“Virosis emergentes:

Gripe e influenza aviar H5N1 e

influenza A H1N1(2009) pandémica”

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Fac. de Medicina. UBA.

Contenidos

- ¿Qué son los virus causantes de la gripe estacional, la gripe aviar y la influenza pandémica?
- ¿Qué se conoce de su patogénesis?
- ¿Cuál es la situación 2010 ante estas virosis emergentes / re-emergentes?
- ¿Cómo se las diagnostica?
- ¿Cómo actúan los antivirales en uso? ¿Qué importancia médica tiene el monitoreo de la resistencia?

Toser y estornudar: se expelen microgotas de Flügge

- Al toser: diseminación de cientos de microgotas más de 900 km/h.
- Al estornudar: diseminación de centenas de miles de microgotas. a más de 100 km / h,

… y al pronunciar la letra "F"…

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Sabía que…

- Bastan 1 a 5 $TCID_{50}$ para infectar un humano…
- El virus Influenza (humano) persiste infeccioso al menos un día.
- El virus Influenza aviar (H5N1) persiste infectivo durante al menos varias semanas en superficies no porosas, dependiendo de la Tº y la humedad.

Relación entre infección y enfermedad

- Patogenicidad:

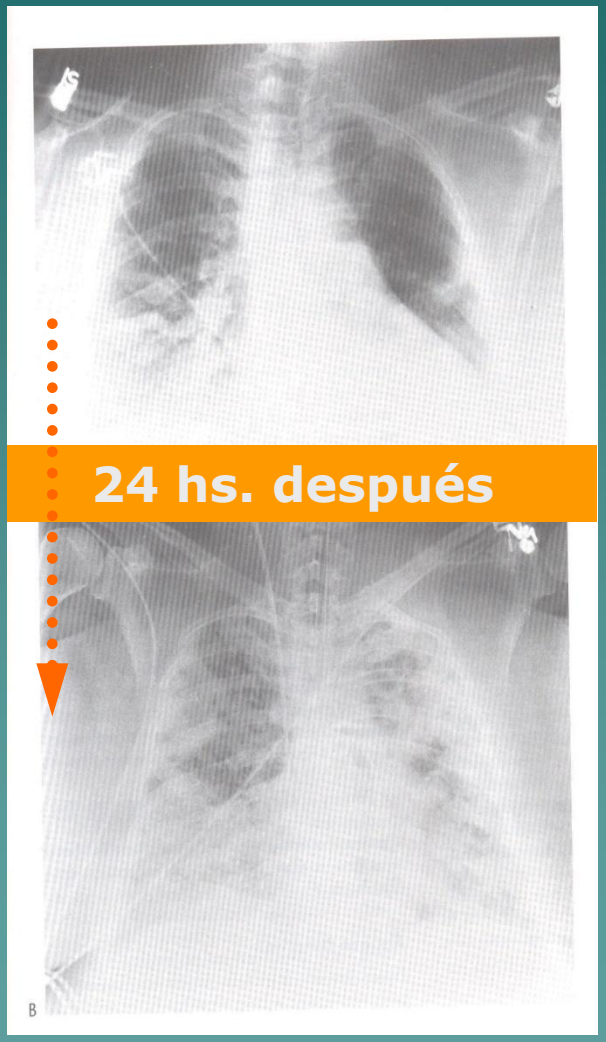
  "capacidad para generar enfermedad"

$$P = \frac{\text{N º de enfermos}}{\text{Nº total de infectados}}$$

- Virulencia:

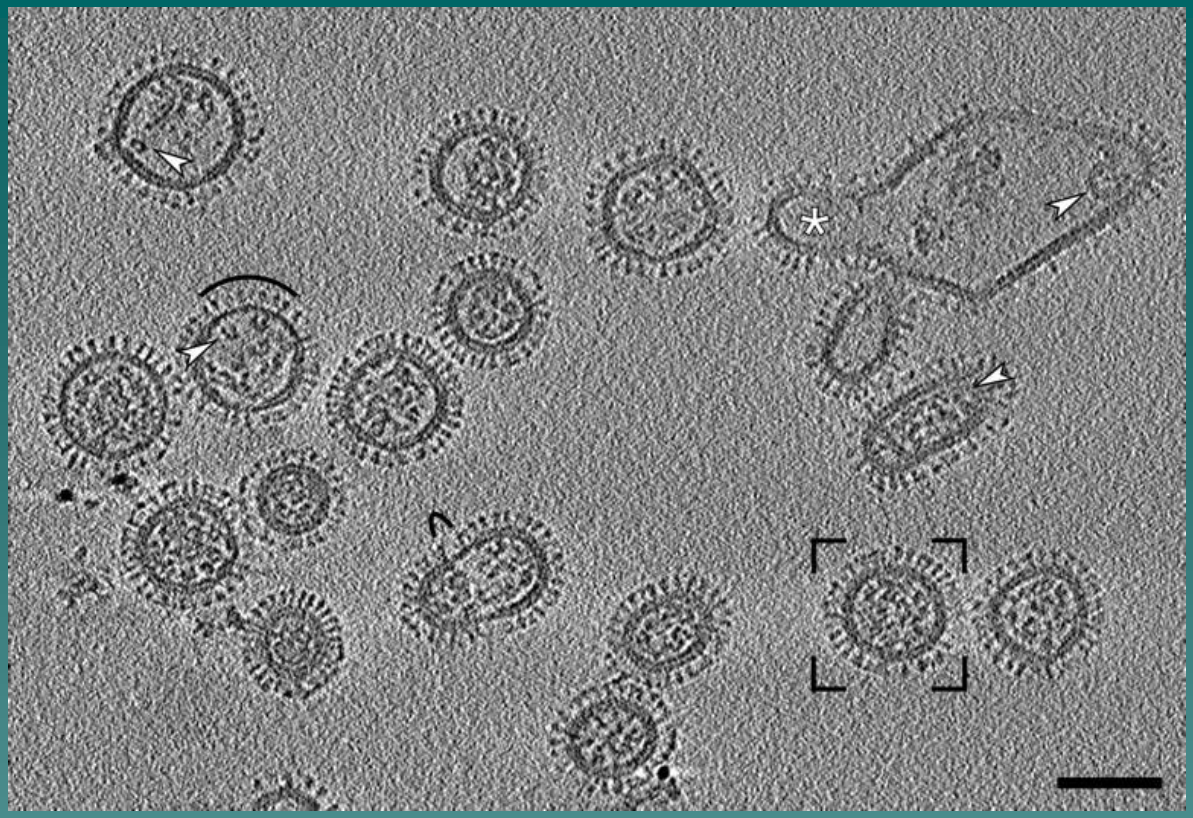
  Gravedad de la enfermedad.

Neumonía severa por virus Influenza A



- Paciente de 30 años inmunocompetente.
- Infiltrados en lóbulo medio derecho e inferiores bilaterales. Requirió asistencia respiratoria mecánica.

Richman y col. 1997



Sección obtenida mediante tomografía crioelectrónica correspondiente a un campo conteniendo partículas de virus Influenza. Las flechas blancas indican ribonucleoproteínas (RNP) típicas del virus. Los arcos negros indican áreas de la capa de matriz con zonas que exhiben gaps o una menor densidad de "paquetes" de glicoproteínas de envoltura. La imagen irregular marcada con un asterisco -probablemente se haya formado en el proceso de disrupción celular- contiene en su interior una partícula brotando. La partícula viral enmarcada se observa también en la figura 2. La barra ubicada en el extremo inferior derecho del panel indica 100 nm.
Fuente Harris A. *et al*. PNAS 2006, 13: 19123-7.

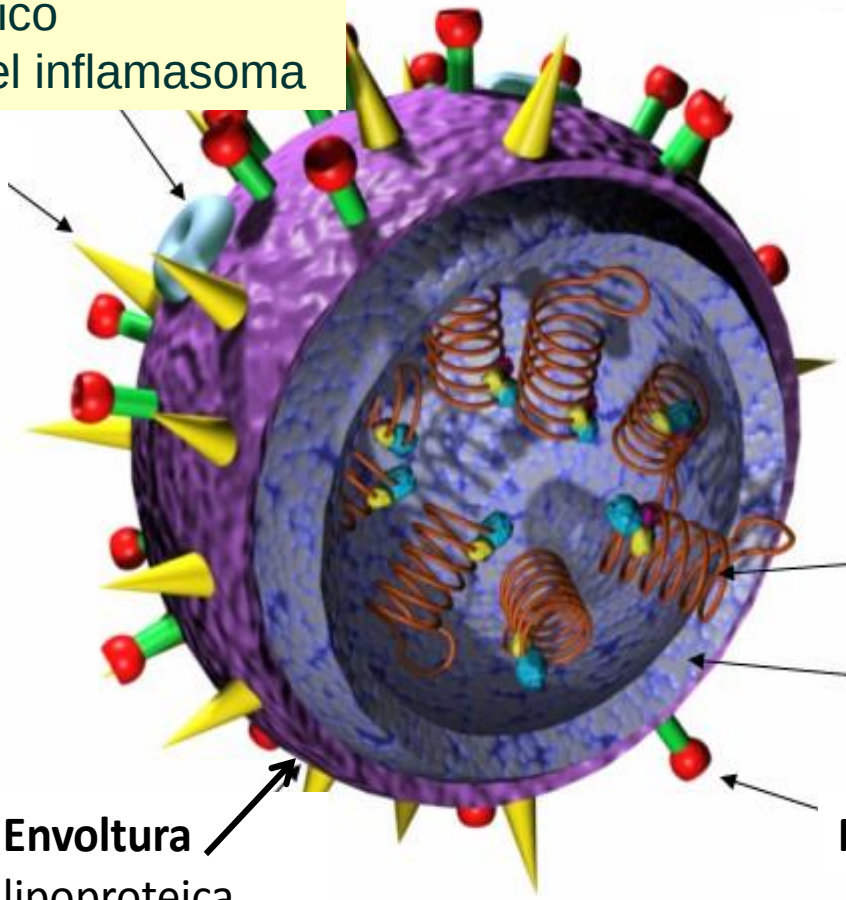
Virus Influenza:

Esquema

M2: canal iónico
y activador del inflamasoma

Hemaglutinina

Dr. Wilson Smith (1933)

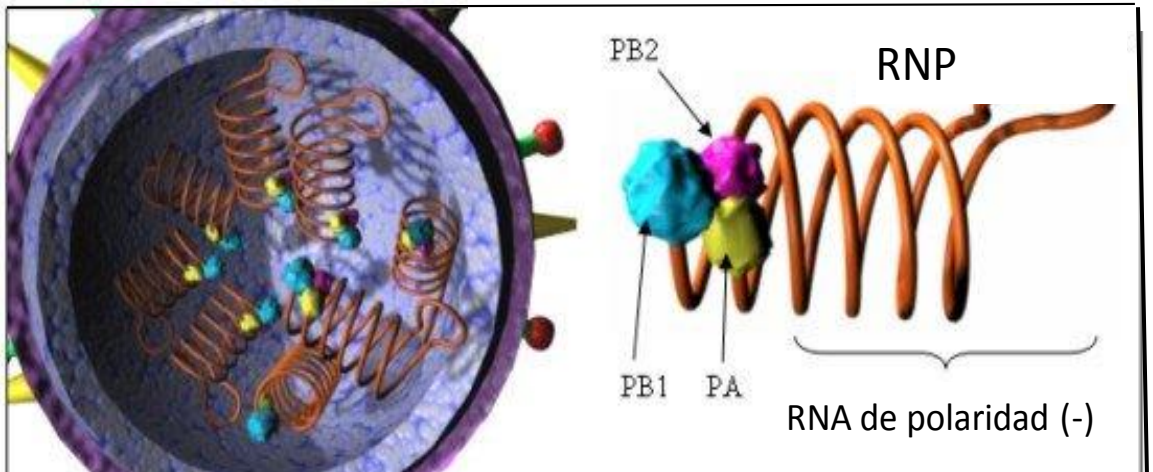


Ribonucleoproteína (RNP)

Cápside

Envoltura lipoproteica

Neuraminidasa



PA: Polimerasa ácida
PB1: Polimerasa básica 1
PB2: Polimerasa básica 2.

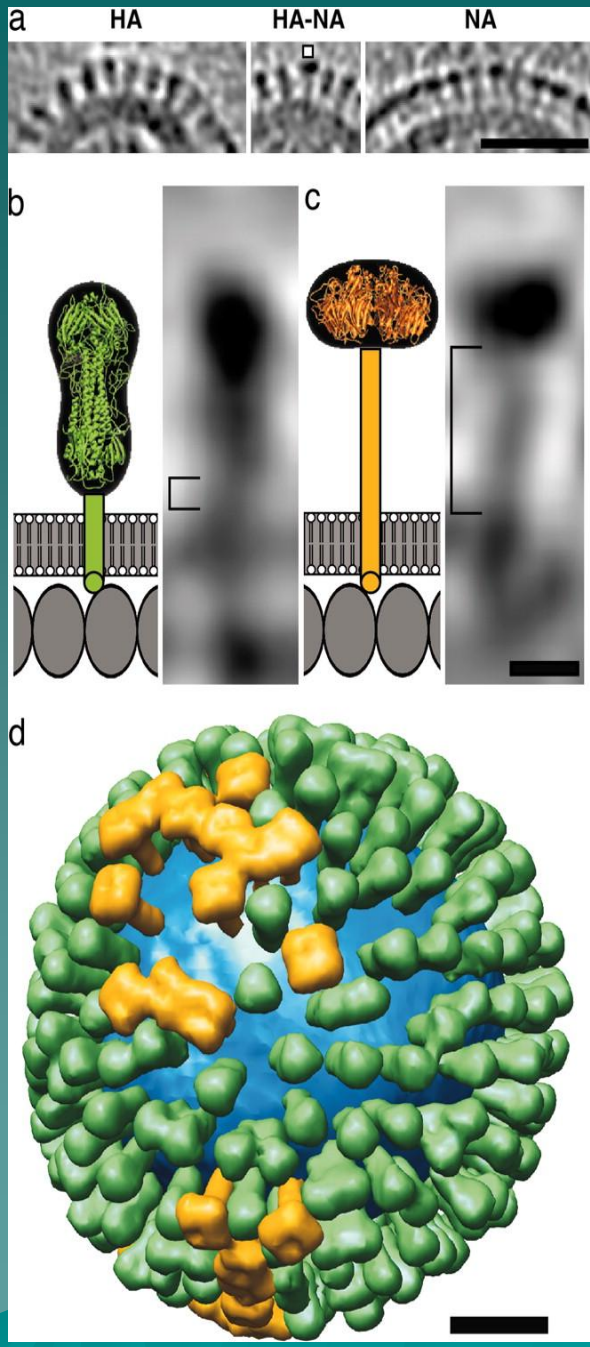
Distribución de las hemaglutininas (HA) y neuraminidasas (NA) en la superficie del virus Influenza, según su disposición espacial.

Se observa un *cluster* de hemaglutininas (panel "a", a la izquierda), una molécula de neuraminidasa en un *cluster* de hemaglutininas (panel "a", al centro) y un *cluster* de neuraminidasas (panel "a", a la derecha).

Los paneles "b" y "c" muestran dentro del recuadro respectivo la estructura de HA y NA. La barra horizontal indica 5 nm.

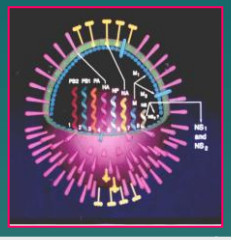
El panel "d" muestra un modelo de distribución de HA (en verde) y NA (en amarillo), así como de la capa lipídica de envoltura (en azul).

La barra indica 20 nm.



Fuente: Harris A. *et al*. PNAS 2006, 13: 19123-7.

3 tipos antigénicos (A, B y C)
según Nucleoproteína (NP) y proteína de matriz (M)



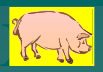
	Tipo A	**Tipo B**	**Tipo C**
Subtipos	Sí (según *Hemaglutinina* (H) y *Neuraminidsa* (N)	No	No
Producen epidemias	Sí	Sí	No
Pueden producir pandemias	Sí	No	No
Huéspedes	**Aves acuáticas, aves de corral, cerdos , caballos, mamíferos acuáticos, visón, humanos**	**Humanos**	**Humanos, cerdos**

Subtipos de hemaglutinina del virus Influenza A

Subtipo

H1
H2
H3
H4
H5
H6
H7
H8
H9
H10
H11
H12
H13
H14
H15
H16

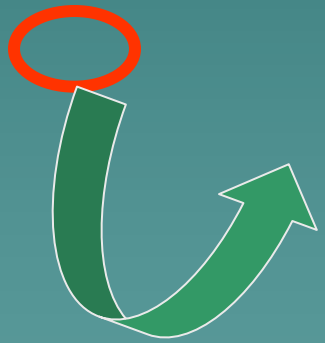
Seres humanos



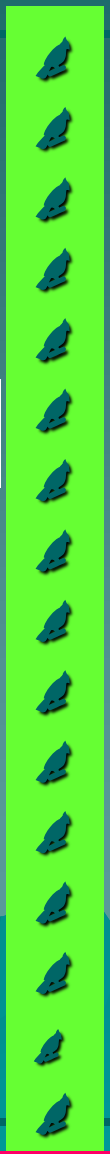
Cerdos

Aves

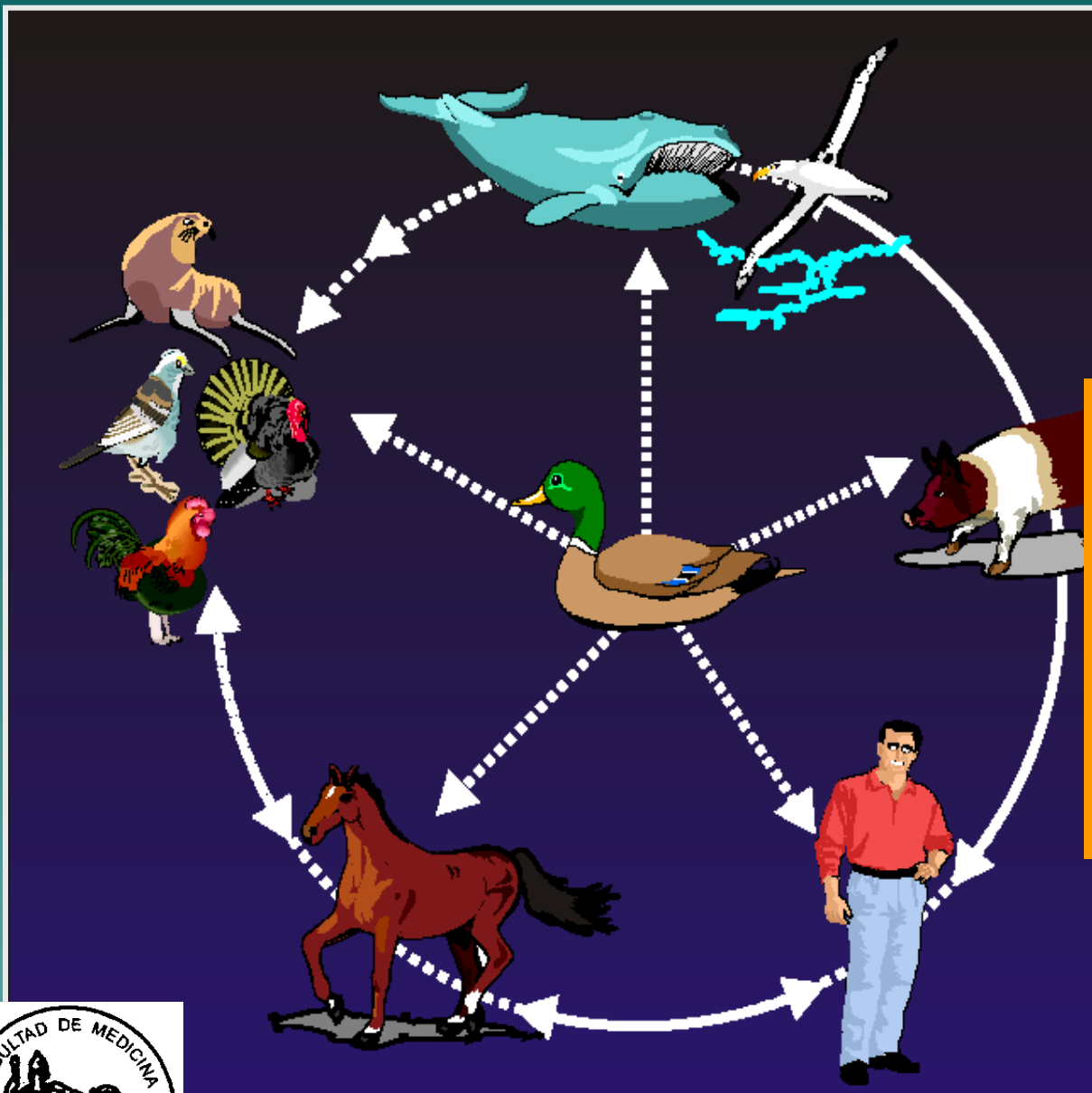
Caballos



Hemaglutininas asociadas a virosis emergentes en años recientes o en la actualidad



Reservorios del virus Influenza A



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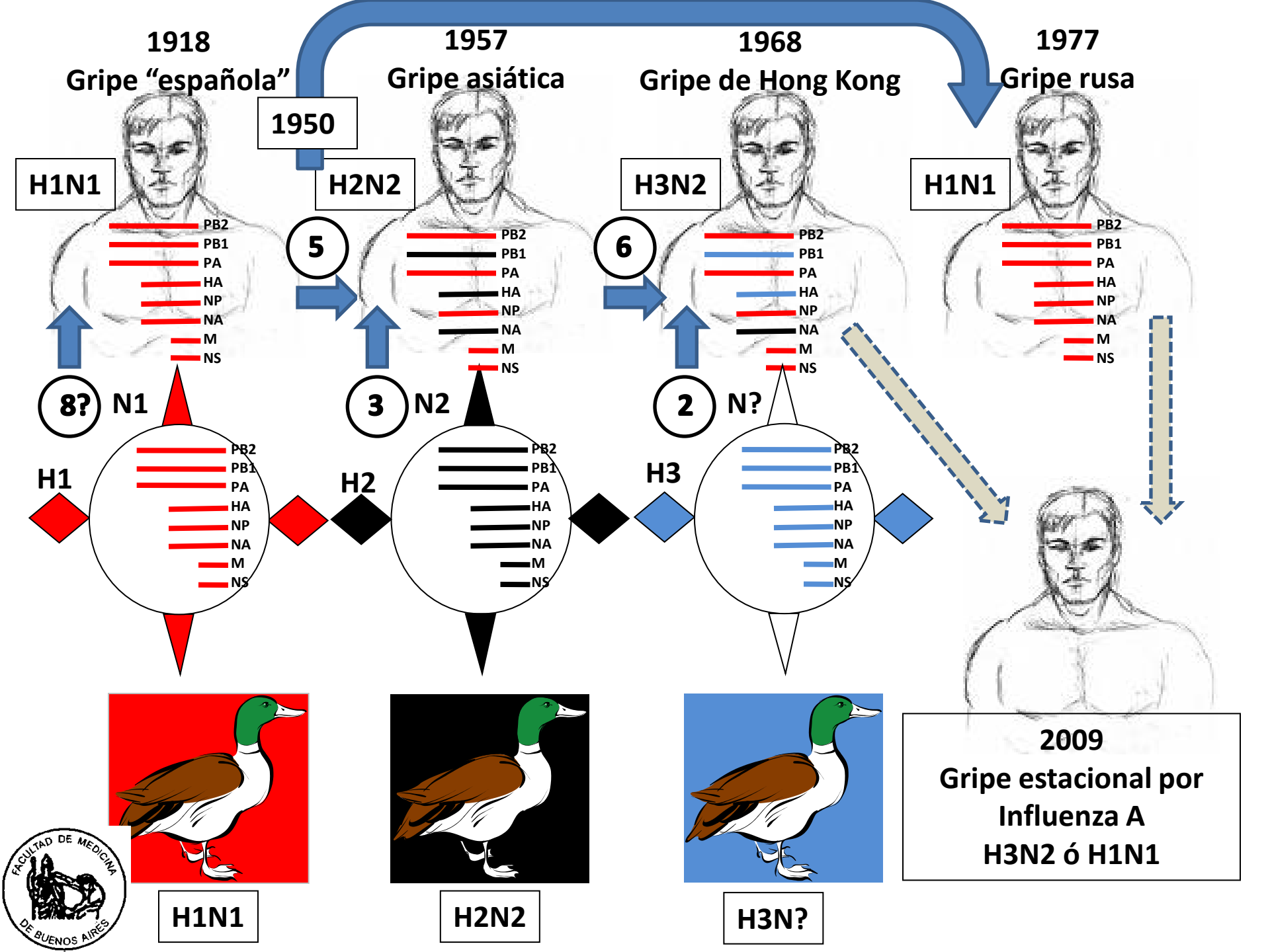
CDC

