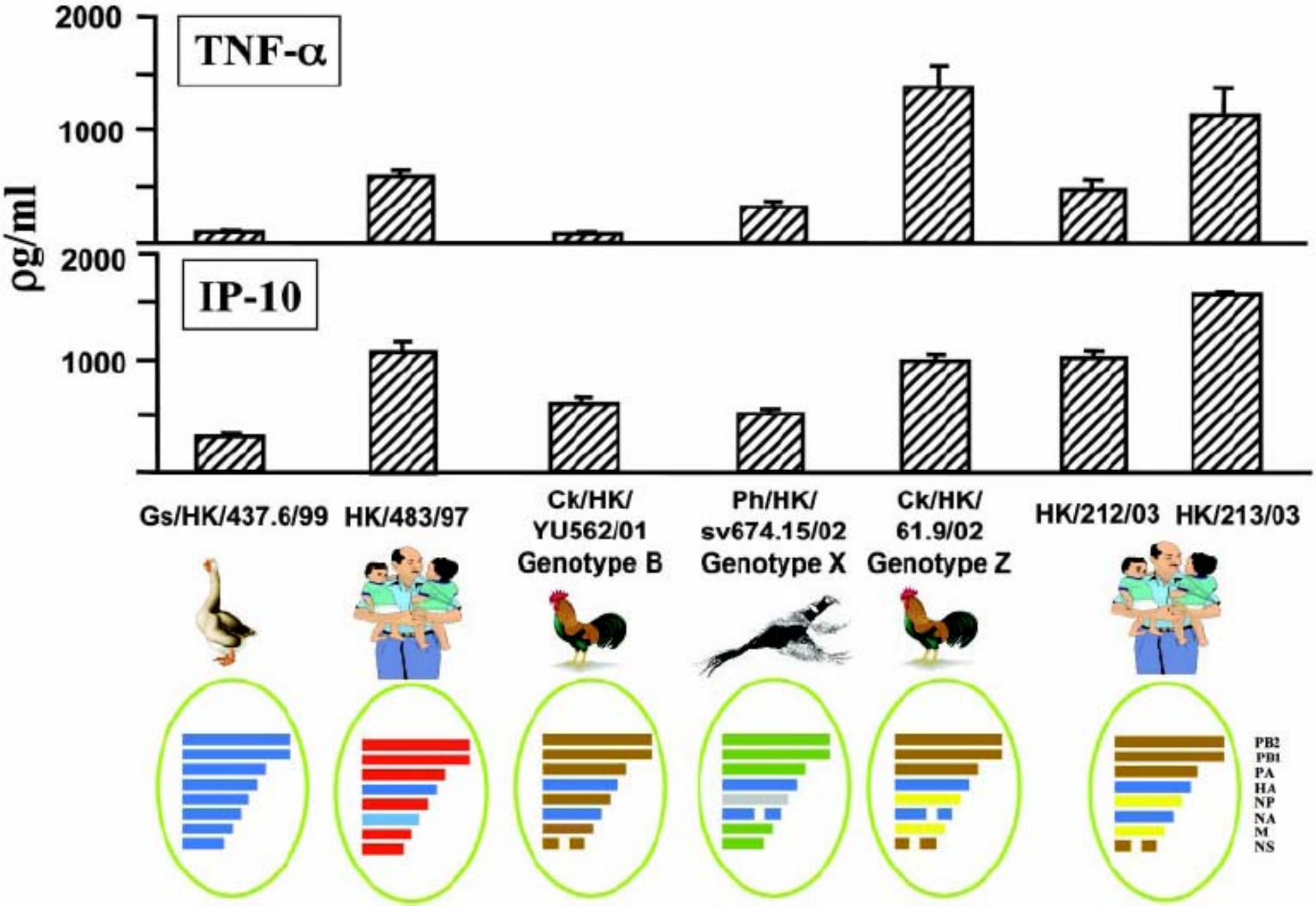
Chotpitayasunondh T, EID 2005

Table 2 Detection of influenza virus in respiratory and non-respiratory sites

					H5N1		
		H5N1	H3/H1	*P*	Fatal	Not fatal	*P*
Nasopharynx							
Virus isolation rate (positive/tested; %)		12/16[a] (75)	NA		8/12 (67)	4/4 (100)	
Detectable RNA (positive/tested; %)	Nose	13/17 (76)	6/8 (75)		10/12 (83)	3/5 (60)	
	Throat	18/18 (100)	8/8 (100)		13/13 (100)	5/5 (100)	