Influenza A en…

H3N2
H5N1
H3N8
(*desde caballos*)

H5N1

Vet Immunol Immunopathol 15:54-60, 2010


1918
Gripe "española"
1957
Gripe asiática
1968
Gripe de Hong Kong
1977
Gripe rusa
1950
H1N1
H2N2
H3N2
H1N1
PB2
PB1
PA
HA
NP
NA
M
NS
5
6
8?
N1
3
N2
2
N?
H1
H2
H3
H1N1
H2N2
H3N?
2009
Gripe estacional por
Influenza A
H3N2 ó H1N1
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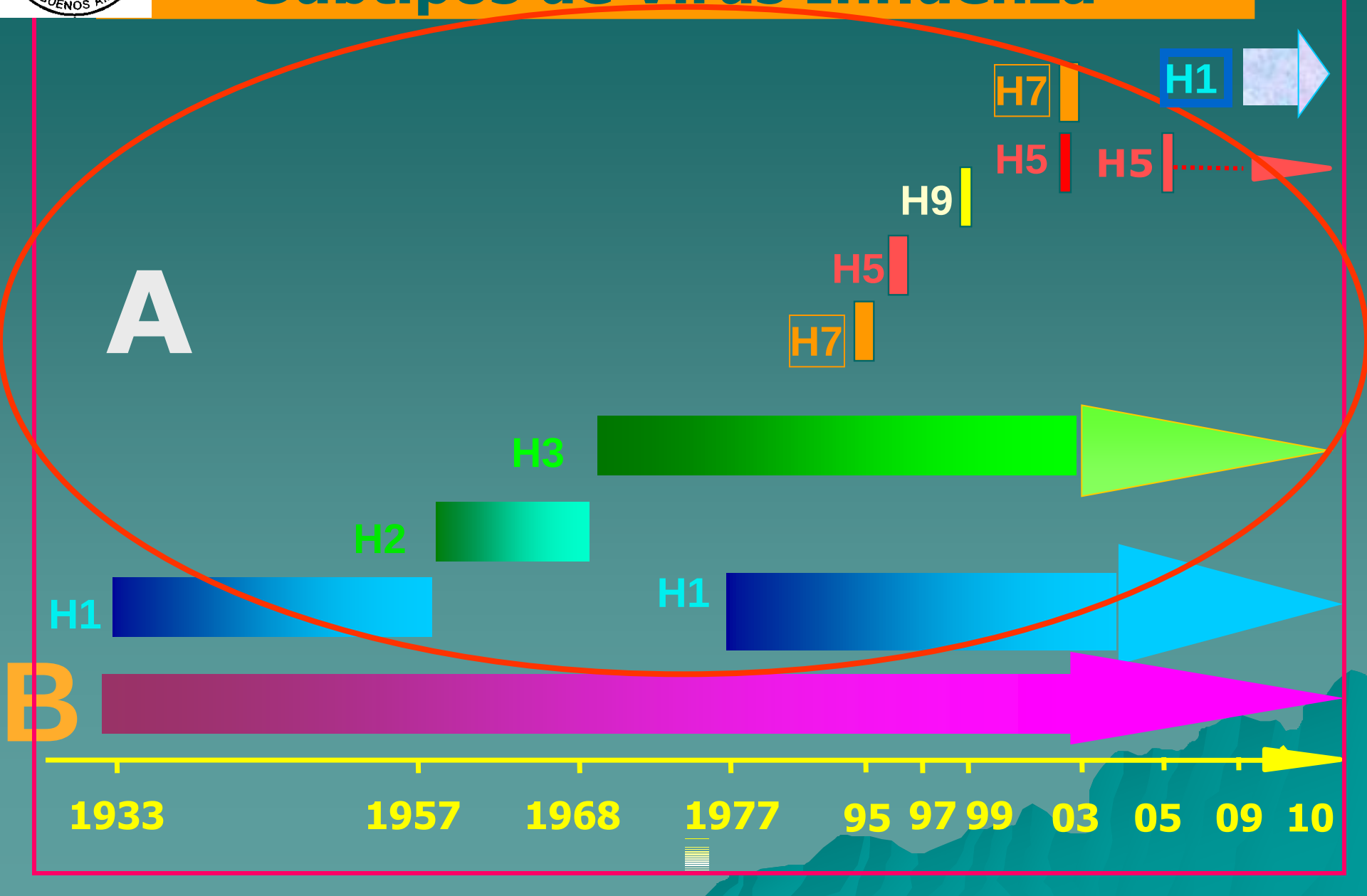
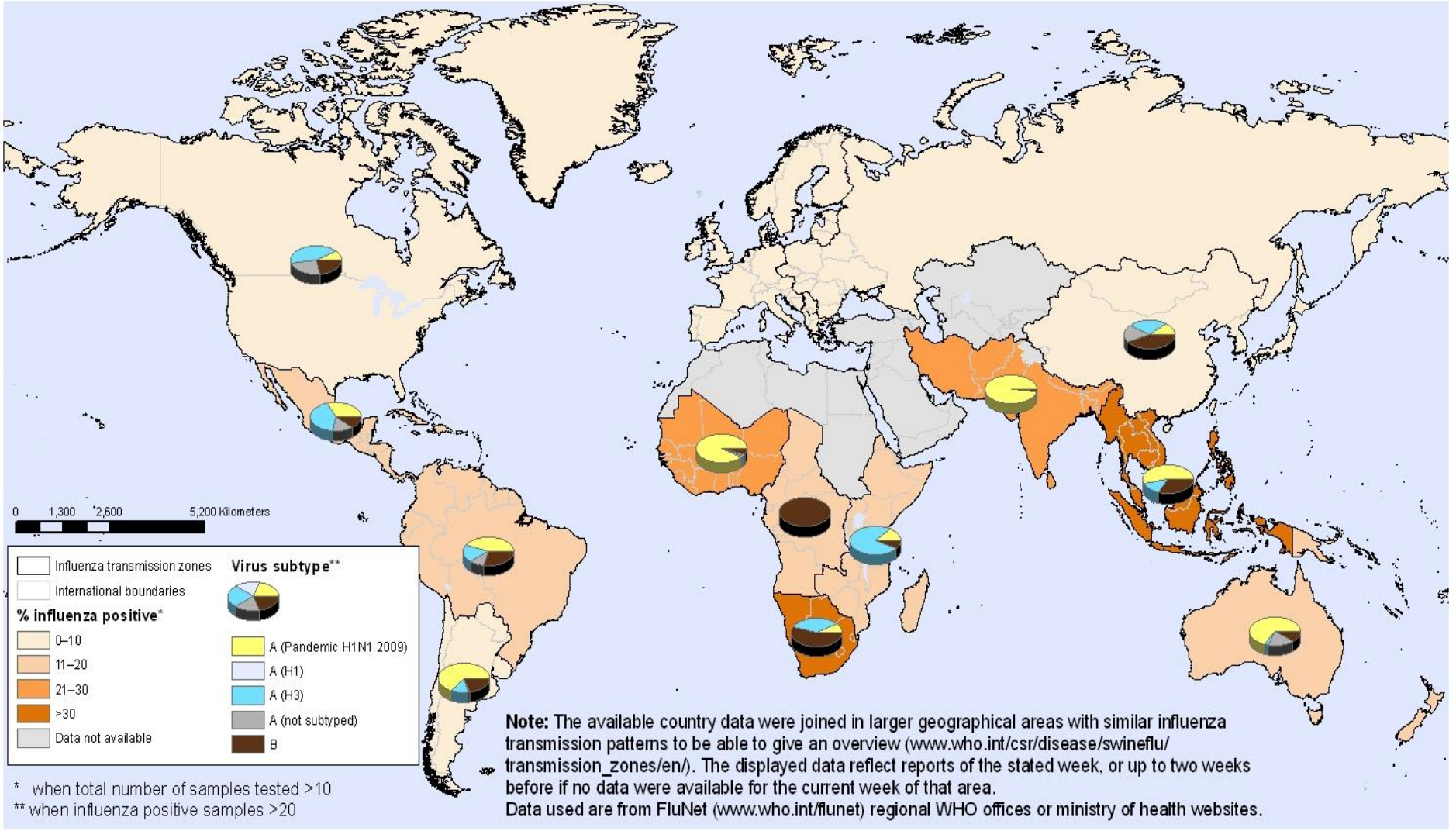
DE BUENOS AIRES

Circulación en Humanos de Tipos y Subtipos de Virus Influenza



Percentage of respiratory specimens that tested positive for influenza

Status as of week 30
25 July – 31 July 2010



* when total number of samples tested >10
** when influenza positive samples >20

Note: The available country data were joined in larger geographical areas with similar influenza transmission patterns to be able to give an overview (www.who.int/csr/disease/swineflu/transmission_zones/en/). The displayed data reflect reports of the stated week, or up to two weeks before if no data were available for the current week of that area.
Data used are from FluNet (www.who.int/flunet) regional WHO offices or ministry of health websites.

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

Fase actual de alerta de la OMS para Influenza A H5N1 (aviar)

Fase inter-pandémica *Nuevo virus circula en animales. No C.H.*	Bajo riesgo de casos humanos (C.H.)	Fase 1
	Mayor riesgo de C.H.	Fase 2
Alerta de Pandemia *Nuevo virus causa C.H.*	Ausencia o limitado Nº de C:H.	Fase 3
	Aumento de la transmisión interhumana	Fase 4
	Significativo aumento de la transmisión interhumana	Fase 5
Pandemia	Eficiente y sostenida transmisión interhumana	Fase 6

Fase actual de alerta de la OMS para Influenza A 2009 (H1N1)

Fase inter-pandémica *Nuevo virus circula en animales. No C.H.*	Bajo riesgo de casos humanos (C.H.)	Fase 1
	Mayor riesgo de C.H.	Fase 2
Alerta de Pandemia *Nuevo virus causa C.H.*	Ausencia o limitado Nº de C:H.	Fase 3
	Aumento de la transmisión interhumana	Fase 4
	Significativo aumento de la transmisión interhumana	Fase 5
Pandemia	Eficiente y sostenida transmisión interhumana	**Hasta el 10-8-2010**

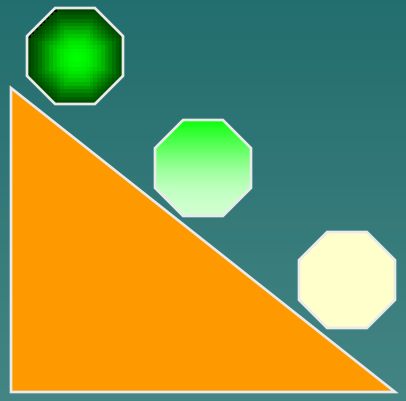
Pandemias de gripe

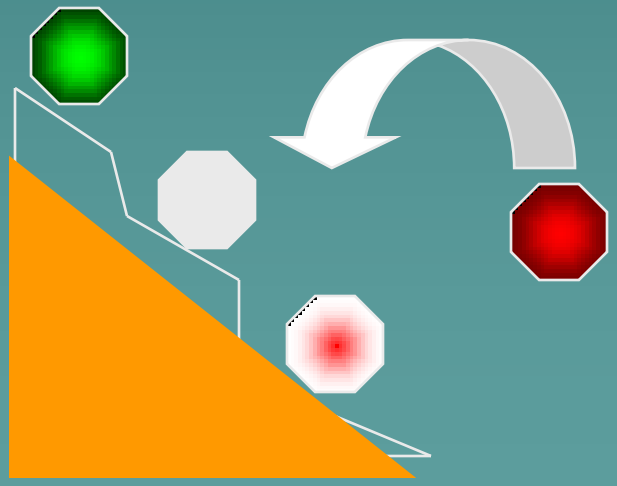
- Aprendamos del pasado…
- Exploremos el presente…
- Preparémonos para el futuro…

Pandemias y alertas de pandemia en los siglos XIX, XX y XXI

- 1891-92 H3
- **1918-19 "Gripe española" H1N1**
- **1957 "Gripe asiática" H2N2**
- **1968 "Gripe de Hong Kong" H3N2**
- 1976 Episodio de Fort Dix "gripe porcina"
- 1977 "Gripe rusa" H1N1
- 1997 Influenza aviar A (H5N1)
- 2004 Influenza aviar A (H5N1)
- 2005 - 2009 Influenza aviar A (H5N1)
- **2009 Influenza A (H1N1/09)**

Variabilidad del virus Influenza





- *Cambios menores:* Falta de lectura de prueba de la Polimerasa viral (no se corrigen errores: se acumulan mutaciones)

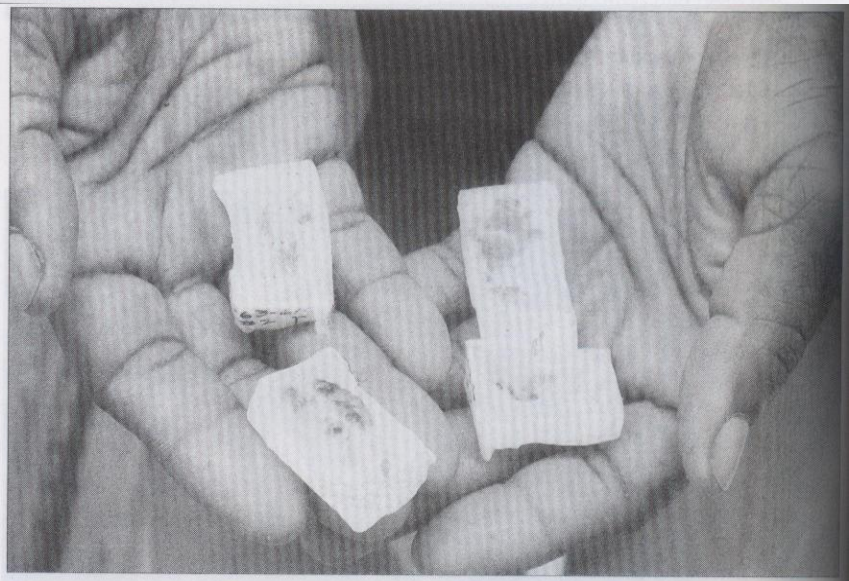
- ***CAMBIOS MAYORES***
  - * **Reasociación** génica
  - * Recombinación génica
  - * Adaptación de una cepa de otra especie al humano

Johan Hultin
analizando cuerpos congelados de esquimales muertos en 1918

(Alaska, 1957;

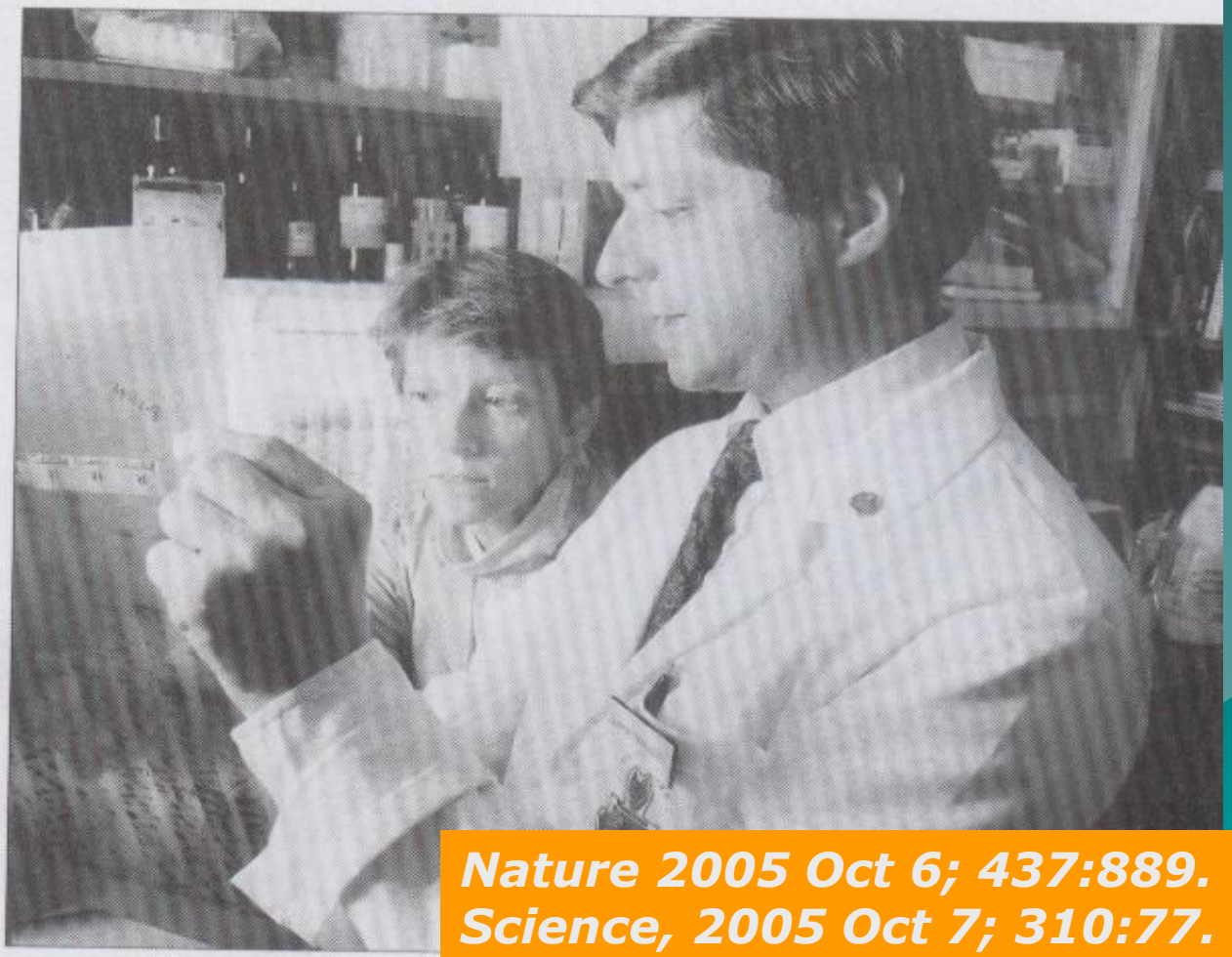
volvería en 1997…)

Johan Hultin and his colleagues in Brevig, Alaska, in June 1951, standing in the mass grave of flu victims whose bodies had been preserved by permafrost since 1918. From left, Hultin, Otto Geist, Jack Layton and Albert McKee (*Courtesy of Johan Hultin*)



Lung tissue samples, preserved in paraffin, from victims of the 1918 flu. These and more than 3 million other tissue samples are stored in the National Tissue Repository maintained by the Armed Forces Institute of Pathology (*Courtesy of Eric Haase*)

Jeffrey Taubenberger Secuenció el genoma completo del virus Influenza H1N1 de 1918



Jeffery Taubenberger and Ann Reid examine DNA readouts at the Armed Forces Institute of Pathology in Washington, D.C. They are studying tissue samples from victims of the 1918 flu to try to determine what made the virus so deadly (*Courtesy of Eric Haase*)

Nature 2005 Oct 6; 437:889.
Science, 2005 Oct 7; 310:77.

El inicio de una enfermedad emergente

Brote de Hong Kong 1997 por Influenza A (H5N1)

Factor principal de infección:
Exposición a aves de granja una semana antes de la enfermedad. (Período de incubación:.2-10 días)

Influenza A (H5N1) en Hong Kong Mayo - Diciembre 1997

- 18 casos confirmados (16 por aislamiento viral, 2 por seroconversión)
- 6 muertos
- Brotes concomitantes de enfermedad por A(H5N1) en aves de corral. A/goose/Guangdong/1/96(H5N1). HPAI para pollos pero no para patos.
- Todos los genes de los virus A (H5N1) eran derivados de virus de influenza aviares.
- No hubo evidencia de reasociación con virus influenza humanos.

Eliminación (sin protección) de pollos probablemente muertos por Influenza H5N1 Tailandia, Febrero de 2004

¿Qué se requiere para la emergencia de una pandemia de influenza ?

1. **Emergencia de una nueva cepa de influenza con una nueva HA *o* una nueva HA y NA (*shift* antigénico).**

2. **Susceptibilidad de la mayoría de la población para la nueva cepa.**

3. **Transmisión humano-humano eficiente del nuevo virus.**

Virus influenza humano

Virus pandémico

Fuente aviar

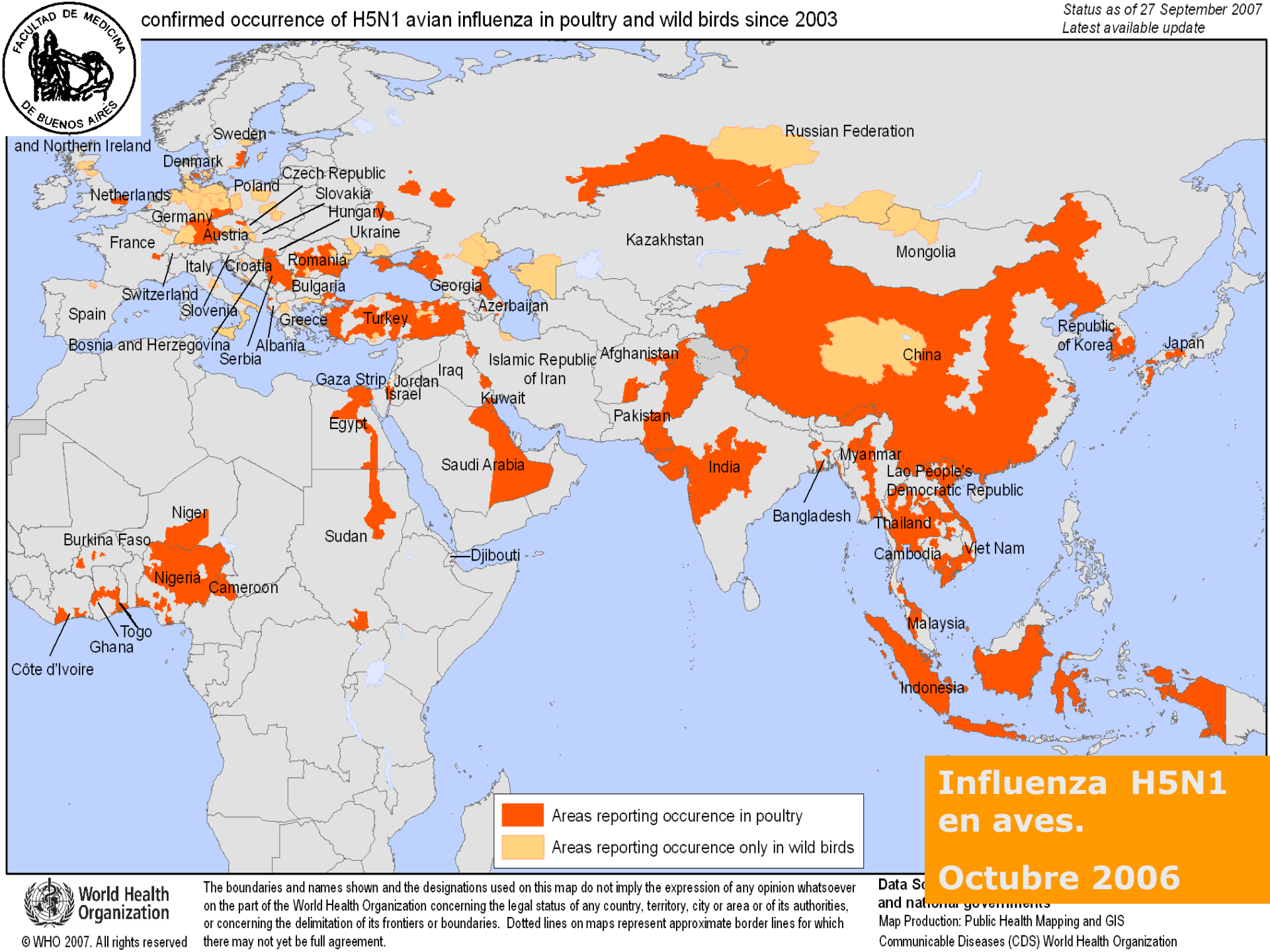
H5N1

Humano infectado

Reasociación

Mutación

Alerta de pandemia


confirmed occurrence of H5N1 avian influenza in poultry and wild birds since 2003
Status as of 27 September 2007
Latest available update
and Northern Ireland
Sweden
Denmark
Netherlands
Poland
Czech Republic
Slovakia
Germany
Hungary
Austria
Ukraine
France
Romania
Italy
Croatia
Bulgaria
Switzerland
Slovenia
Spain
Greece
Turkey
Bosnia and Herzegovina
Albania
Serbia
Russian Federation
Kazakhstan
Mongolia
Georgia
Azerbaijan
China
Republic of Korea
Japan
Afghanistan
Islamic Republic of Iran
Iraq
Gaza Strip
Jordan
Israel
Kuwait
Pakistan
Egypt
Saudi Arabia
India
Myanmar
Lao People's Democratic Republic
Bangladesh
Thailand
Cambodia
Viet Nam
Malaysia
Indonesia
Niger
Burkina Faso
Nigeria
Cameroon
Sudan
Djibouti
Togo
Ghana
Côte d'Ivoire
Areas reporting occurence in poultry
Areas reporting occurence only in wild birds
Influenza H5N1 en aves.
Octubre 2006
World Health Organization
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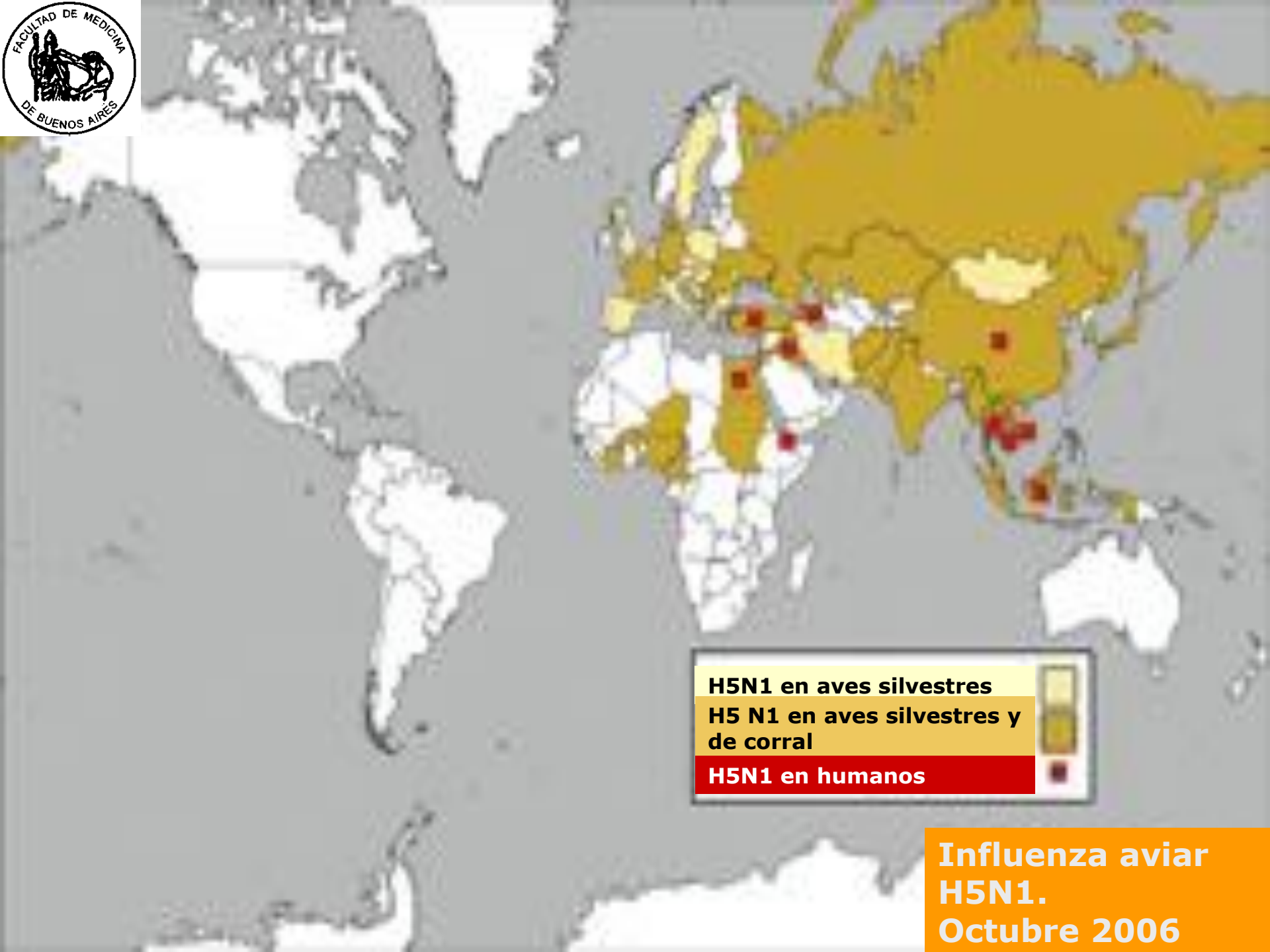
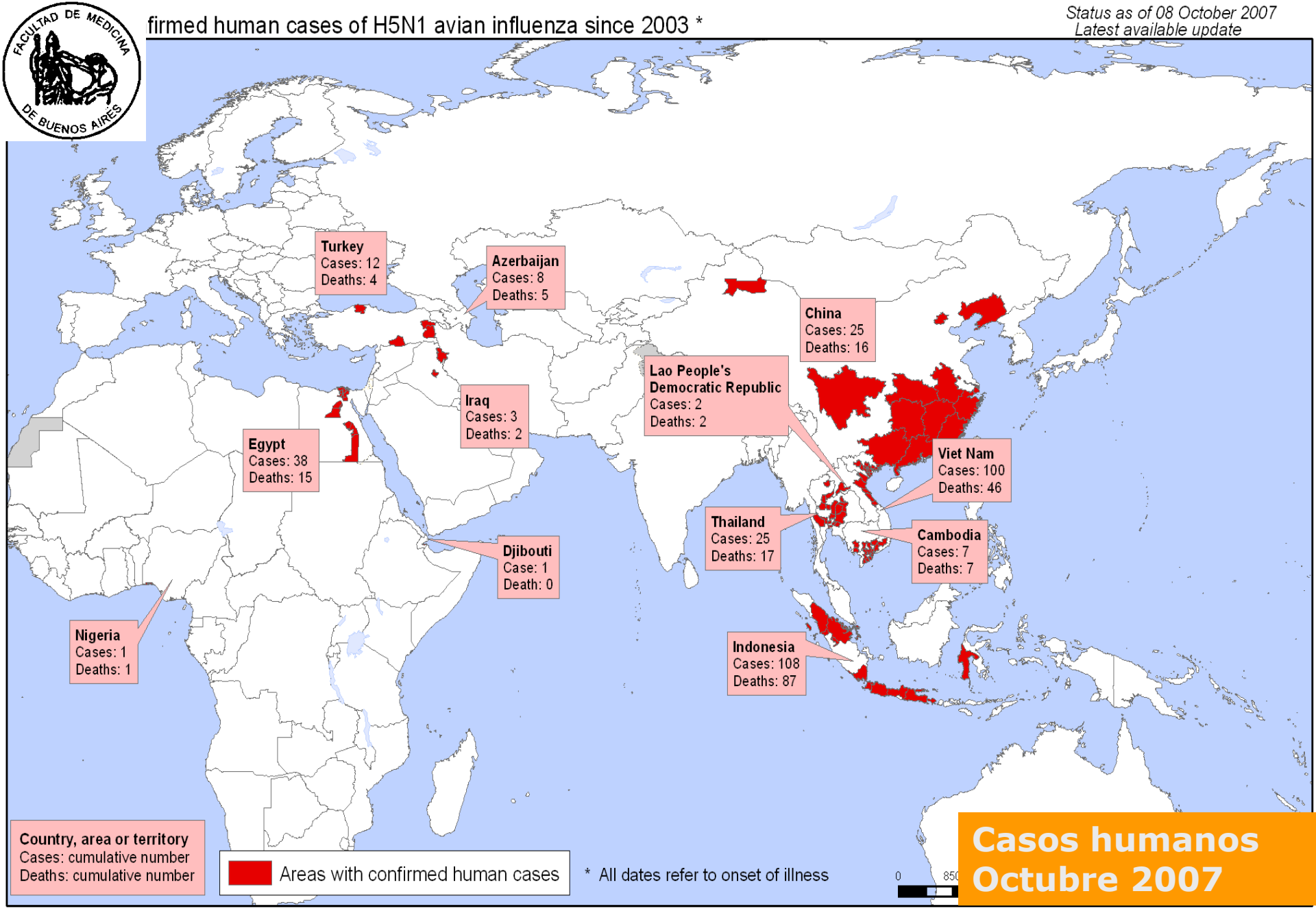
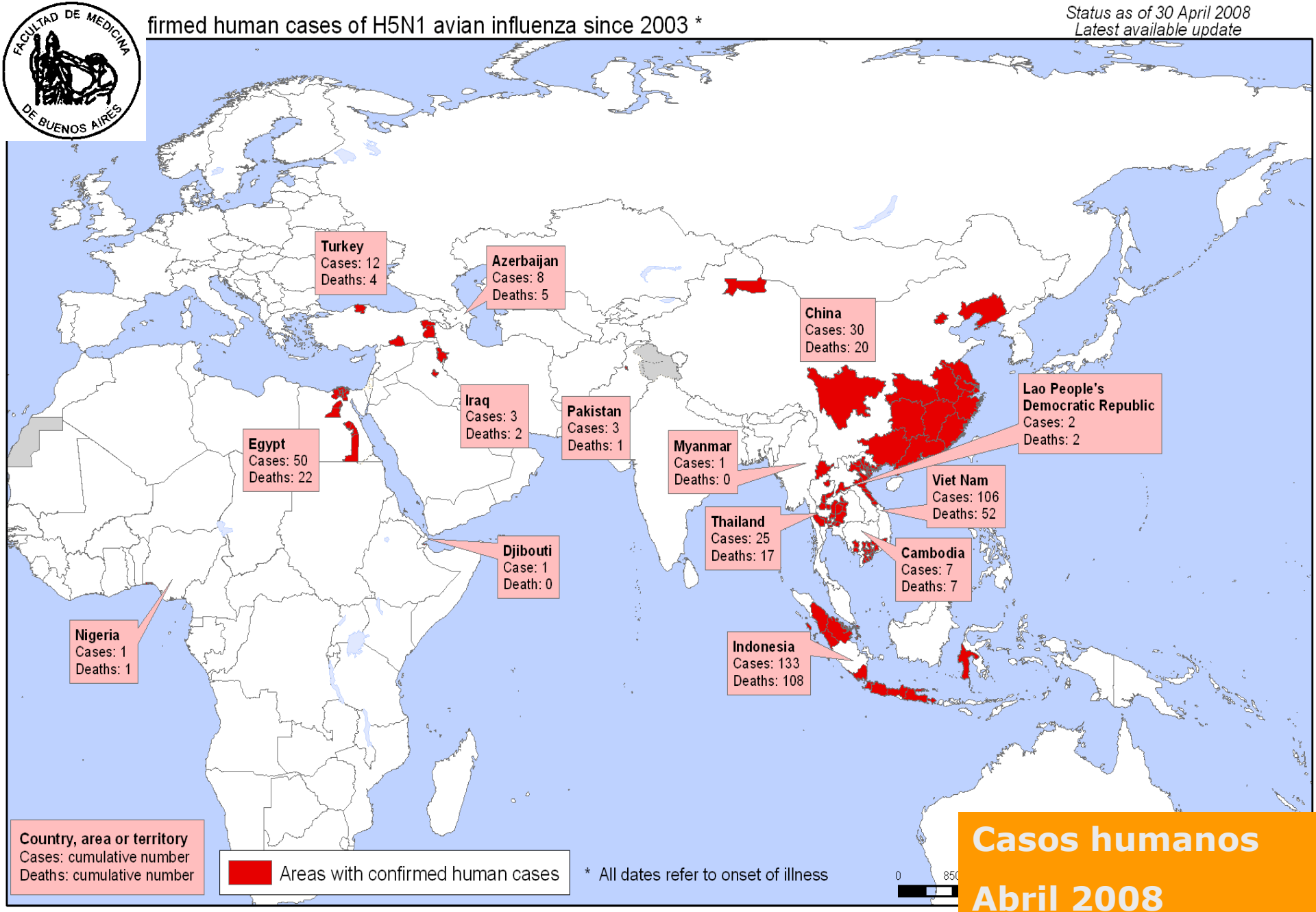
Data Sc
and national governments
Map Production: Public Health Mapping and GIS
Communicable Diseases (CDS) World Health Organization



Influenza aviar H5N1. Octubre 2006



Data Source: WHO / Map Production: Public Health Mapping and GIS
Communicable Diseases (CDS) World Health Organization



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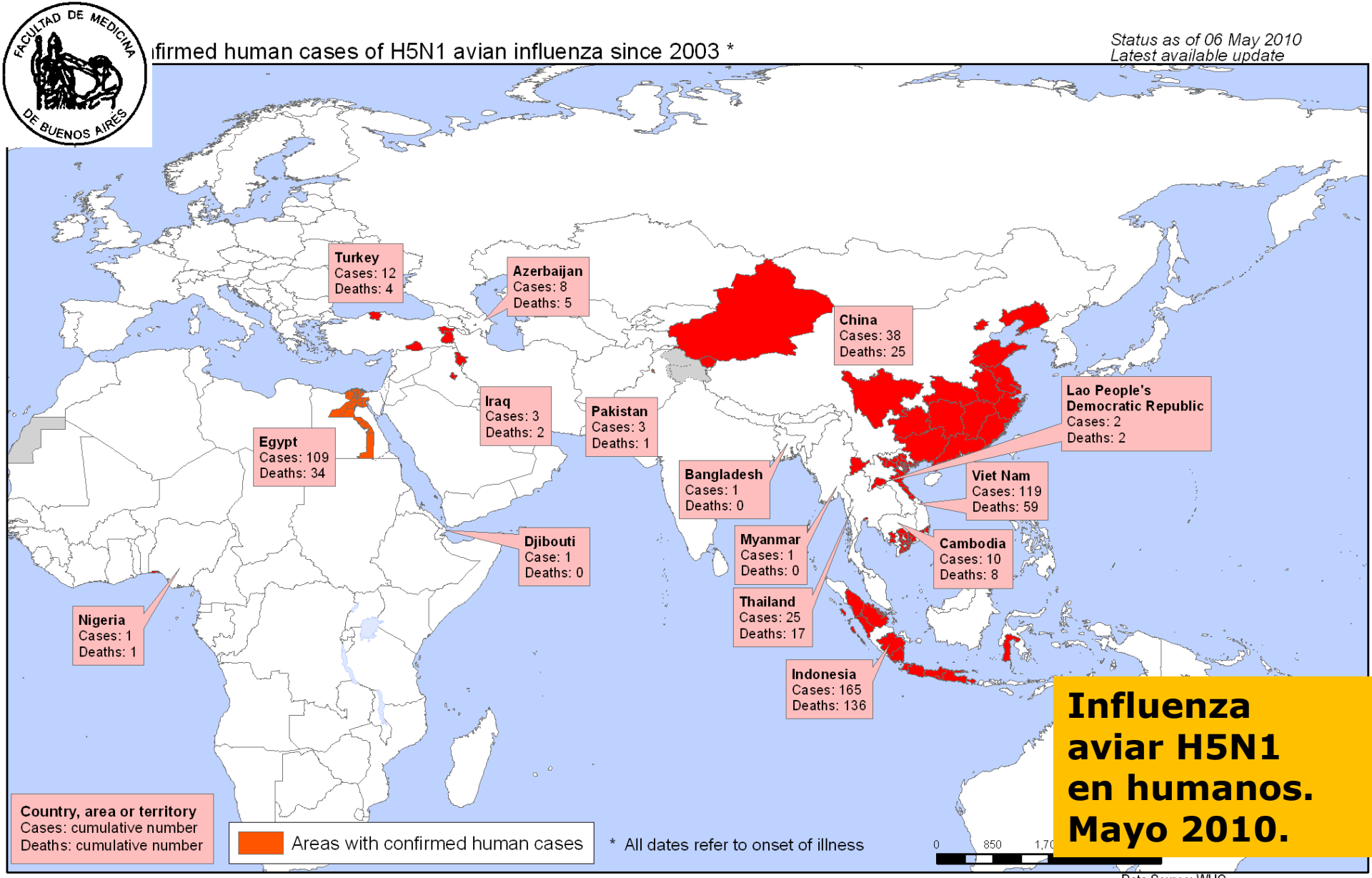
Map Production: Public Health Mapping and GIS
World Health Organization



World Health Organization

Data Source: WHO
Map Production: Public Health Information and Geographic Information System (GIS)
World Health Organization

Global Alert and Response (GAR)

Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO

12 August 2010

Country	2003		2004		2005		2006		2007		2008		2009		2010		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Azerbaijan	0	0	0	0	0	0	8	5	0	0	0	0	0	0	0	0	8	5
Bangladesh	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Cambodia	0	0	0	0	4	4	2	2	1	1	1	0	1	0	1	1	10	8
China	1	1	0	0	8	5	13	8	5	3	4	4	7	4	1	1	39	26
Djibouti	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Egypt	0	0	0	0	0	0	18	10	25	9	8	4	39	4	21	8	111	35
Indonesia	0	0	0	0	20	13	55	45	42	37	24	20	21	19	6	5	168	139
Iraq	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	3	2
Lao People's Democratic Republic	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	2	2
Myanmar	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Nigeria	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1
Pakistan	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	3	1
Thailand	0	0	17	12	5	2	3	3	0	0	0	0	0	0	0	0	25	17
Turkey	0	0	0	0	0	0	12	4	0	0	0	0	0	0	0	0	12	4
Viet Nam	3	3	29	20	61	19	0	0	8	5	6	5	5	5	7	2	119	59
Total	4	4	46	32	98	43	115	79	88	59	44	33	73	32	36	17	504	299

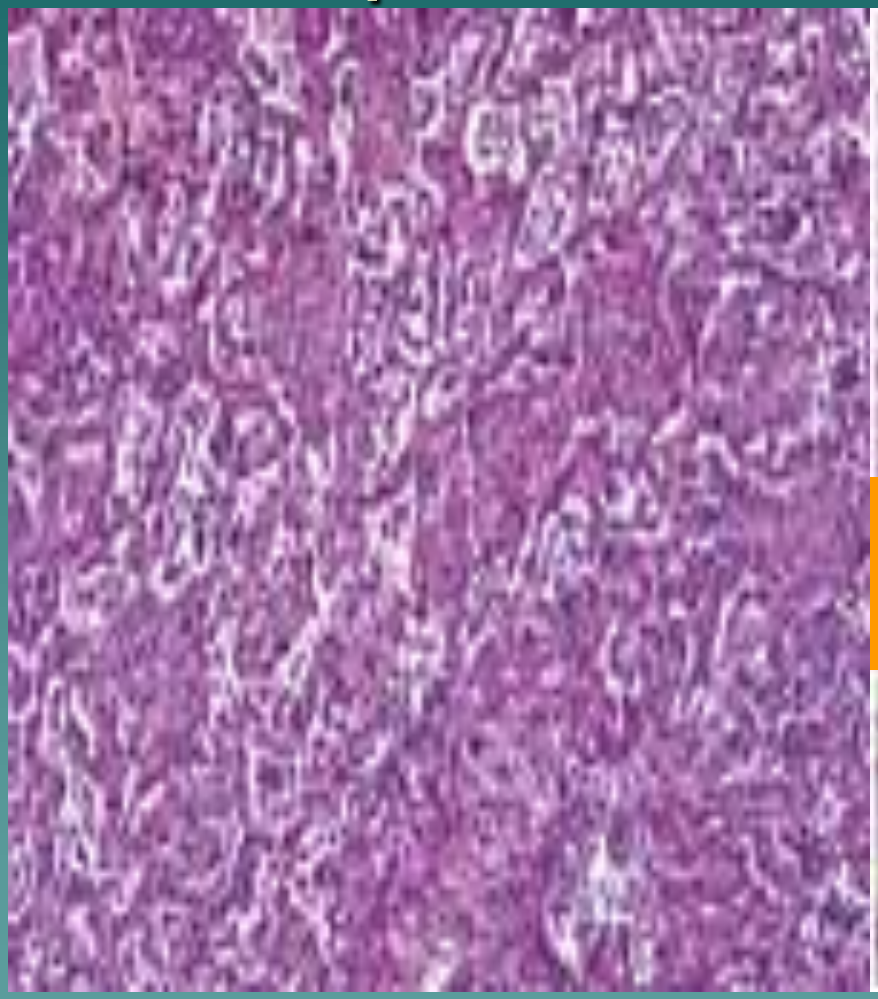
Total number of cases includes number of deaths.
WHO reports only laboratory-confirmed cases.
All dates refer to onset of illness.
Indonesia numbers indicate cumulative total of sporadic cases and deaths which occurred during 2009.