Viral load (median; range)	Nose	5.5 (und.–8.1)	4.5 (und.–7.7)	0.59	5.8 (und.–8.1)	4.5 (und.–6.4)	0.20
	Throat	7.0 (4.3–8.2)	4.8 (4.2–5.8)	0.003	7.5 (4.7–8.2)	5.9 (4.3–7.0)	0.058
	P	0.001	0.87				
Rectum[b]							
Virus isolation rate (positive/tested; %)		1/7 (14)	NA		1/7 (14)	NA	
Detectable RNA (positive/tested; %)		5/7 (71)	NA		5/7 (71)	NA	
Viral load (median; range)		4.8 (3.6–5.8)	NA		4.8 (3.6–5.8)	NA	
Blood[c]							
Virus isolation rate (positive/tested; %)		1/6 (17)	NA		1/6 (17)	NA	
Detectable RNA (positive/tested; %)		9/16 (56)	0/6 (0)	0.046	9/11 (82)	0/5 (0)	0.005
Viral load (median; range)		4.5 (3.2–-5.7)	Und.		4.5 (3.2–5.7)	Und.	

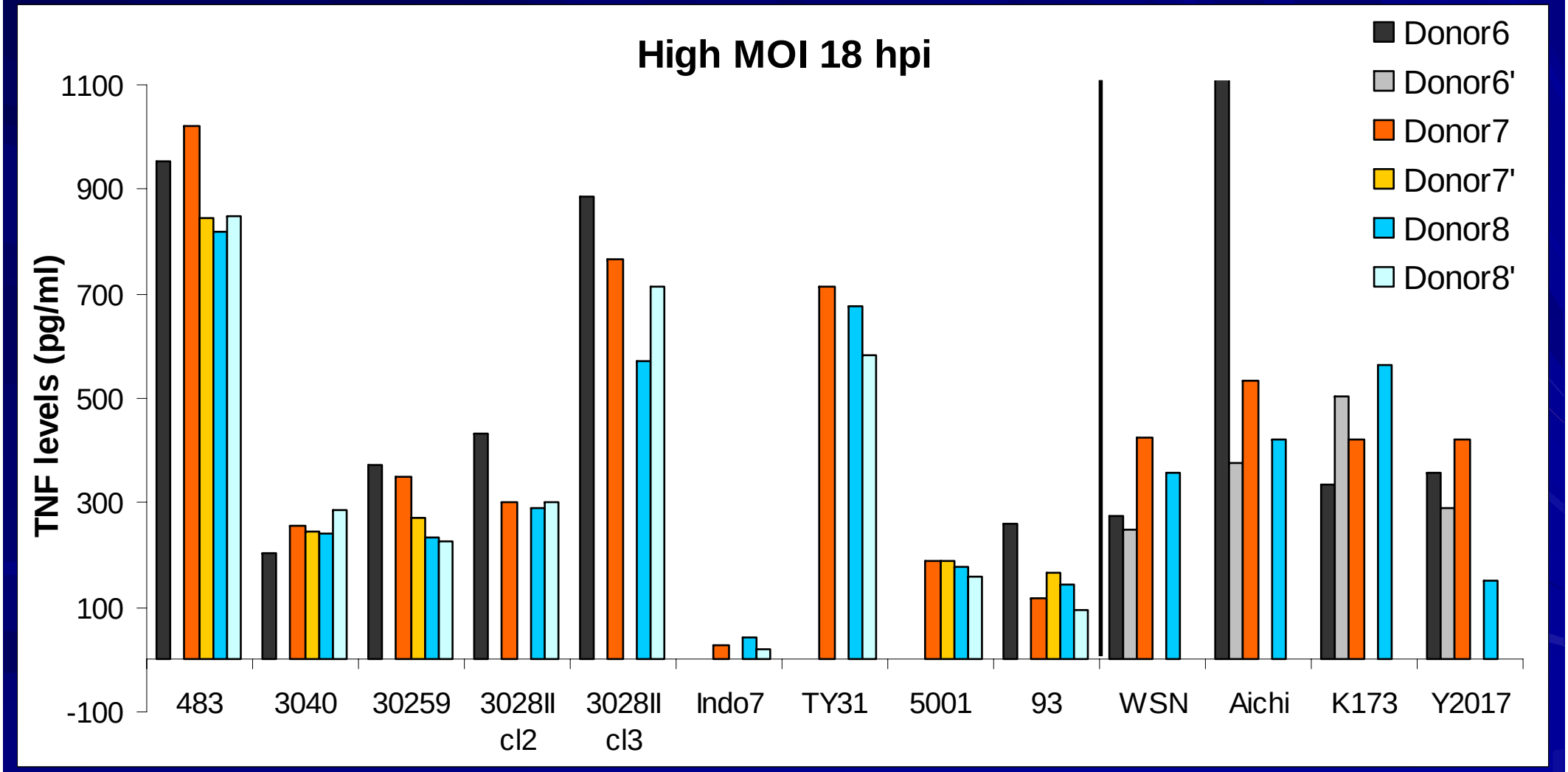
Rates of virus isolation, detection of viral RNA and viral loads in nasopharyngeal, rectal and blood specimens of patients with influenza H5N1 and H3N2 or H1N1. Viral loads are given as $\log_{10}$ cDNA copies per ml of viral transport medium. Und., below detection limit; NA, not assessed.