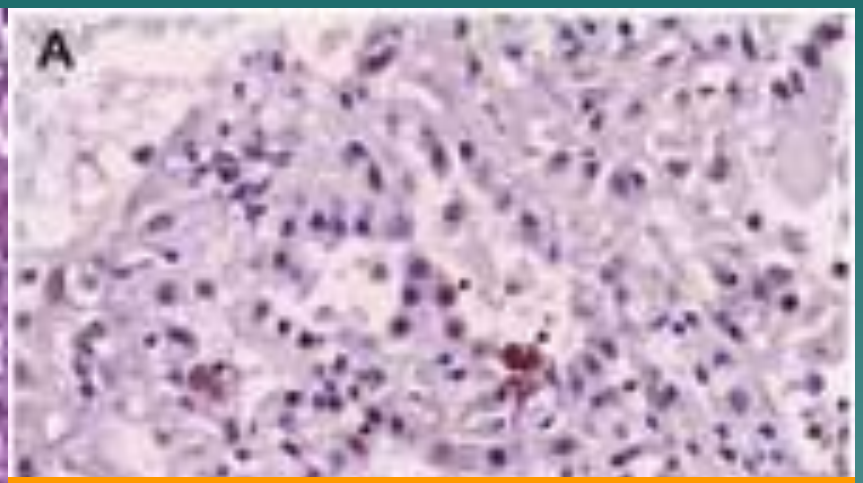
Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO

16 June 2011

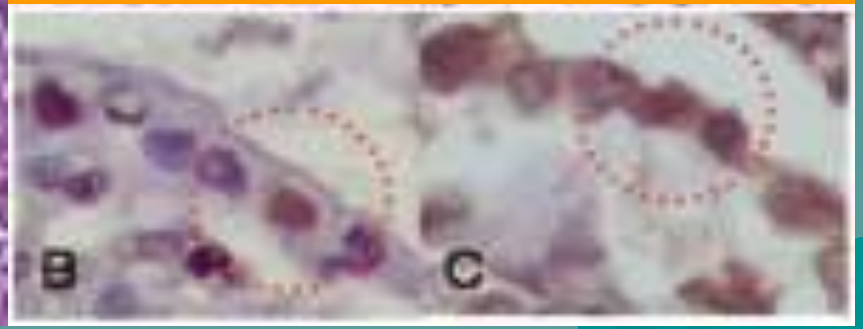
Country	2003		2004		2005		2006		2007		2008		2009		2010		2011		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Azerbaijan	0	0	0	0	0	0	8	5	0	0	0	0	0	0	0	0	0	0	8	5
Bangladesh	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	3	0
Cambodia	0	0	0	0	4	4	2	2	1	1	1	0	1	0	1	1	6	6	16	14
China	1	1	0	0	8	5	13	8	5	3	4	4	7	4	2	1	0	0	40	26
Djibouti	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Egypt	0	0	0	0	0	0	18	10	25	9	8	4	39	4	29	13	30	11	149	51
Indonesia	0	0	0	0	20	13	55	45	42	37	24	20	21	19	9	7	7	5	178	146
Iraq	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	3	2
Lao People's Democratic Republic	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	0	0	2	2
Myanmar	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Nigeria	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	1	1
Pakistan	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	3	1
Thailand	0	0	17	12	5	2	3	3	0	0	0	0	0	0	0	0	0	0	25	17
Turkey	0	0	0	0	0	0	12	4	0	0	0	0	0	0	0	0	0	0	12	4
Viet Nam	3	3	29	20	61	19	0	0	8	5	6	5	5	5	7	2	0	0	119	59
Total	4	4	46	32	98	43	115	79	88	59	44	33	73	32	48	24	45	22	561	328

Neumonía intersticial por Influenza A H5N1





Infiltrado intersticial y expresión de antígenos virales



Mecanismos patogénicos de Influenza

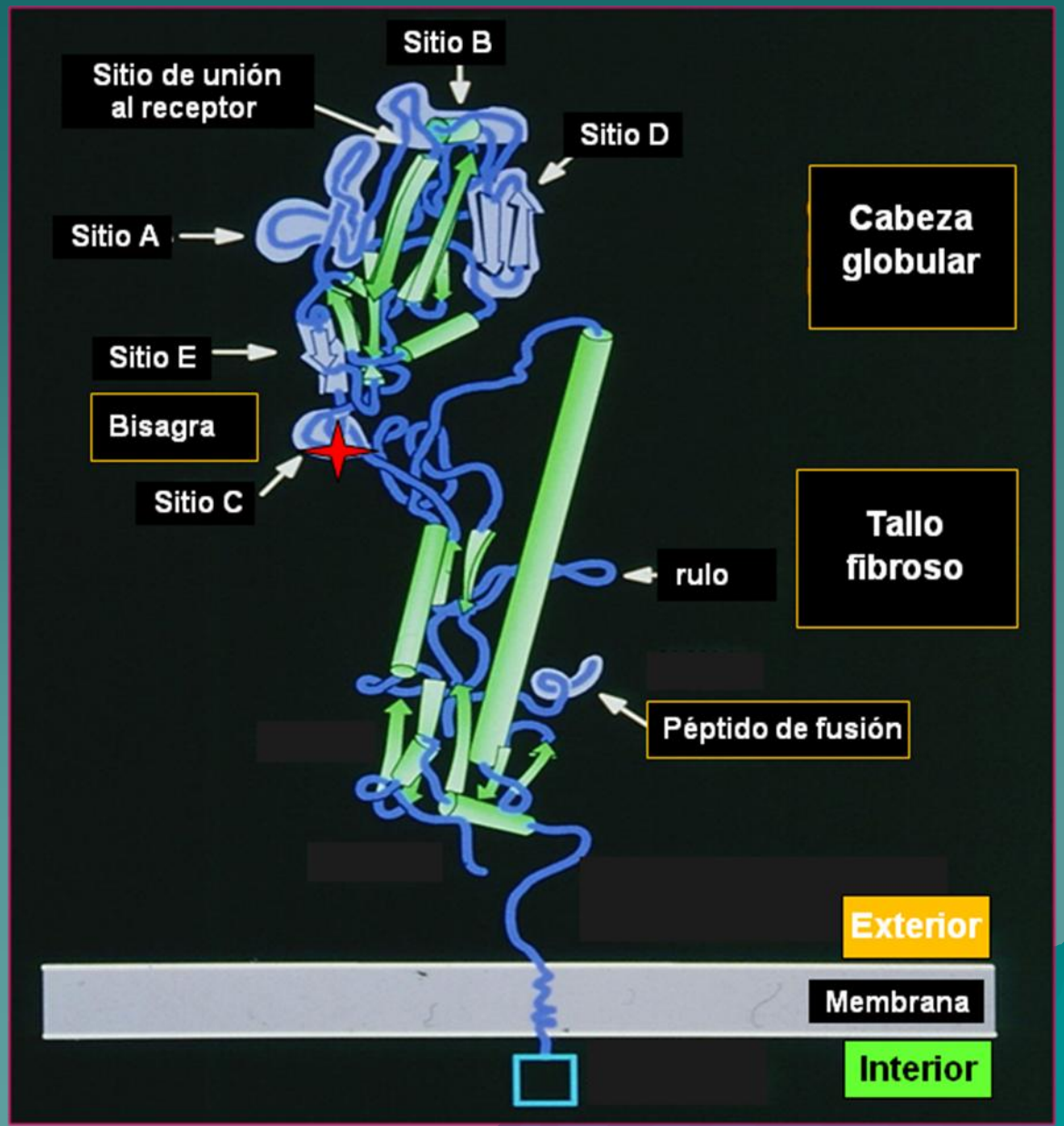
Estrategias

- **Resistencia**
- **Contraataque**
- **Escape**

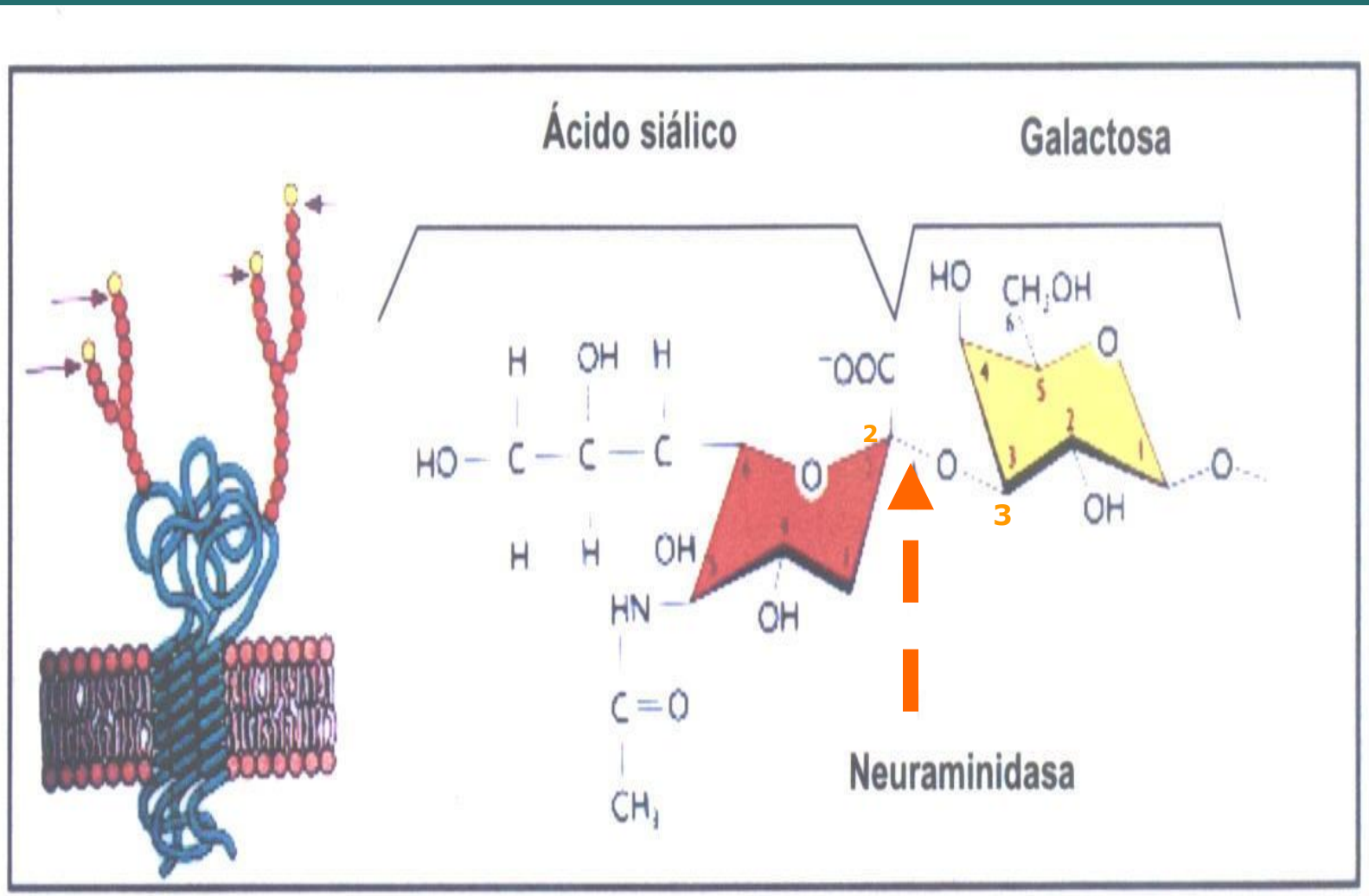
Escape a la respuesta inmune (por modificación viral)

- Modificación de epítopes T y B (cambios menores)
- **Introducción (por reasociación o adaptación génica) de nuevos antígenos (cambios mayores) con alta virulencia**

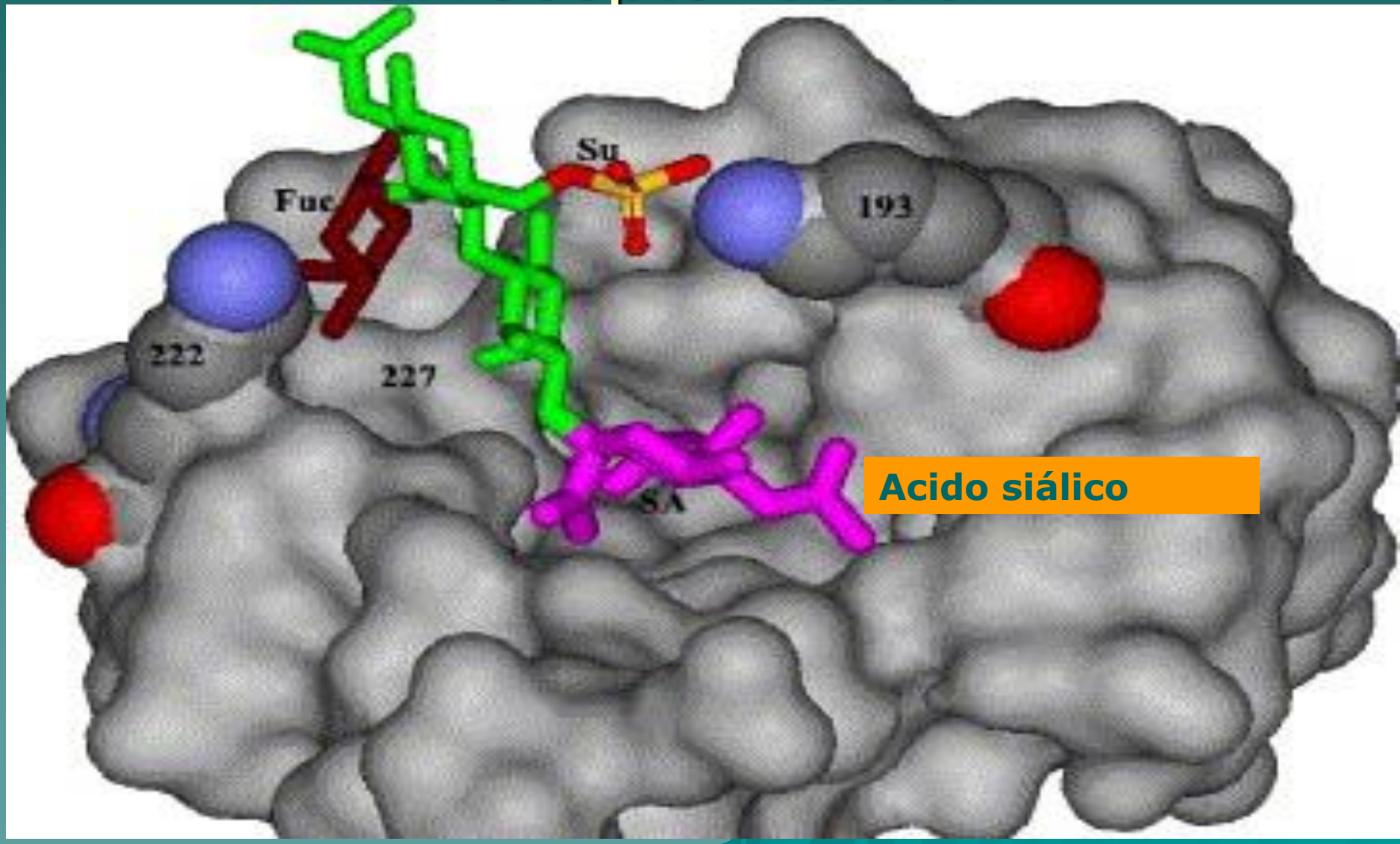
ESQUEMA DE UN MONÓMERO DE HA



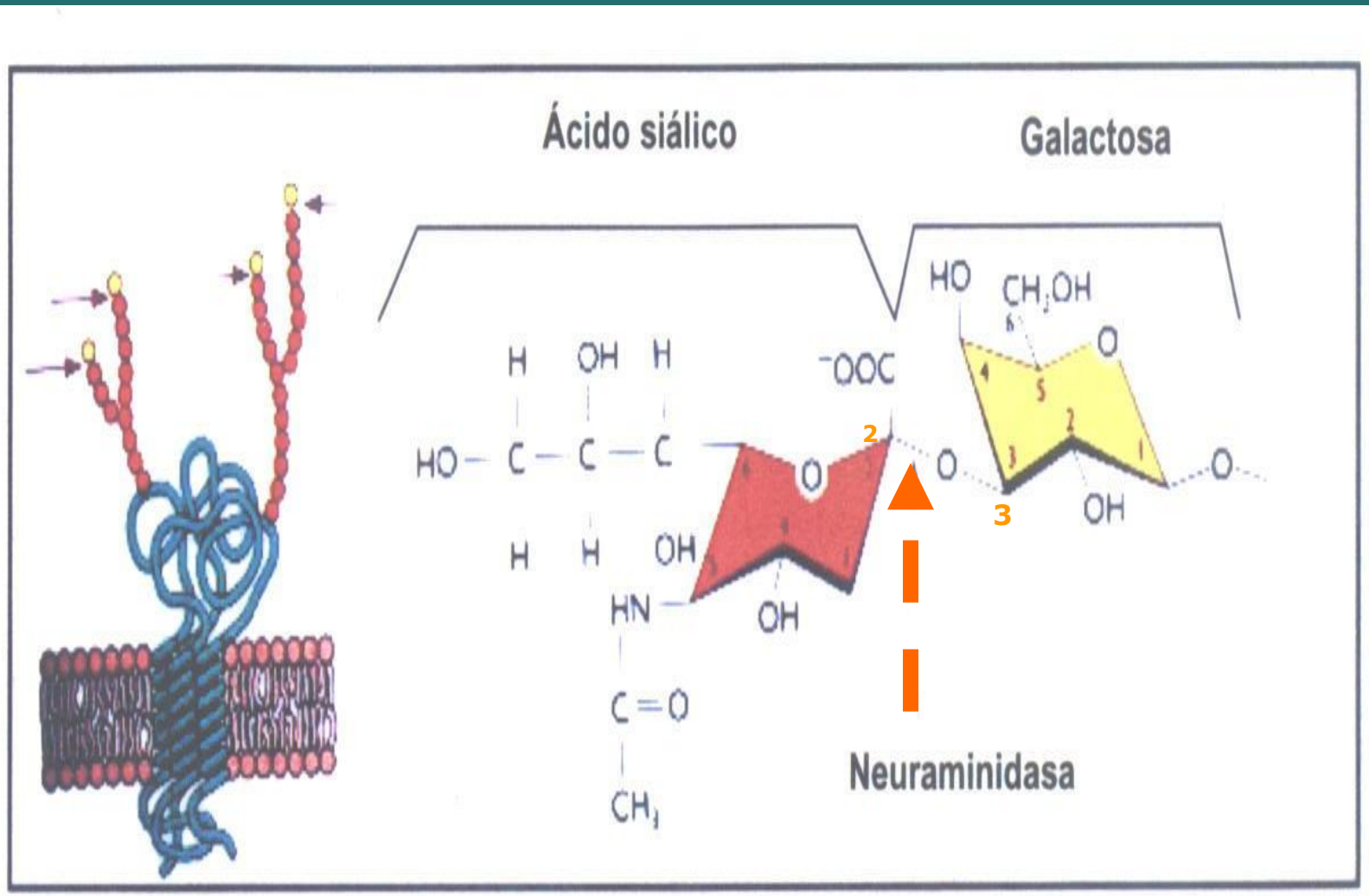
Reconocimiento del receptor de ácido siálico con 2-3 Galactosa

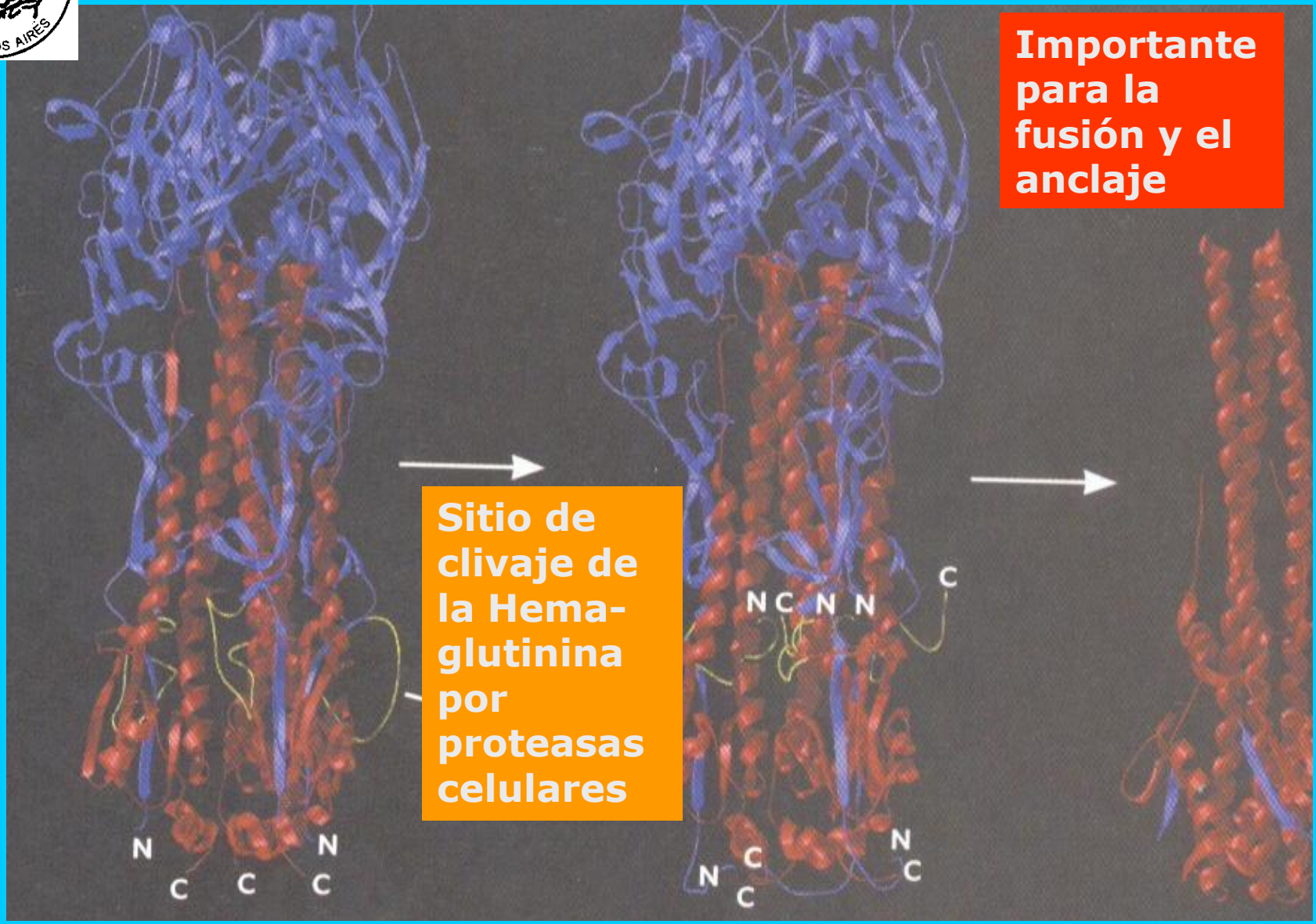


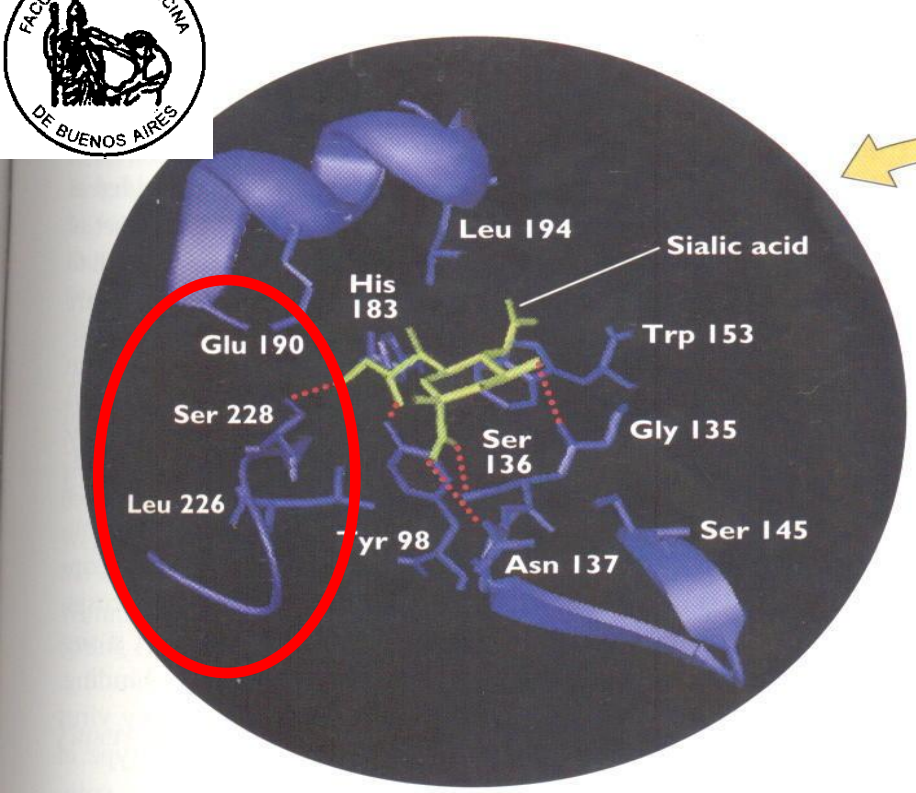
Unión de la hemaglutinina viral al receptor celular

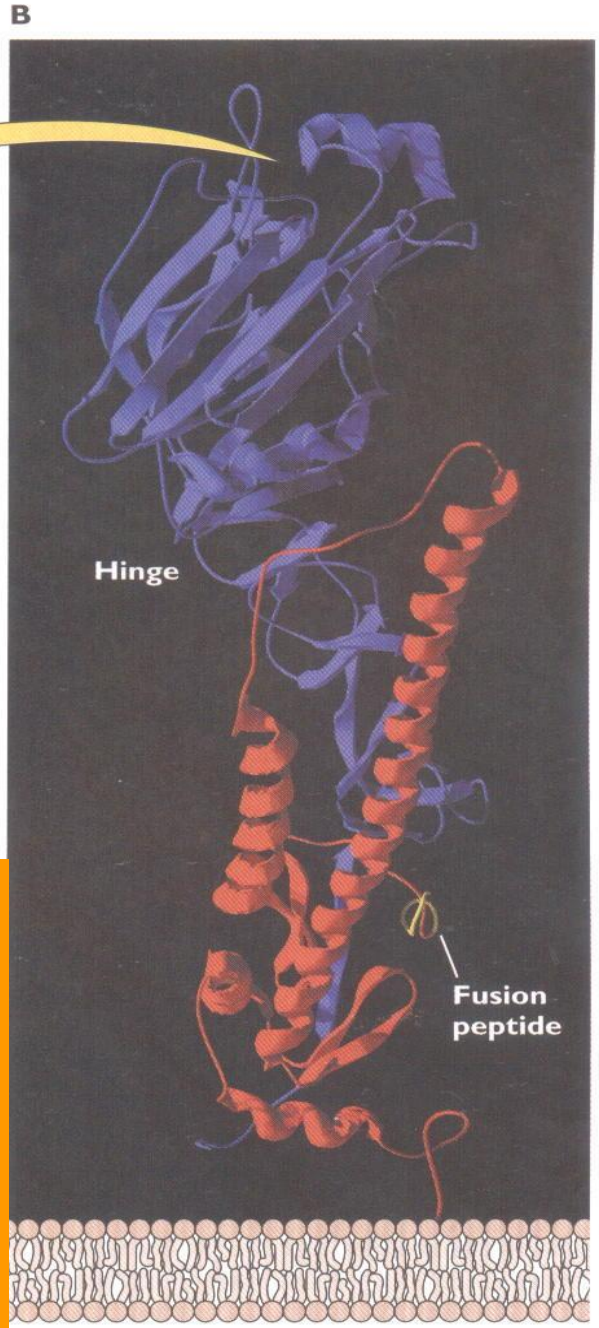


Clivaje de ácido siálico por la Neuraminidasa viral




Importante para la fusión y el anclaje
Sitio de clivaje de la Hema-glutinina por proteasas celulares
N
C
C
N
C
N C N N
C
N
C
C
N
C





Cabeza globular

Asa fibrosa

Membrana

Unión al receptor de Ácido siálico,2-6 Galactosa y sitio de clivaje de la hemaglutinina

Hemaglutinina de Influenza H5

Avirulenta	….RETR*GLF
Altamente patógena	….RKKR*GLF
HK humanos	…RERRRKKR*GLF

La presencia de múltiples aminoácidos básicos Arg –R- o Lys –K- adyacentes al sitio de clivaje de la HA aumenta el tropismo para diferentes tejidos de los virus aviares.

Factores virales en la infección por Influenza H5 N1

- **Patogenicidad:** multigénica

HA

NA

PB1

PB2 (Lisina 627 y Asparagina 701): aumentan capacidad replicativa;

NS1 (Ac. Glutámico 92: bloquea inhibición por IFN)

- **Transmisibilidad** multigénica

HA

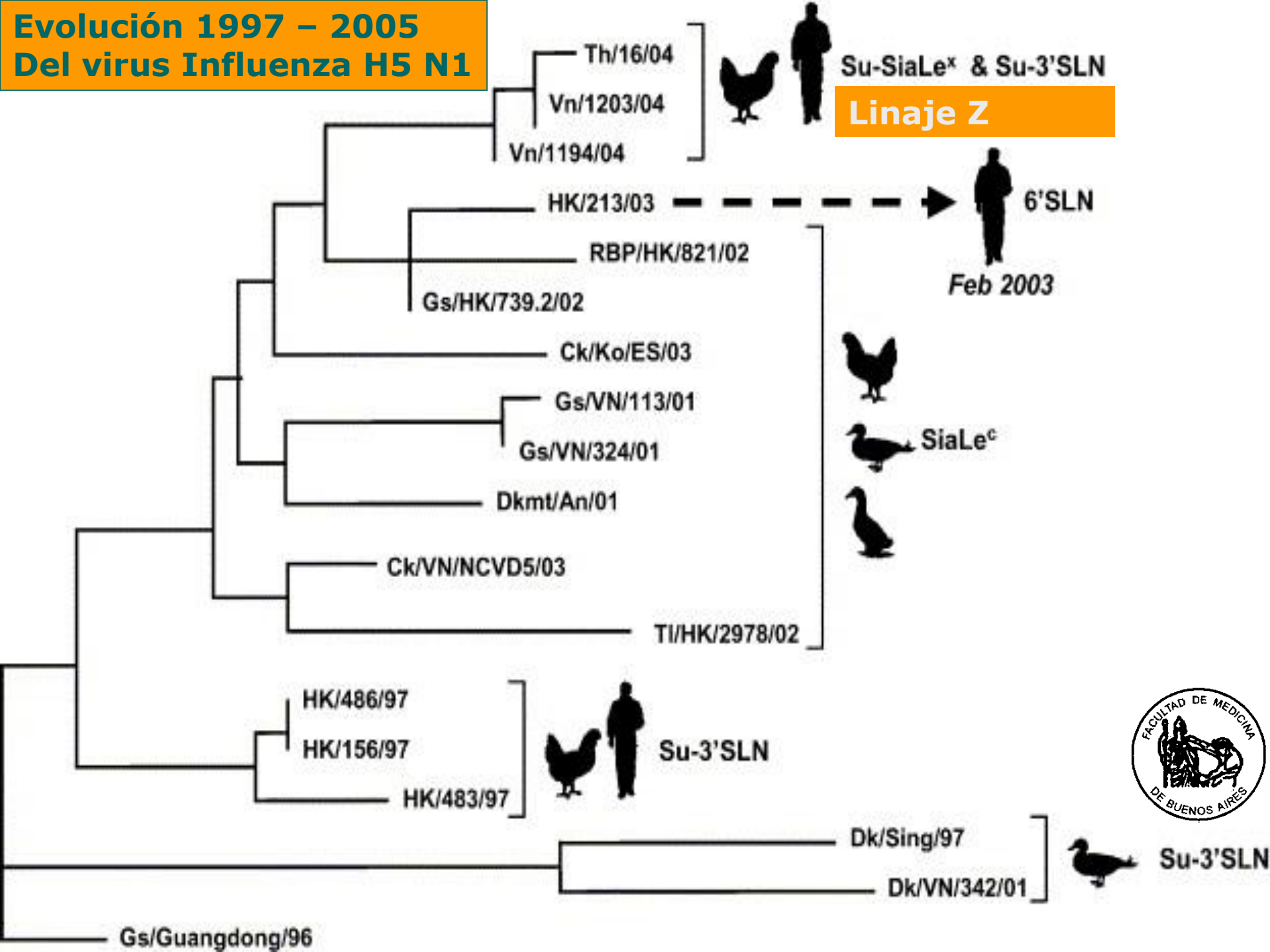
NA

PB1

Restricción de especie del virus Influenza

Influenza	AA en Hemaglutinina	Unión a receptor
Humana	226 Leu 228 Ser (bolsillo ancho)	Humano Ac. Siálico α-2,6-Galactosa (tracto alto)
Aviar (H5N1)	226 Gln 228 Gly (bolsillo estrecho) E190	Aviar Ac. Siálico α-2,3-Galactosa (también *en células cuboidales no ciliadas bronquiolares y en neumonocitos tipo II humanos*)
H1N1 (gripe española)	226 Gln 228 Gly + D190	Humano Ac. Siálico α-2,6-galactosa

Evolución 1997 – 2005
Del virus Influenza H5 N1



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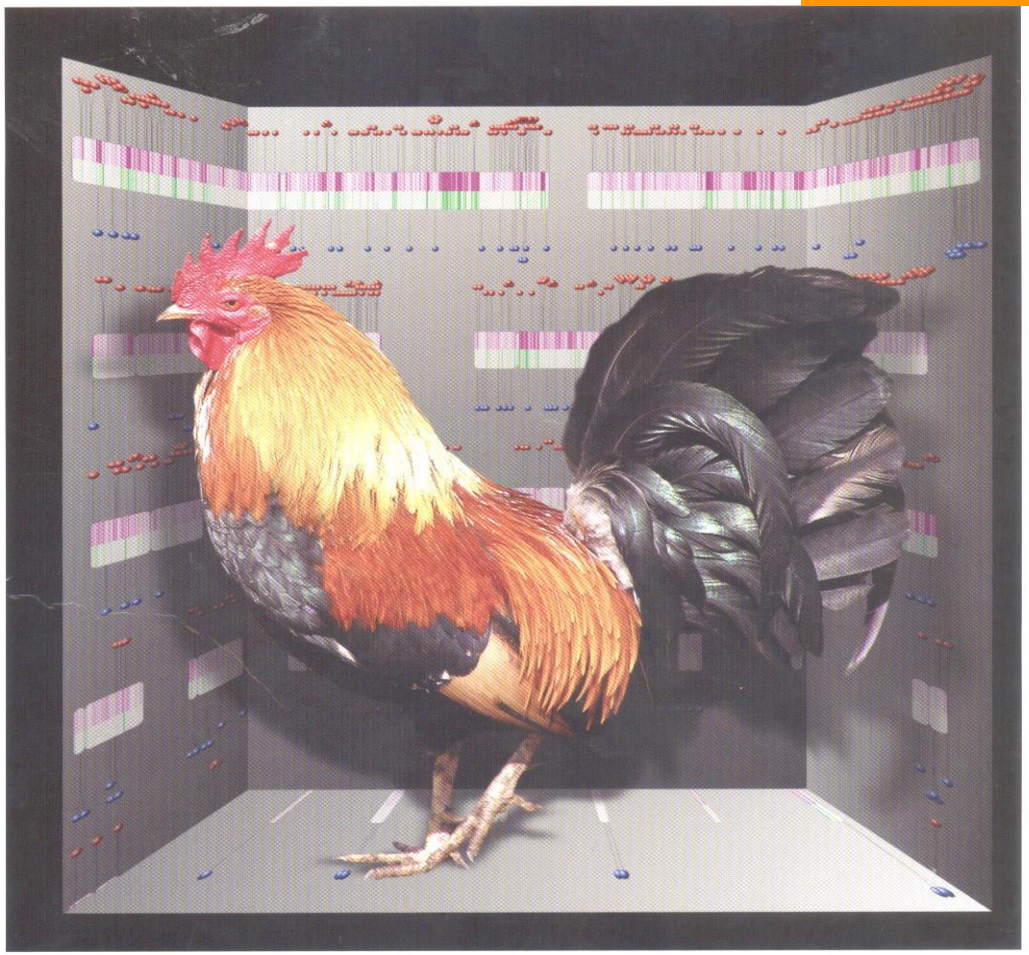
Septiembre 2005



Journal of Virology

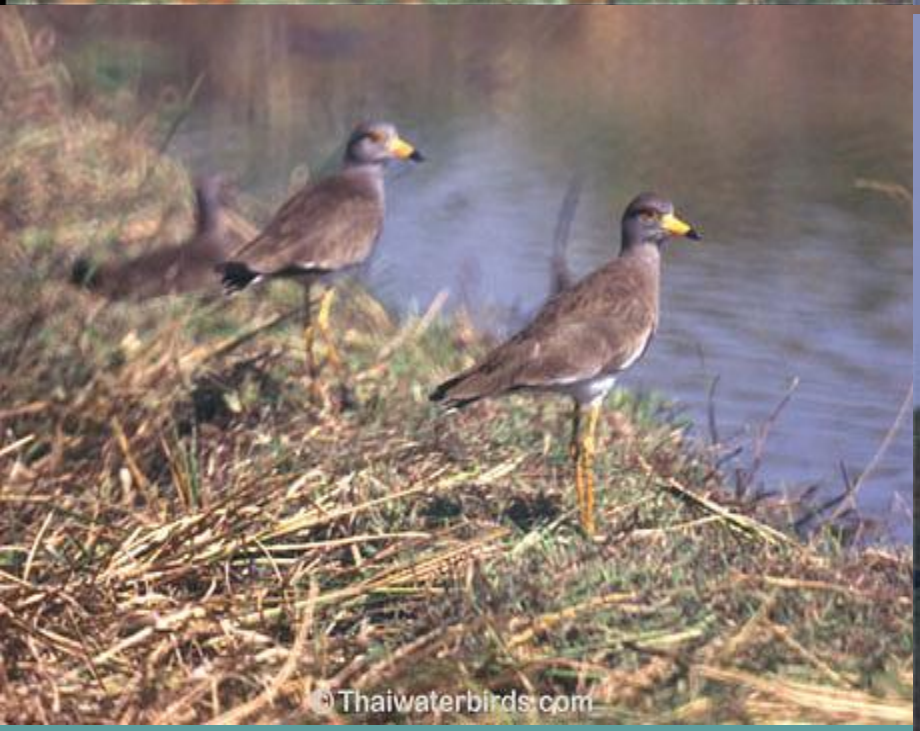
Molecular Basis of Replication of Duck H5N1 Influenza Viruses in a Mammalian Mouse Model

Zejun Li,[1] Hualan Chen,[1]* Peirong Jiao,[1] Guohua Deng,[1] Guobin Tian,[1] Yanbing Li,[1] Erich Hoffmann,[2] Robert G. Webster,[2] Yumiko Matsuoka,[3] and Kangzhen Yu[1]*

Animal Influenza Laboratory, Ministry of Agriculture, and National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin 150001, People's Republic of China[1]; Division of Virology, Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee 38105[2]; and Influenza Branch, Centers for Disease Control, 1600 Clifton Road, Atlanta, Georgia 30333[3]

Received 27 January 2005/Accepted 10 June 2005

We recently analyzed a series of H5N1 viruses isolated from healthy ducks in southern China since 1999 and found that these viruses had progressively acquired the ability to replicate and cause disease in mice. In the present study, we explored the genetic basis of this change in host range by comparing two of the viruses that are genetically similar but differ in their ability to infect mice and have different pathogenicity in mice. A/duck/Guangxi/22/2001 (DKGX/22) is nonpathogenic in mice, whereas A/duck/Guangxi/35/2001 (DKGX/35) is highly pathogenic. We used reverse genetics to create a series of single-gene recombinants that contained one gene from DKGX/22 and the remaining seven gene segments from DKGX/35. We find that the PA, NA, and NS genes of DKGX/22 could attenuate DKGX/35 virus to some extent, but PB2 of DKGX/22 virus attenuated the DKGX/35 virus dramatically, and an Asn-to-Asp substitution at position 701 of PB2 plays a key role in this function. Conversely, of the recombinant viruses in the DKGX/22 background, only the one that contains the PB2 gene of DKGX/35 was able to replicate in mice. A single amino acid substitution (Asp to Asn) at position 701 of PB2 enabled DKGX/22 to infect and become lethal for mice. These results demonstrate that amino acid Asn 701 of PB2 is one of the important determinants for this avian influenza virus to cross the host species barrier and infect mice, though the replication and lethality of H5N1 influenza viruses involve multiple genes and may result from a constellation of genes. Our findings may help to explain the expansion of the host range and lethality of the H5N1 influenza viruses to humans.



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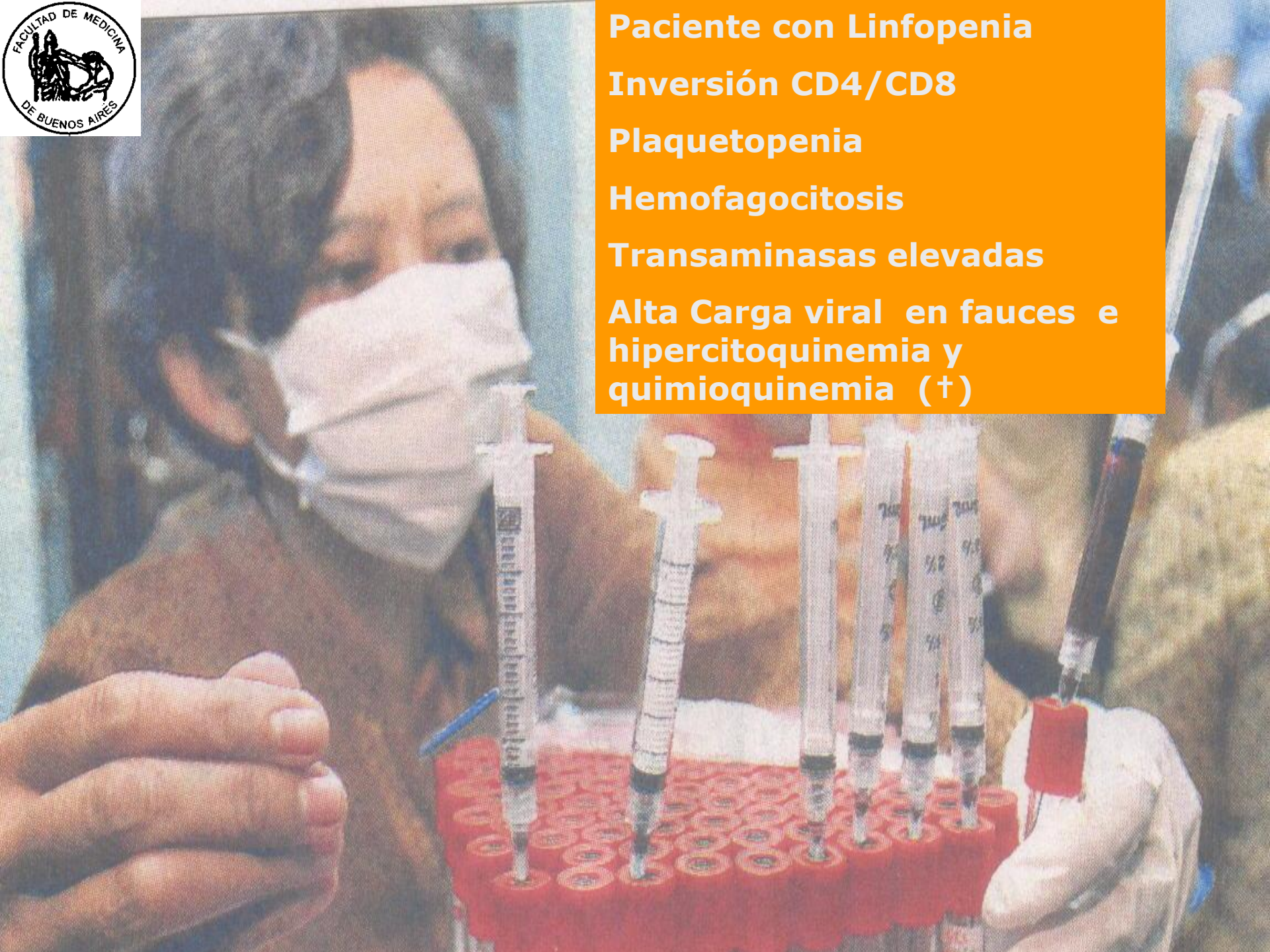
DE BUENOS AIRES

Gendarmes rumanos trasladan un ganso muerto posiblemente por gripe aviar.