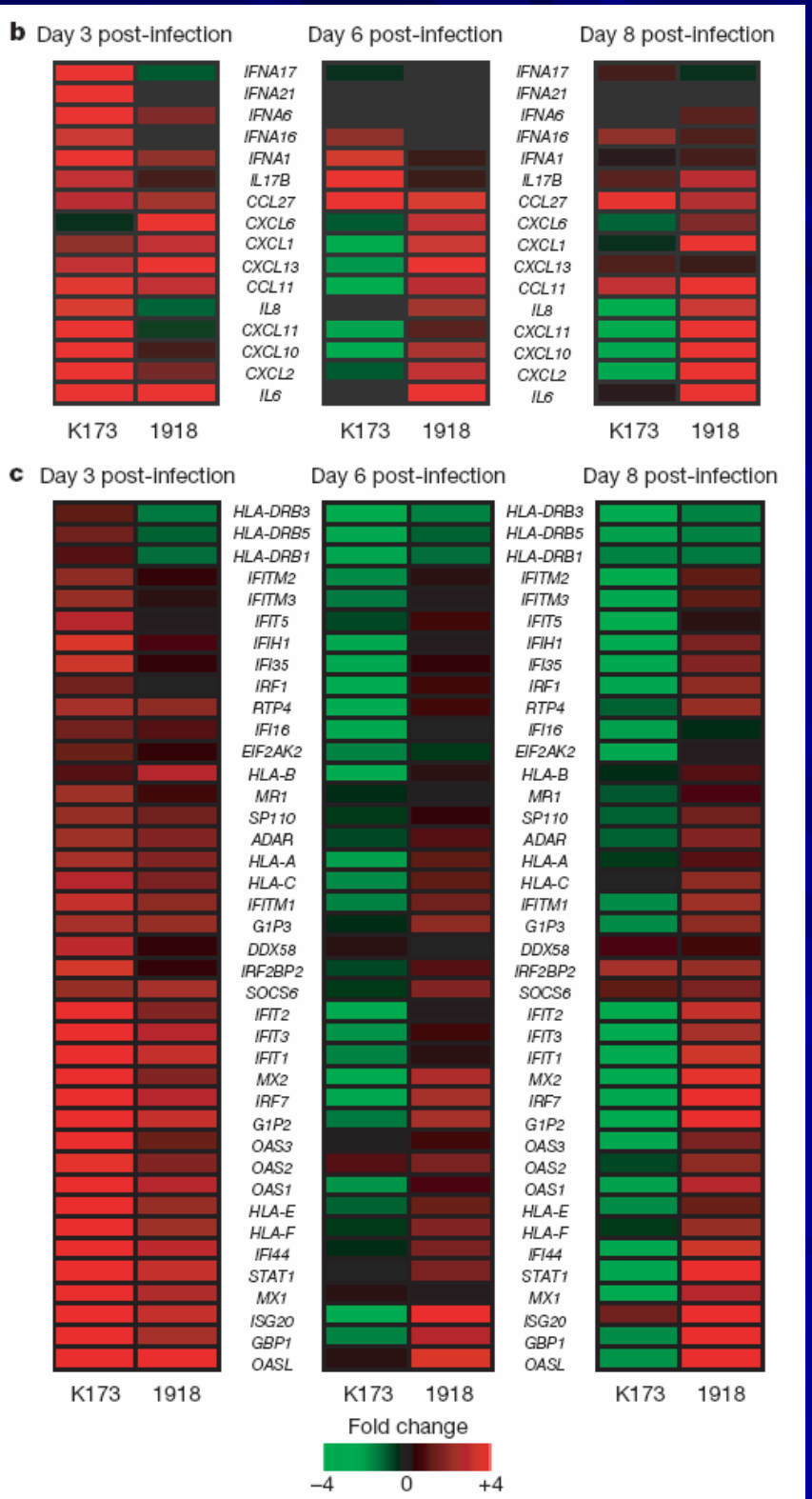
de Jong, 2006


TNF-α
IP-10
pg/ml
2000
1000
2000
1000
Gs/HK/437.6/99
HK/483/97
Ck/HK/
YU562/01
Genotype B
Ph/HK/
sv674.15/02
Genotype X
Ck/HK/
61.9/02
Genotype Z
HK/212/03
HK/213/03
PB2
PB1
PA
HA
NP
NA
M
NS