Bucarest, Octubre de 2005

Influenza aviar H5N1
Período de incubación: 2 - 10 días



Paciente con Linfopenia

Inversión CD4/CD8

Plaquetopenia

Hemofagocitosis

Transaminasas elevadas

Alta Carga viral en fauces e hipercitoquinemia y quimioquinemia (†)

El diagnóstico virológico

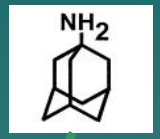
Métodos directos

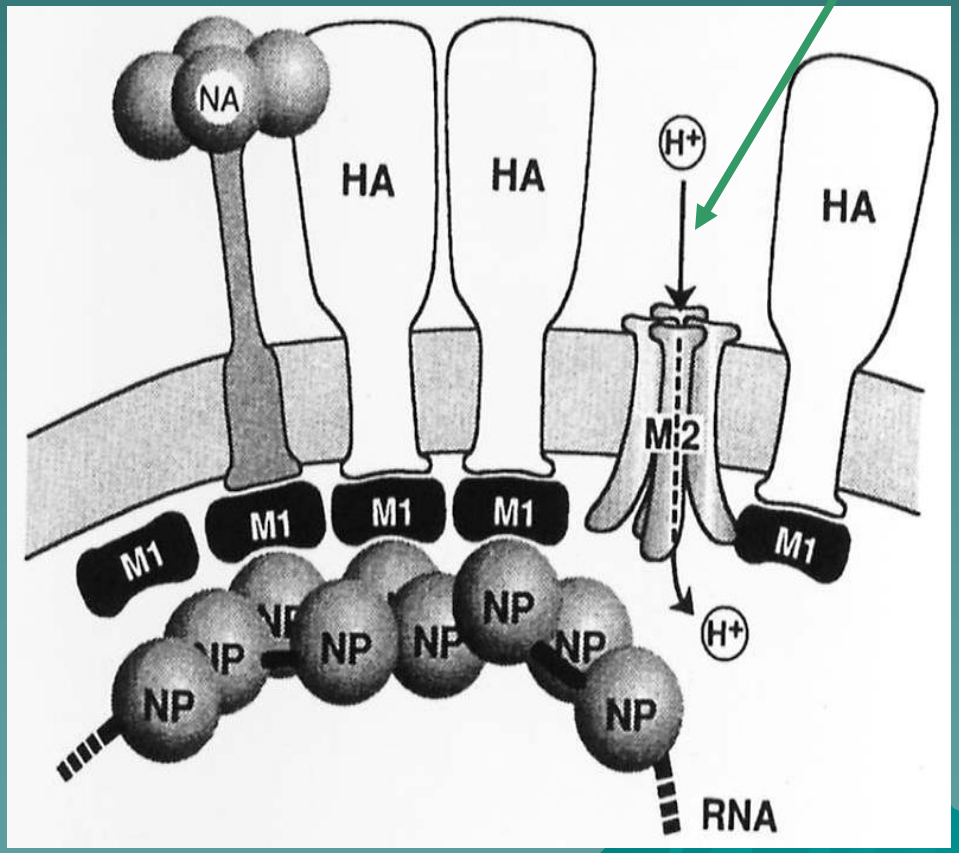
- Aislamiento viral
- Detección de antígeno
- **Detección genómica por RT-PCR y tipificación por RFLP o RT-PCR en tiempo real**

Métodos indirectos

- Serología (con fines epidemiológicos)

AMANTADINA
Bloqueo del canal iónico M2

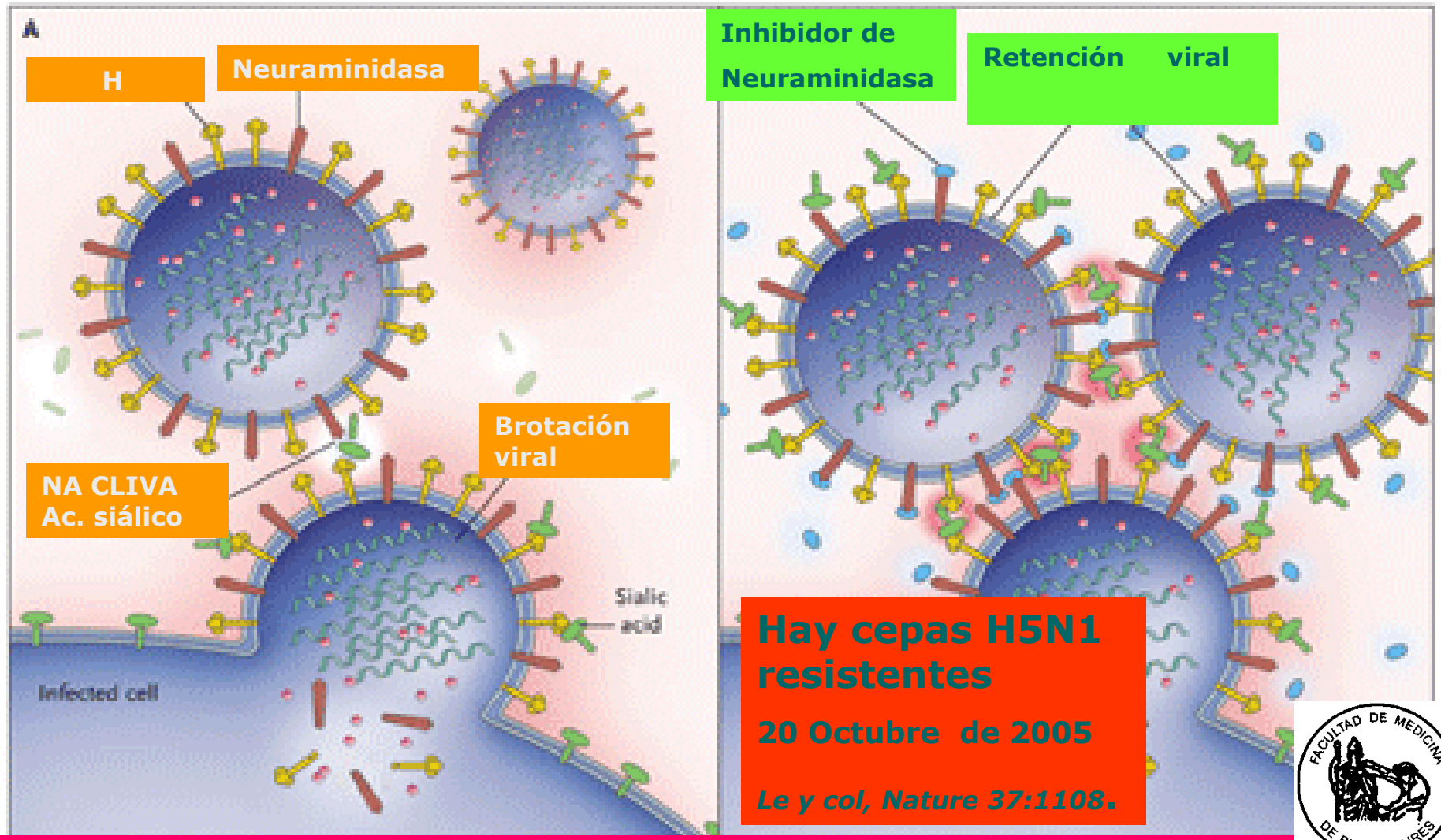




Ya hay cepas H5N1 resistentes

(2004)

ACCION DE INHIBIDORES DE LA NEURAMINIDASA (*Oseltamivir, Zanamivir*)



Vacunas de Influenza humana (*no aviar H5N1*)

Vacuna a virus completo inactivado:
cultivado en huevos embrionados.

Para H5 N1

- **Se investigan vacunas a DNA**
- **Vacuna producida mediante genética reversa (4 plásmidos)**
- **Inactivadas preparadas en cultivos celulares**
- **Atenuadas H5N1**
- **H5 recombinante en vectores adenovirales**
- **Vacunas conteniendo NP**
- ***VLP (VIRAL LIKE PARTICLES)***

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A Clinical Trial of a Whole-Virus H5N1 Vaccine Derived from Cell Culture

- Hartmut J. et al. N Engl J Med 2008;358:2573-84. (12 June 2008)

Abstr act

Background

Widespread infections of avian species with avian influenza H5N1 virus and its limited spread to humans suggest that the virus has the potential to cause a human influenza pandemic. An urgent need exists for an H5N1 vaccine that is effective against divergent strains of H5N1 virus.

Methods

In a randomized, dose-escalation, phase 1 and 2 study involving six subgroups, we investigated the safety of an H5N1 whole-virus vaccine produced on Vero cell cultures and determined its ability to induce antibodies capable of neutralizing various H5N1 strains. In two visits 21 days apart, 275 volunteers between the ages of 18 and 45 years received two doses of vaccine that each contained 3.75 µg, 7.5 µg, 15 µg, or 30 µg of hemagglutinin antigen with alum adjuvant or 7.5 µg or 15 µg of hemagglutinin antigen without adjuvant. Serologic analysis was performed at baseline and on days 21 and 42.

A Clinical Trial of a Whole-Virus H5N1 Vaccine Derived from Cell Culture (II)

Results

The vaccine induced a neutralizing immune response not only against the clade 1 (A/Vietnam/1203/2004) virus strain but also against the clade 2 and 3 strains. The use of adjuvants did not improve the antibody response. Maximum responses to the vaccine strain were obtained with formulations containing 7.5 µg and 15 µg of hemagglutinin antigen without adjuvant. Mild pain at the injection site (in 9 to 27% of subjects) and headache (in 6 to 31% of subjects) were the most common adverse events identified for all vaccine formulations.

Conclusions

A two-dose vaccine regimen of either 7.5 µg or 15 µg of hemagglutinin antigen without adjuvant induced neutralizing antibodies against diverse H5N1 virus strains in a high percentage of subjects, suggesting that this may be a useful

Nuevas vacunas recombinantes conteniendo H5 y N1

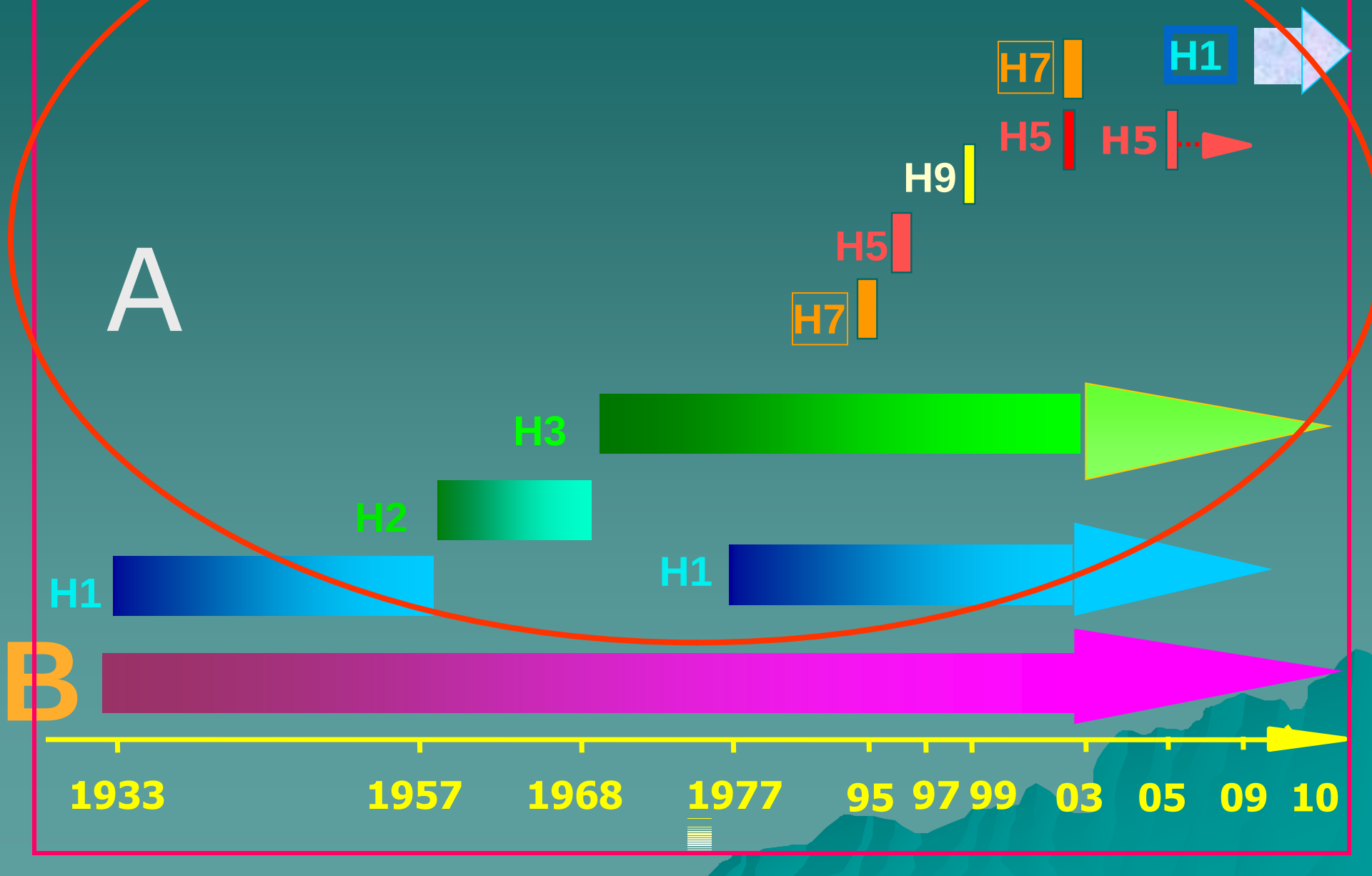
- A/duck/Laos/3295/2006 (H5N1) (clado 2.3.4; FDA; 10 Setiembre **2008**)
- A/ Egypt /2321-NAMRU3/2007 (H5N1) (clado 2.2.1 WHO/CDC; Mayo **2009**)
- A/ Egypt /3300-NAMRU3/2008 (H5N1)-PR8-IDCDC-RG13 (WHO/CDC, 3 Agosto **2010**)

Síntesis

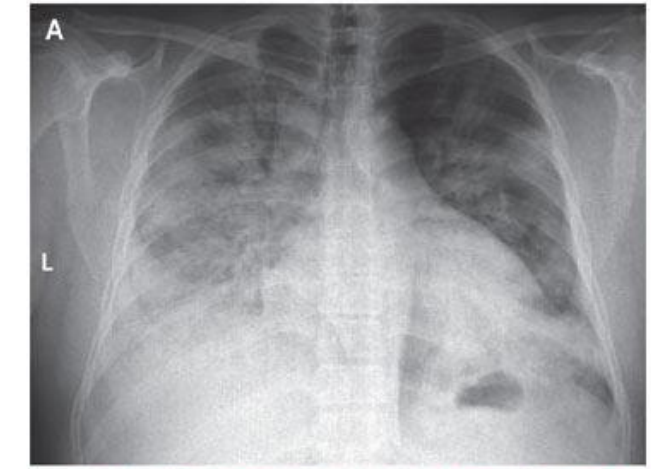
- Influenza H5N1 ha sido extremadamente difícil de controlar desde 1997.
- La relajación prematura de medidas de control facilitará la persistencia de la epizootia por virus H5N1.
- Hay persistencia y aumento del riesgo de adaptación de las cepas H5N1 al humano mediante reasociación o mutación génica.

Proteo

Circulación en Humanos de Tipos y Subtipos de virus *Influenza*

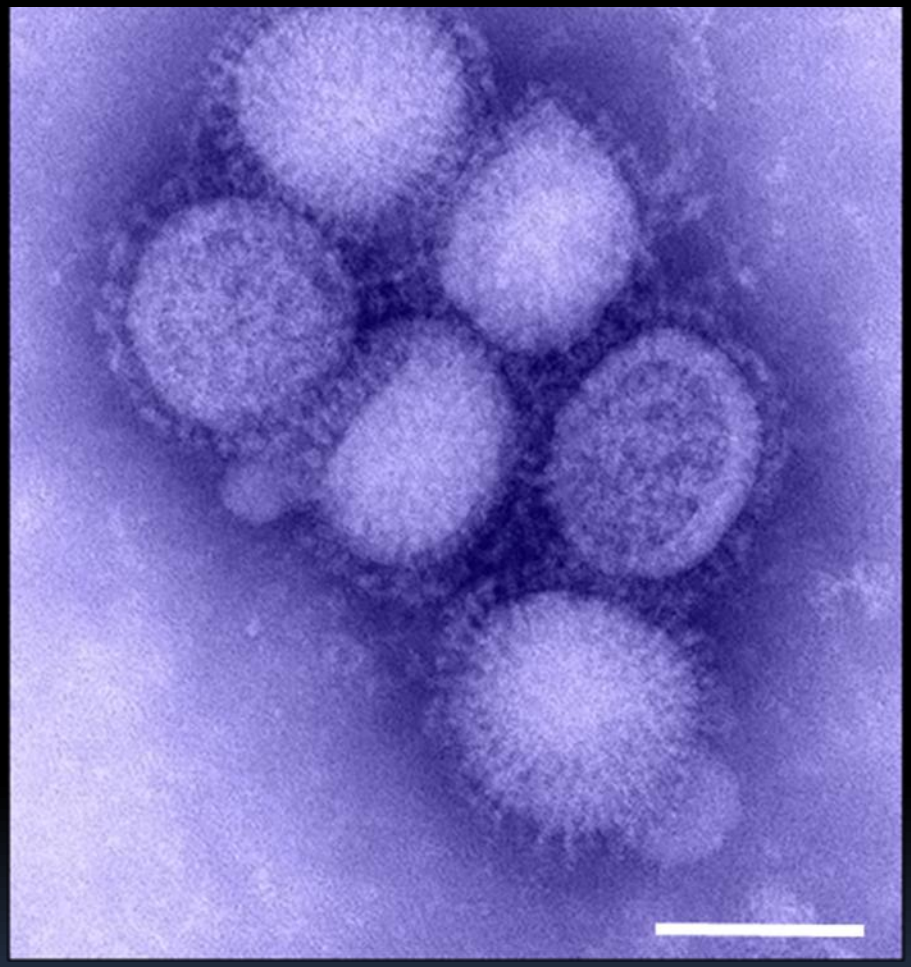


Radiografía inicial de pulmón e histología de una muestra pulmonar de un paciente infectado con Influenza A (H1N1/09)



Perez-Padilla R et al. N Engl J Med 2009;10.1056/NEJMoa0904252

Virus *Influenza A(H1N1/09)*
Agente etiológico de la pandemia 2009-2010



Virus Influenza A(H1N1) causante del actual brote de influenza de origen porcino en 2009. Fuente: CDC (*Centers for Disease Control and Prevention*, EE.UU.). Se observan partículas ovales o esféricas con espículas correspondientes a la expresión en su superficie de moléculas de hemaglutinina (HA) y neuraminidasa (NA).
La barra blanca indica 100 nm.

Reasociación génica en el genoma del virus causante de la pandemia de gripe A 2009

1

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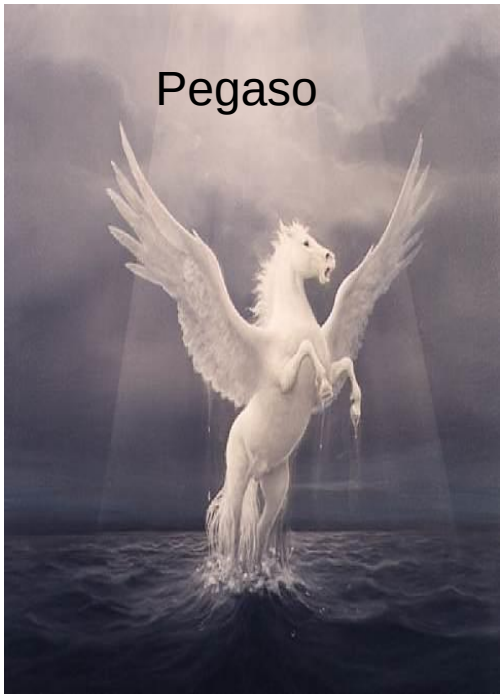
?

Influenza A(H1N1): triple reasociación génica ocurrida en 1998.

Influenza A(H1N1) causante de la pandemia de 2009

PB2
PB1
PA
HA
NP
NA
M
NS



PB2
PB1
PA
HA
NP
NA
M
NS

Genes del virus influenza aviar. linaje norteamericano

Gen del virus influenza estacional

Genes del virus influenza porcino, linaje norteamericano

Genes del virus influenza porcino, linaje eurasiático

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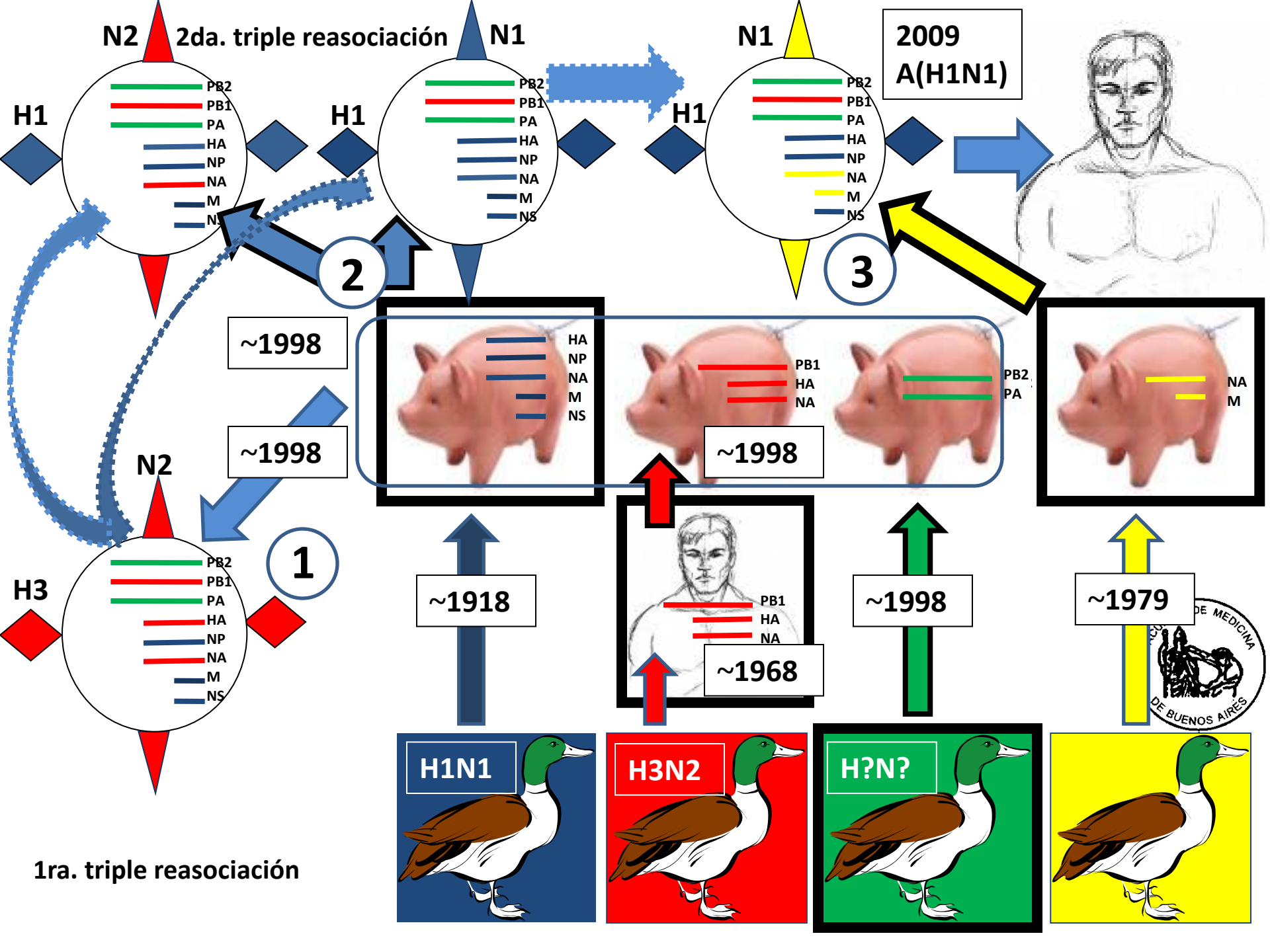

N2
2da. triple reasociación
N1
N1
2009
A(H1N1)
H1
H1
H1
PB2
PB1
PA
HA
NP
NA
M
NS
2
3
~1998
~1998
HA
NP
NA
M
NS
PB1
HA
NA
~1998
PB2
PA
NA
M
N2
1
H3
~1918
~1968
~1998
~1979
PB1
HA
NA
H1N1
H3N2
H?N?
1ra. triple reasociación

¡Primer pandemia del siglo XXI!