Guan, 2004

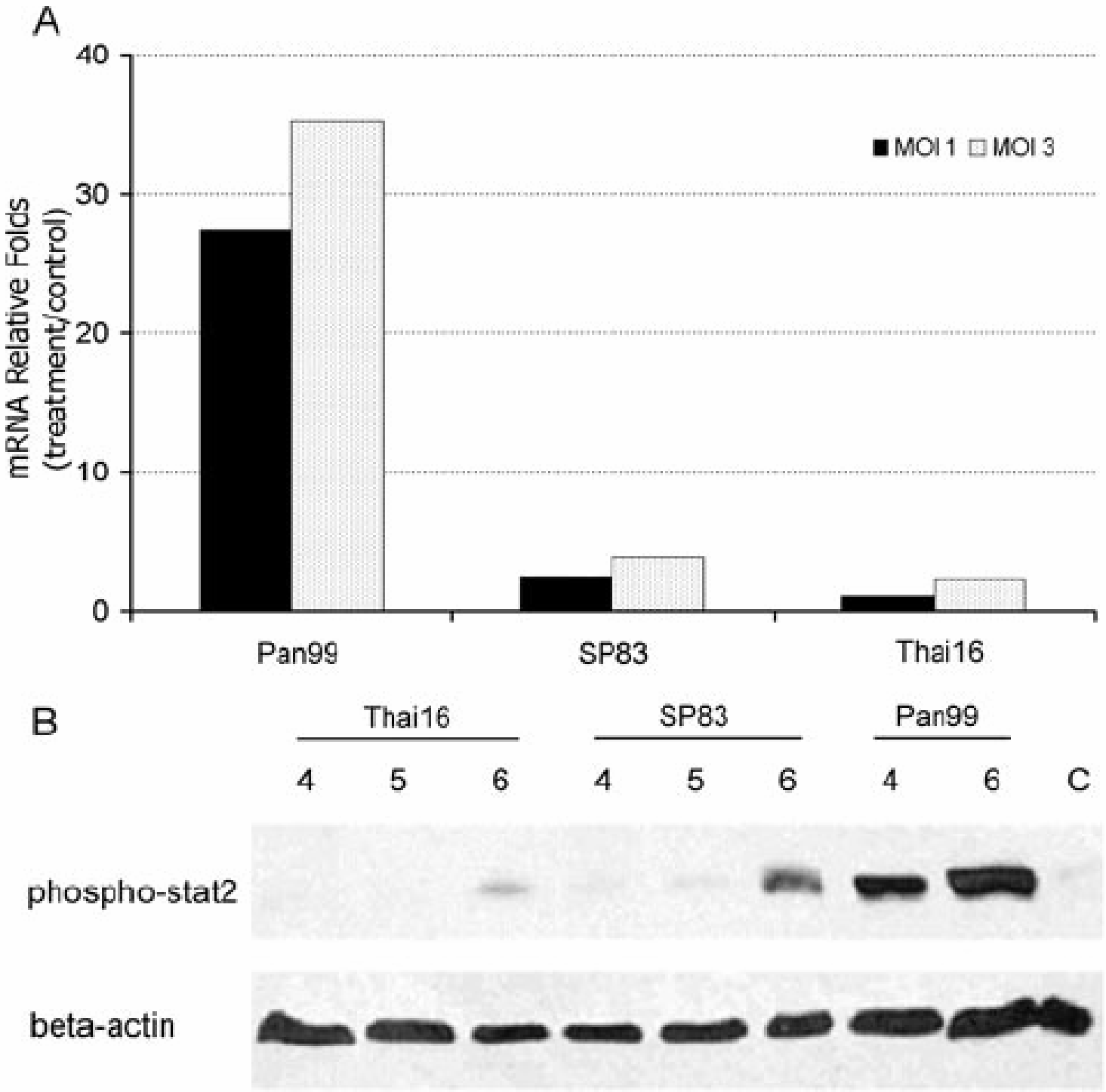
Cytokine production in human macrophage




b
Day 3 post-infection
Day 6 post-infection
Day 8 post-infection
IFNA17
IFNA21
IFNA6
IFNA16
IFNA1
IL17B
CCL27
CXCL6
CXCL1
CXCL13
CCL11
IL8
CXCL11
CXCL10
CXCL2
IL6
K173
1918
c
Day 3 post-infection
Day 6 post-infection
Day 8 post-infection
HLA-DRB3
HLA-DRB5
HLA-DRB1
IFITM2
IFITM3
IFIT5
IFIH1
IFI35
IRF1
RTP4
IFI16
EIF2AK2
HLA-B
MR1
SP110
ADAR
HLA-A
HLA-C
IFITM1
G1P3
DDX58
IRF2BP2
SOCS6
IFIT2
IFIT3
IFIT1
MX2
IRF7
G1P2
OAS3
OAS2
OAS1
HLA-E
HLA-F
IFI44
STAT1
MX1
ISG20
GBP1
OASL
K173
1918
Fold change
−4
0
+4

Kobasa, 2007

Delayed IFN



Zeng, 2007

Conclusions

- Main target cells are type II pneumocytes, and infected cells showed apoptosis.
- Viral RNA is found in many organs despite the relative lack of inflammatory response.
- Delayed or reduced IFN response and enhanced pro-inflammatory cytokine response may be involved in the viral pathogenesis.