- 213 países
- 425.650 casos confirmados mediante estudios de laboratorio
- 6.813 muertes

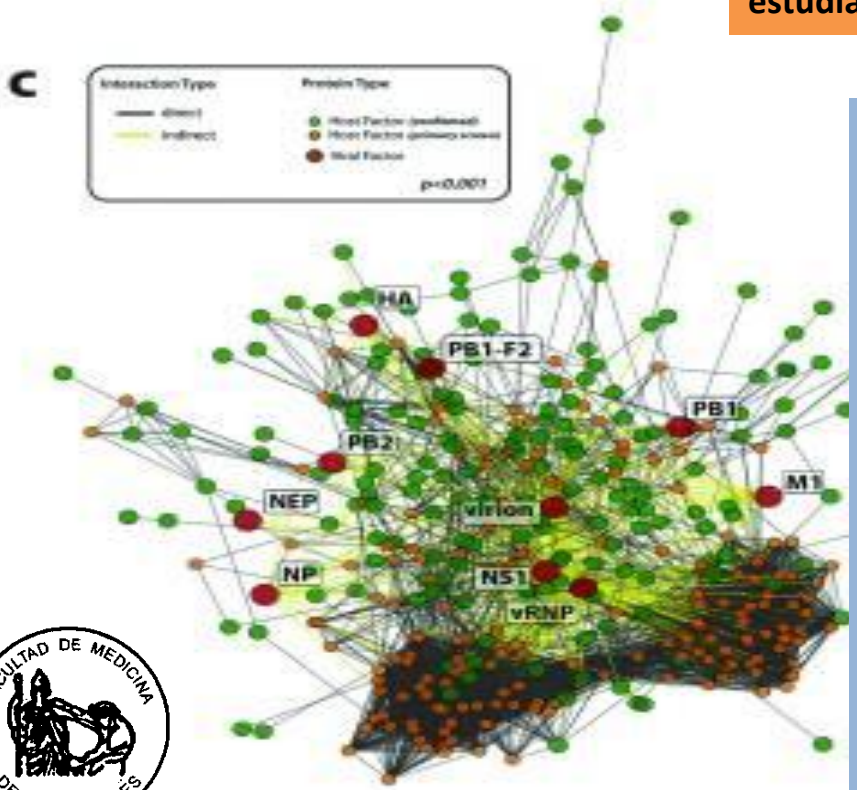
Requerimiento de factores celulares para la replicación de Influenza

Virus con HA delecionada conteniendo en su lugar el código de la luciferasa (el virus no replica)

Biblioteca de RNAi celulares (19000 genes celulares estudiados)

Transfección de alto rendimiento en células epiteliales alveolares

Infección con Virus con HA delecionada (+ luciferasa)



23 factores necesarios para el ingreso viral (incluyedno miembros de la ATPasa vacuolar)y la familia de proteínas endosomales COPI, factor de crecimiento fibroblástico, glucógeno sintetasa kinasa3-beta). Además 10 proteínas están involucradas en la etapa post-ingreso, coponentes de la importación nuclear, proteasas, proteína-quinasa calcio/calmodulina-dependiente II beta.

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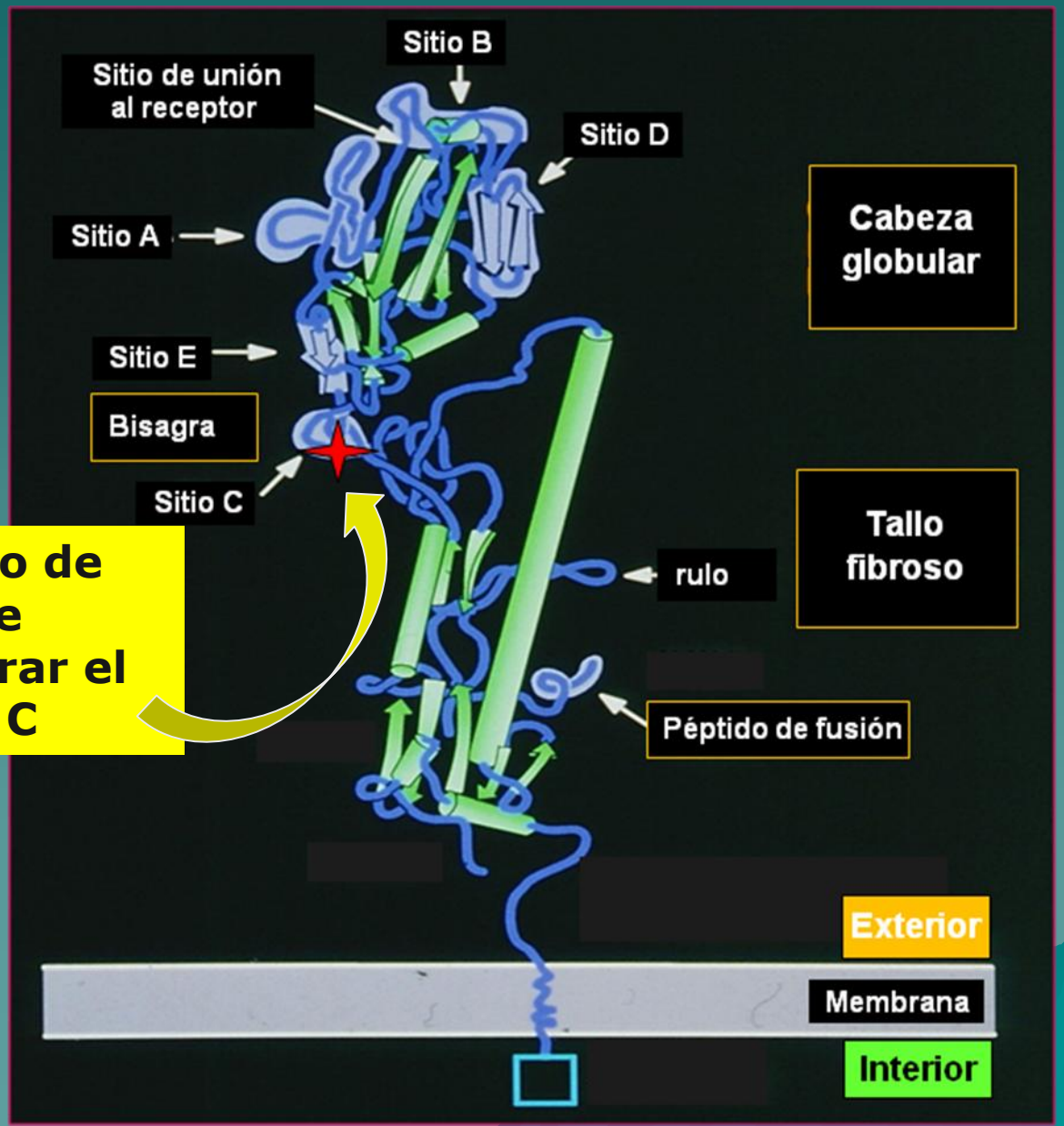
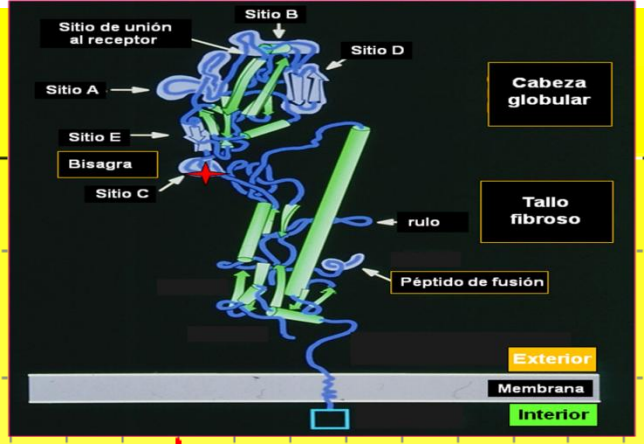
ESQUEMA DE UN MONÓMERO DE HA



Asparagina: sitio de glicosilación que puede enmascarar el sitio antigénico C

A



Comparación del perfil de hidrofilicidad (asociado a antigenicidad) entre la Hemaglutinina del virus estacional de Influenza A (H1N1) circulante en 2008 e Influenza A (H1N1/2009)

Hidrofilicidad

1,6
1,2
0,8
0,4
0
0,4
0,8
-1,2

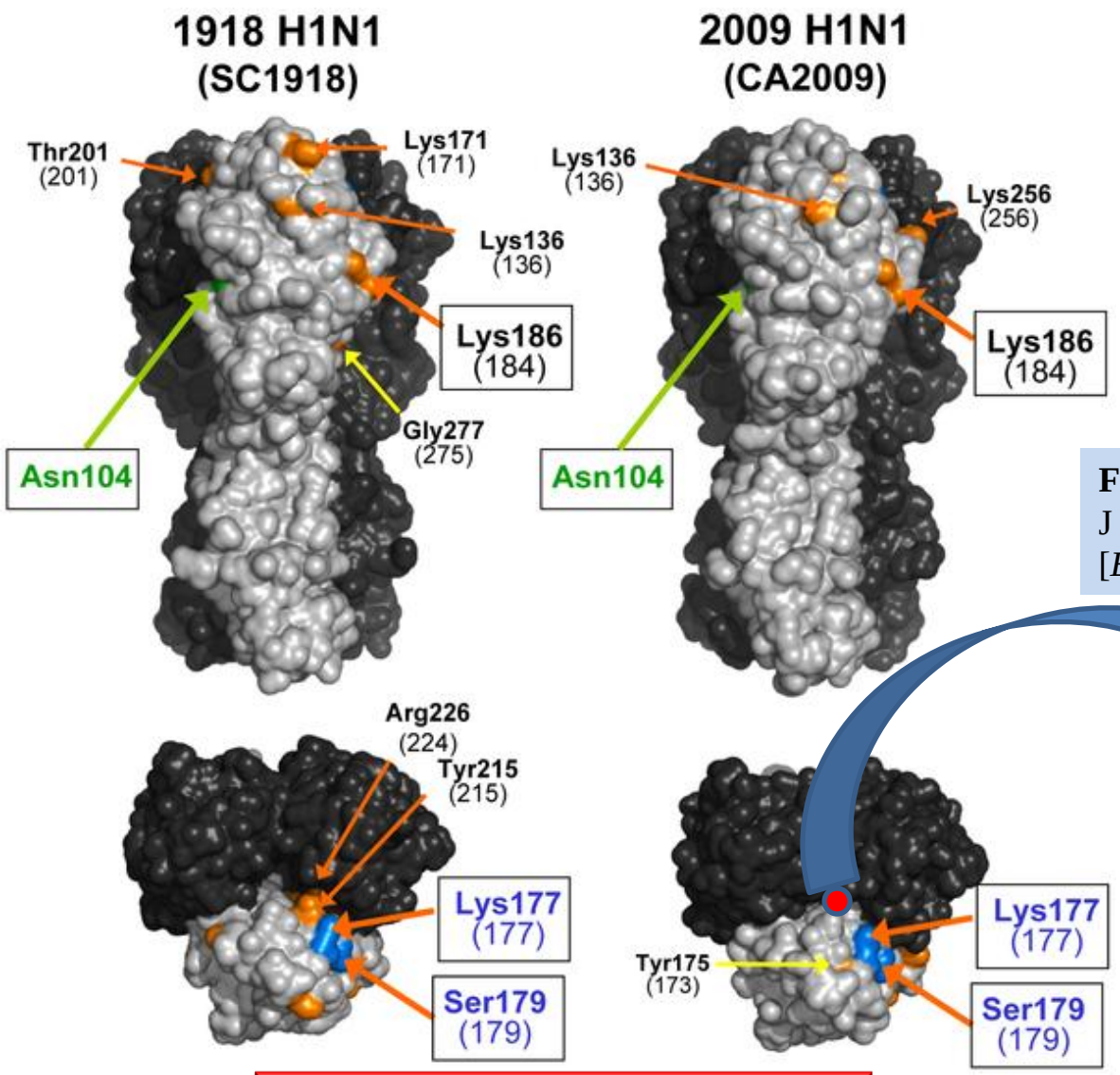
Sitio A
Sitio E
Sitio C
Sitio B
Sitio B
Sitio D
Sitio C

0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320

Posición aminoacídica

2008

2009



Modelo tridimensional de trímeros de Hemaglutinina

Fuente: Chen *et al.* J Infect Dis. 2010 Apr 2. [*Epub ahead of print*]

Asp225→**Gly**

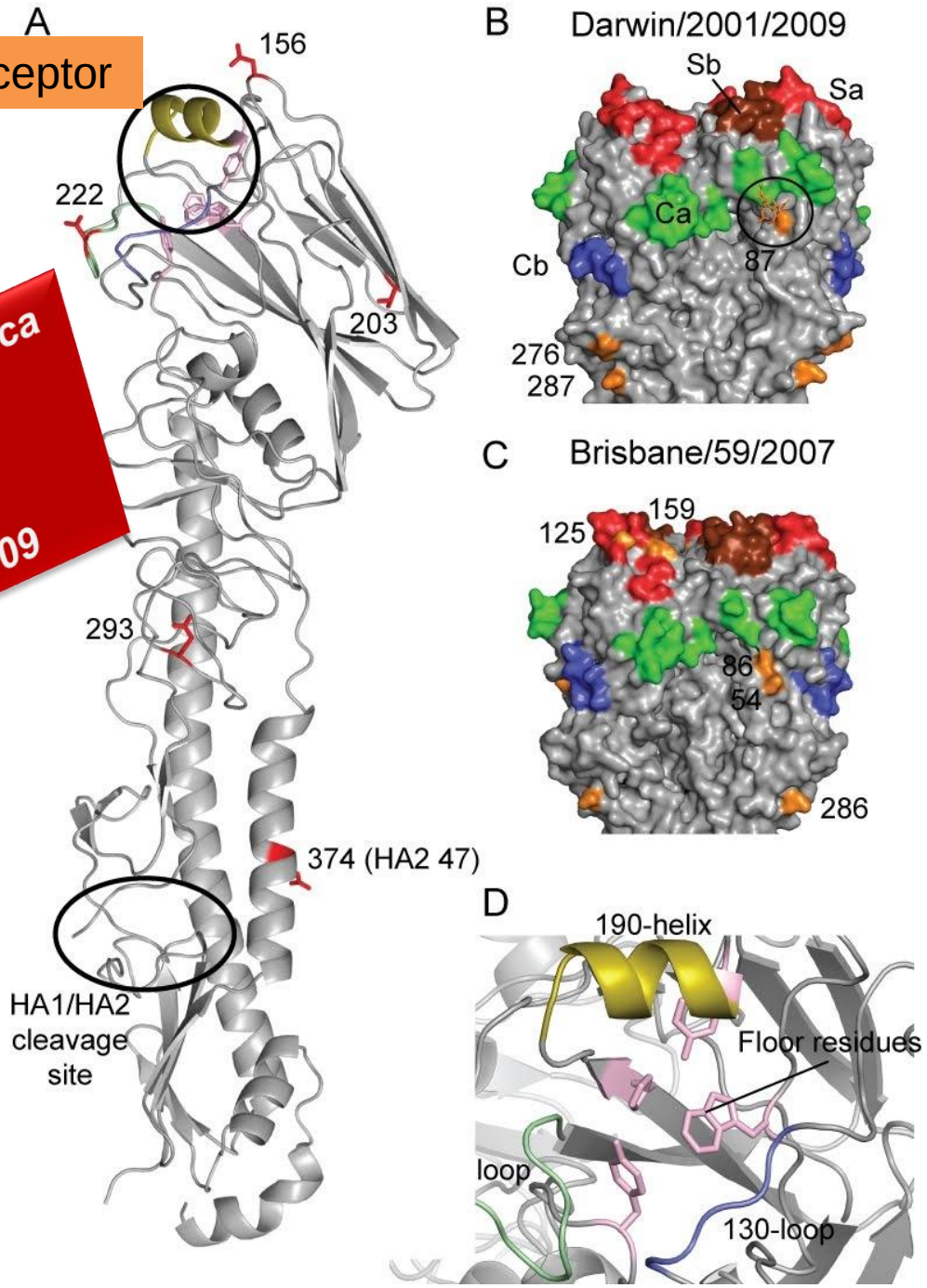
Modifica tropismo

12,5% en casos graves (n=57) vs 0% en casos leves (n=60)

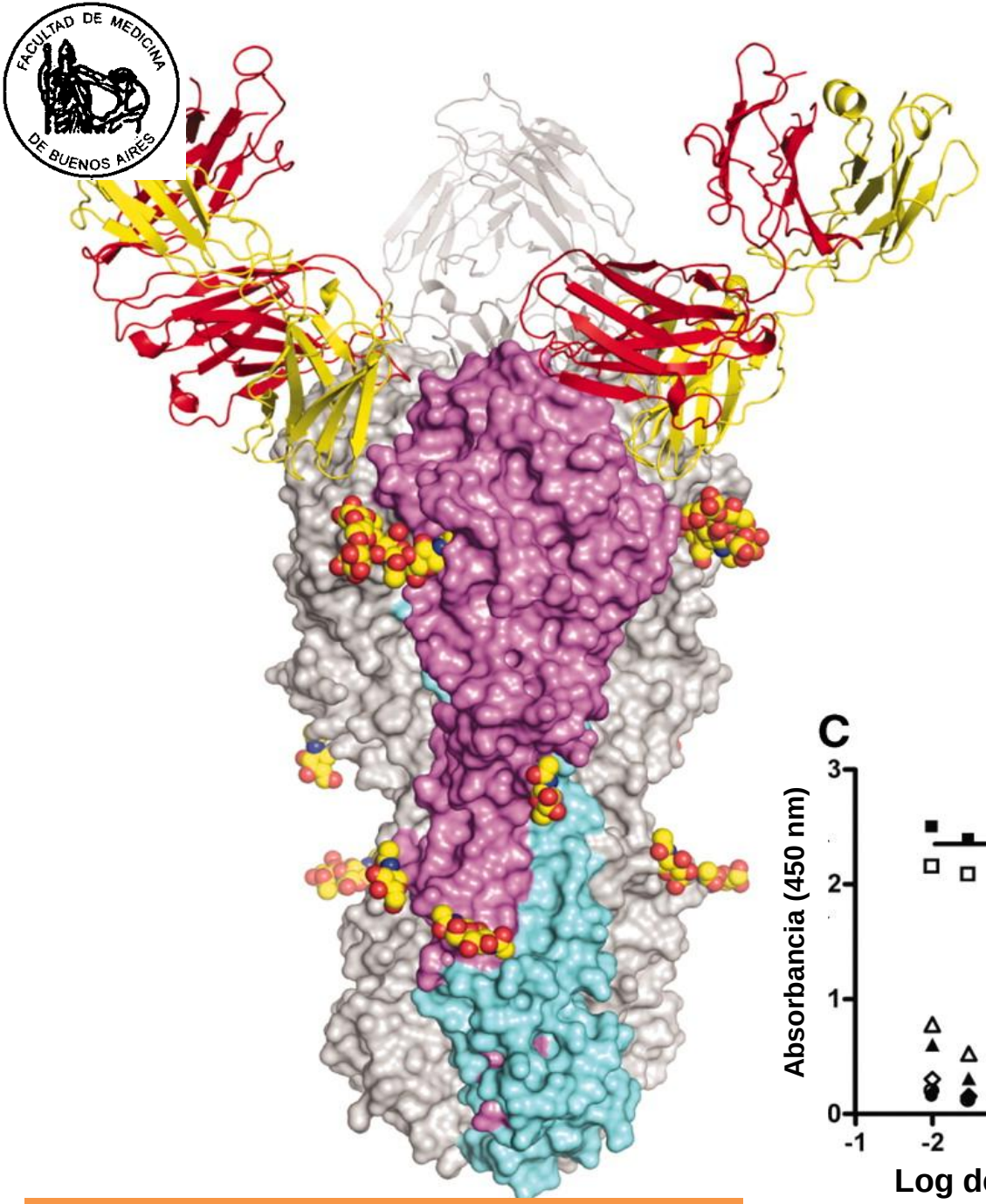
Fuente: Igarashi *et al*. PLoS ONE 5: e8553. doi:10.1371/journal.pone.0008553. 1 Jan 2010.

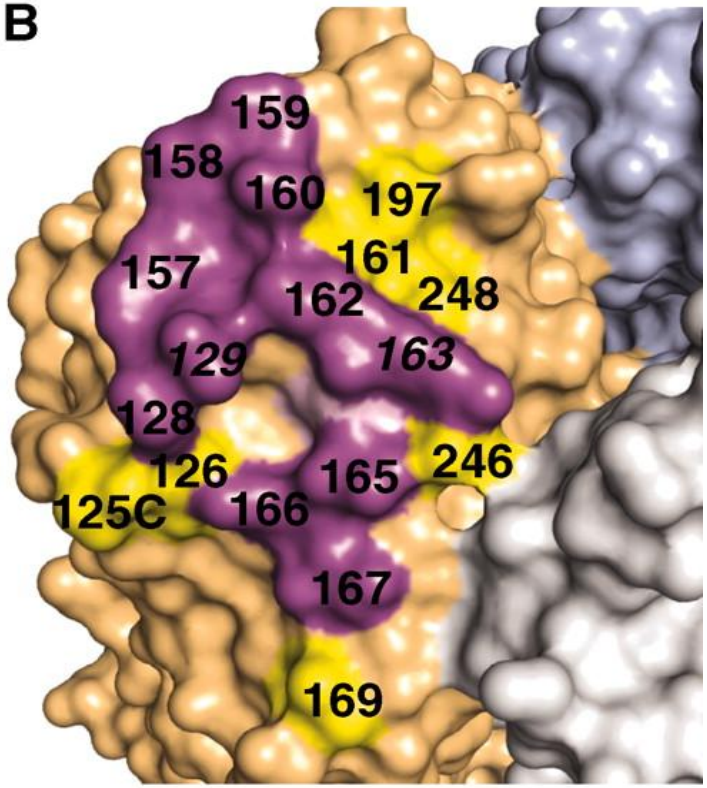
Sitio de unión al receptor

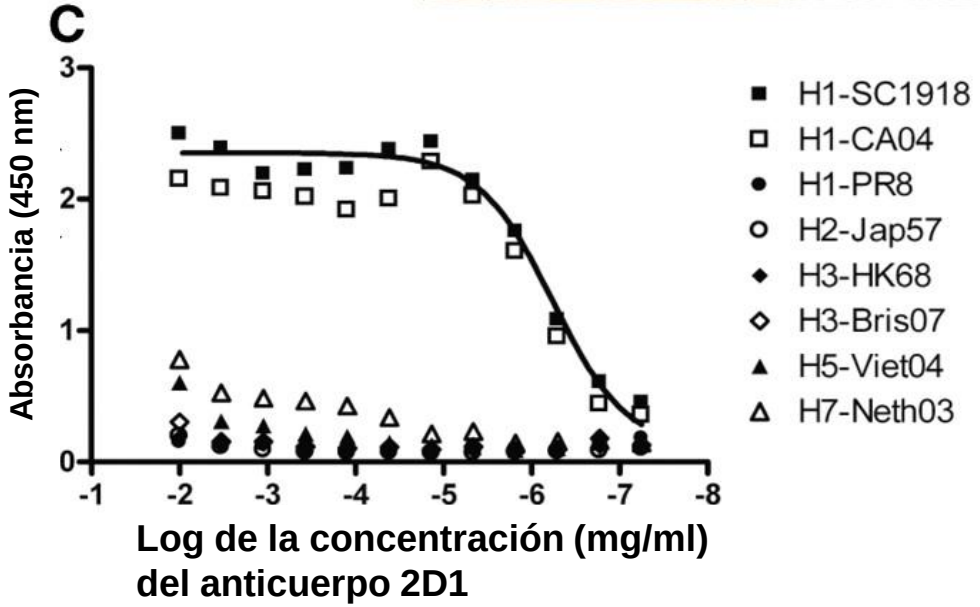
Estructura cristalográfica de un monómero de Hemaglutinina del virus pandémico *Influenza* A H1N1/2009



Fuente: Yang H, *et al*. PLoS Curr Influenza. 2010 Mar 22:RRN1152.







Xu *et al*; Science 328:357-60, 2010.

†

Structural Basis of Preexisting Immunity to the 2009 H1N1 Pandemic Influenza Virus Science. *Epub ahead of print 25 March 2010. En prensa.*

Xu R, Ekiert DC, Krause JC, Hai R, Crowe Jr JE, Wilson IE,

The 2009 H1N1 swine flu is the first influenza pandemic in decades. The crystal structure of the hemagglutinin from the A/California/04/2009 H1N1 virus shows that its antigenic structure, particularly within the Sa antigenic site, is extremely similar to human H1N1 viruses circulating early in the 20th century.

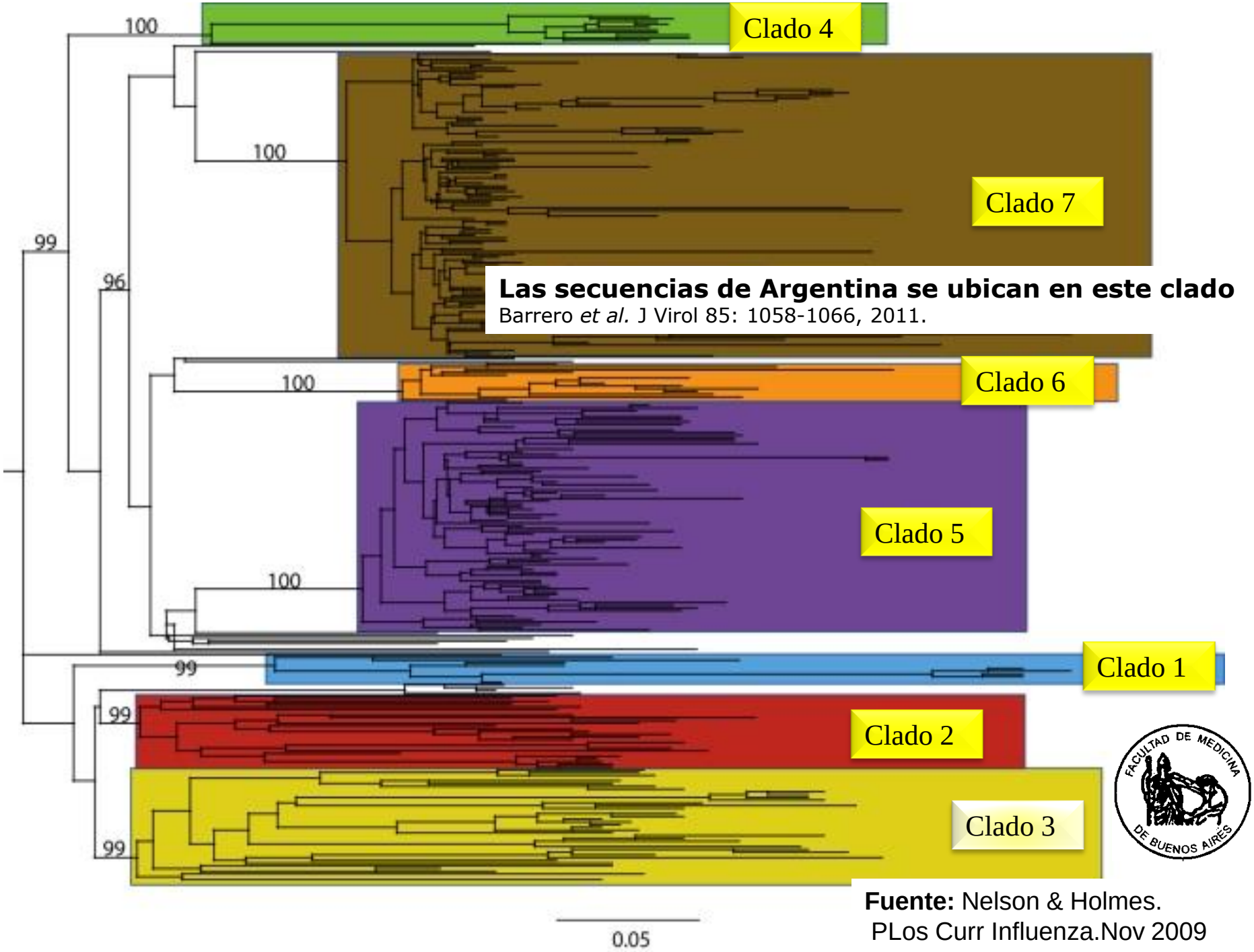
The co-crystal structure of the 1918 HA with 2D1, an antibody from a survivor of the 1918 Spanish flu that neutralizes both 1918 and 2009 H1N1 viruses, reveals an epitope that is conserved in both pandemic viruses.

Thus, antigenic similarity between the 2009 and 1918-like viruses provides an explanation for the age-related immunity to the current influenza pandemic.

Los anticuerpos anti-hemaglutinina generados por vacunación reciente contra la gripe estacional inducen poca o nula reacción cruzada contra el virus pandémico, pero 34% de adultos mayores de 59 años SÍ los evidencian.

- **Cross-Reactive Antibody Responses to the 2009 Pandemic H1N1 Influenza Virus** ive antibodies. Copyright 2009 Massachusetts Medical Society.
- **BACKGROUND:** A new pandemic influenza A (H1N1) virus has emerged, causing illness globally, primarily in younger age groups. To assess the level of preexisting immunity in humans and to evaluate seasonal vaccine strategies, we measured the antibody response to the pandemic virus resulting from previous influenza infection or vaccination in different age groups. METHODS: Using a microneutralization assay, we measured cross-reactive antibodies to pandemic H1N1 virus (2009 H1N1) in stored serum samples from persons who either donated blood or were vaccinated with recent seasonal or 1976 swine influenza vaccines.
- **RESULTS:** A total of 4 of 107 persons (4%) who were born after 1980 had preexisting cross-reactive antibody titers of 40 or more against 2009 H1N1, whereas 39 of 115 persons (34%) born before 1950 had titers of 80 or more. Vaccination with seasonal trivalent inactivated influenza vaccines resulted in an increase in the level of cross-reactive antibody to 2009 H1N1 by a factor of four or more in none of 55 children between the ages of 6 months and 9 years, in 12 to 22% of 231 adults between the ages of 18 and 64 years, and in 5% or less of 113 adults 60 years of age or older. Seasonal vaccines that were formulated with adjuvant did not further enhance cross-reactive antibody responses. Vaccination with the A/New Jersey/1976 swine influenza vaccine substantially boosted cross-reactive antibodies to 2009 H1N1 in adults.
- **CONCLUSIONS:** **Vaccination with recent seasonal nonadjuvanted or adjuvanted influenza vaccines induced little or no cross-reactive antibody response to 2009 H1N1 in any age group.** Persons under the age of 30 years had little evidence of cross-reactive antibodies to the pandemic virus. However, a proportion of older adults had preexisting cross-reac
- Hancoock K, et al. NEJM **Published at www.nejm.org September 10, 2009.**

The NEW ENGLAND JOURNAL of MEDICINE



Las secuencias de Argentina se ubican en este clado
Barrero *et al.* J Virol 85: 1058-1066, 2011.

Fuente: Nelson & Holmes.
PLos Curr Influenza.Nov 2009

Característica	*Influenza* A (H1N1) causante de pandemia en 1918	*Influenza* A (H2N2) causante de pandemia en 1957	*Influenza* A (H5N1) aviar	*Influenza* A (H1N1) e *Influenza* A (H3N2) estacionales	*Influenza* A (H1N1) de origen porcino (2009)
Transmisión interhumana	+++ / ++++	+++ / ++++	- → ± (extremadamente restringida)*	++	+++
Gravedad clínica	++++	+++	++++	++	++ / +++

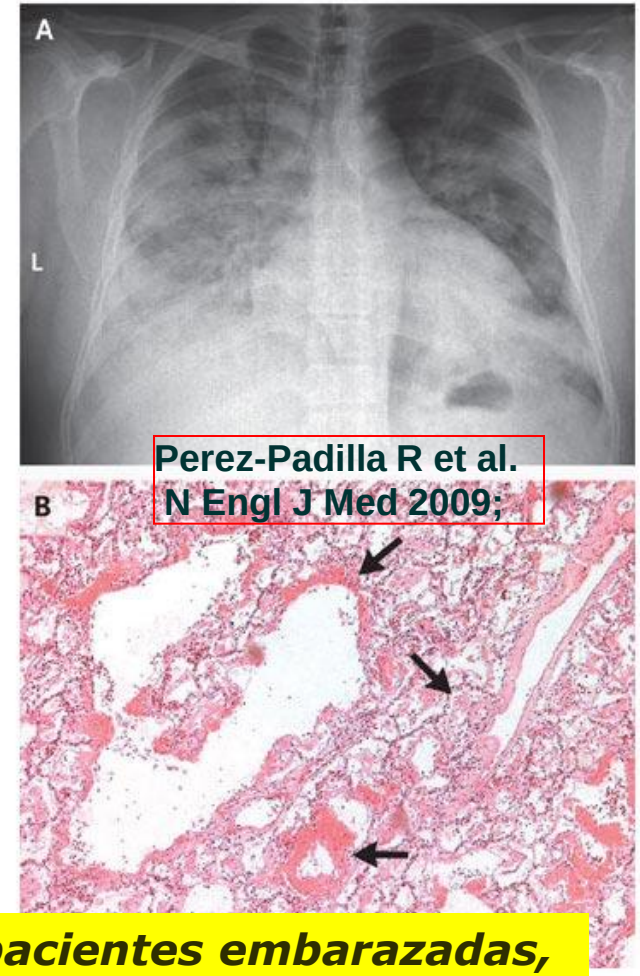
Proteína	Posición	Virus con Patogenicidad baja	Virus con Patogenicidad alta	Influenza A(H1N1) de origen porcino (2009)	Función
PB2	627 701	**Glu** **Asp**	**Lys** **Asn**		Capacidad para replicar en algunos mamíferos, incluido el hombre Importación nuclear; afecta la capacidad replicativa en el ratón
PB1-F2	66	**Asn**	**Ser**		Inducción de apoptosis
HA	Sitio de clivaje	**Único aminoácido básico**	**Múltiples aminoácidos básicos**		Clivaje de la hemaglutinina (ciertas proteasas de localización extrapulmonar reconocen múltiples aminoácidos básicos)
NS1	92 C-terminal	**Asp** **Deleción Arg – Ser- Glu- Val**	**Glu** **Glu–Ser- Glu- Val**		Desconocida (¿diferente respuesta al Interferón?) Desconocida

Fuente: Neumann *et al*; Nature 459: 931-9, 2009.

Radiografía inicial de pulmón e histología de una muestra pulmonar de un paciente infectado con Influenza A (H1N1/09)

Primates: Estudios de patogénesis

El virus replica en neumonocitos tipo II (epitelio respiratorio bajo) con receptores alfa 2-3 y alfa 2-6 Gal unidos al Ac. siálico: *Lesiones más graves que con Influenza estacional*



Perez-Padilla R et al.
N Engl J Med 2009;

La neumonía viral primaria puede evolucionar a la dificultad respiratoria (*distress*) y eventualmente a la muerte.

Hurón: estudios de patogénesis

¡El virus también replica en epitelio intestinal!

No demostrado aún en humanos

Curso más grave en pacientes embarazadas, en obesos y en niños menores de 5 años. Hasta 40% de los pacientes padecen síntomas gastrointestinales.

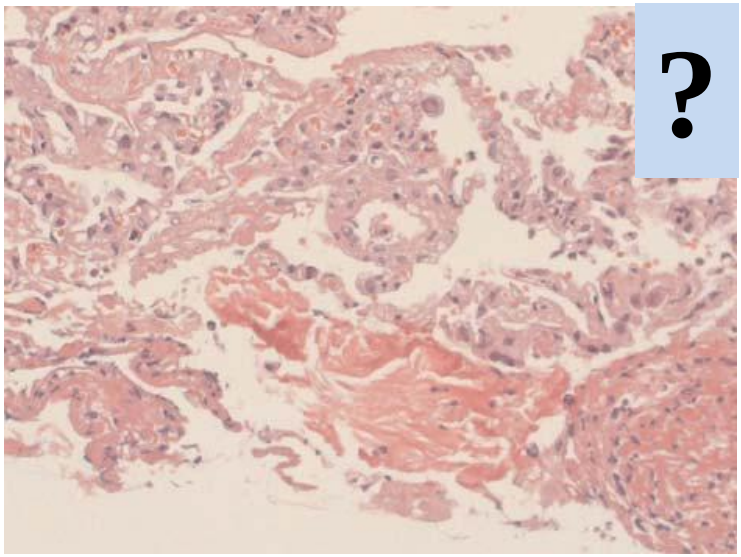
¿?

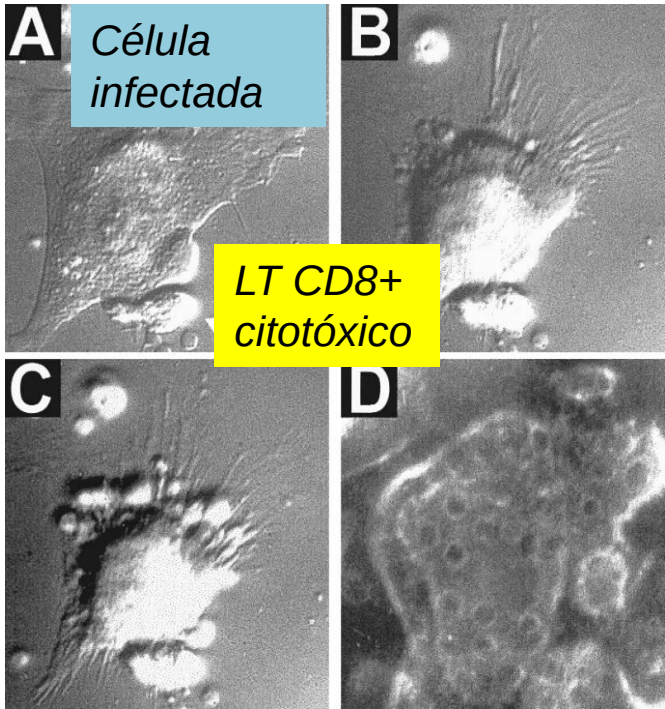
RESPUESTA INMUNE ANTIVIRAL DE LOS LINFOCITOS T CD8^{+} CITOTÓXICOS

DÉBIL OLIGOCLONAL

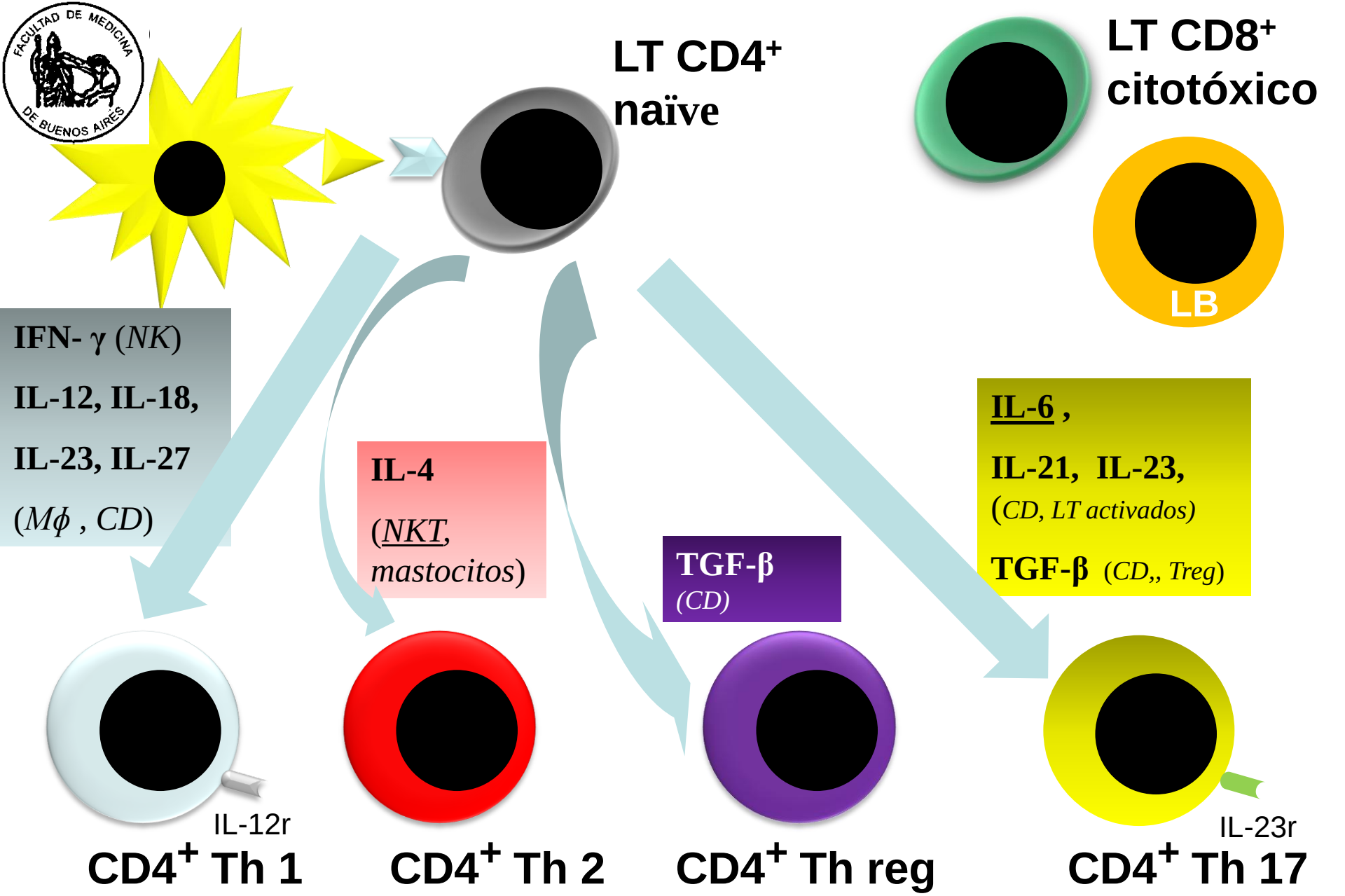
VIGOROSA POLICLONAL MULTIESPECÍFICA (Th1)

INFECCIÓN AGUDA LIMITADA





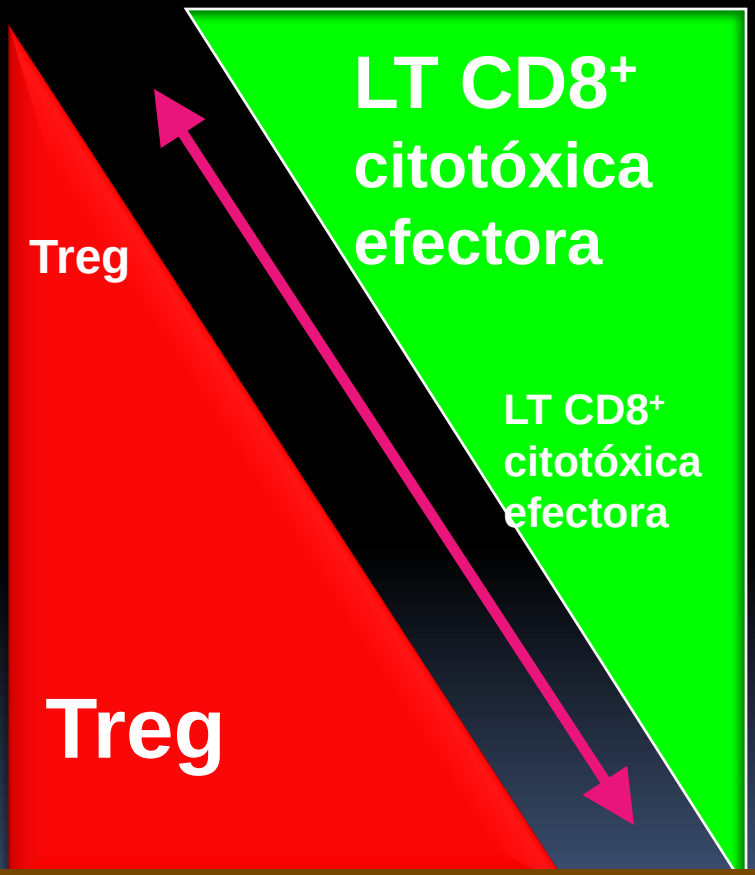
Fuente: Comisión para la Contingencia de Influenza A (H1N1), Hospital Nacional Profesor Alejandro Posadas. Medicina. (Bs. As.). 69: 393- 423. 2009.


LT CD4+ naïve
LT CD8+ citotóxico
LB
IFN- γ (NK)
IL-12, IL-18,
IL-23, IL-27
(Mϕ , CD)
IL-4
(NKT, mastocitos)
TGF-β
(CD)
IL-6 ,
IL-21, IL-23,
(CD, LT activados)
TGF-β (CD,, Treg)
IL-12r
CD4+ Th 1
CD4+ Th 2
CD4+ Th reg
IL-23r
CD4+ Th 17

Un delicado balance …

ELIMINACIÓN VIRAL

DAÑO CELULAR

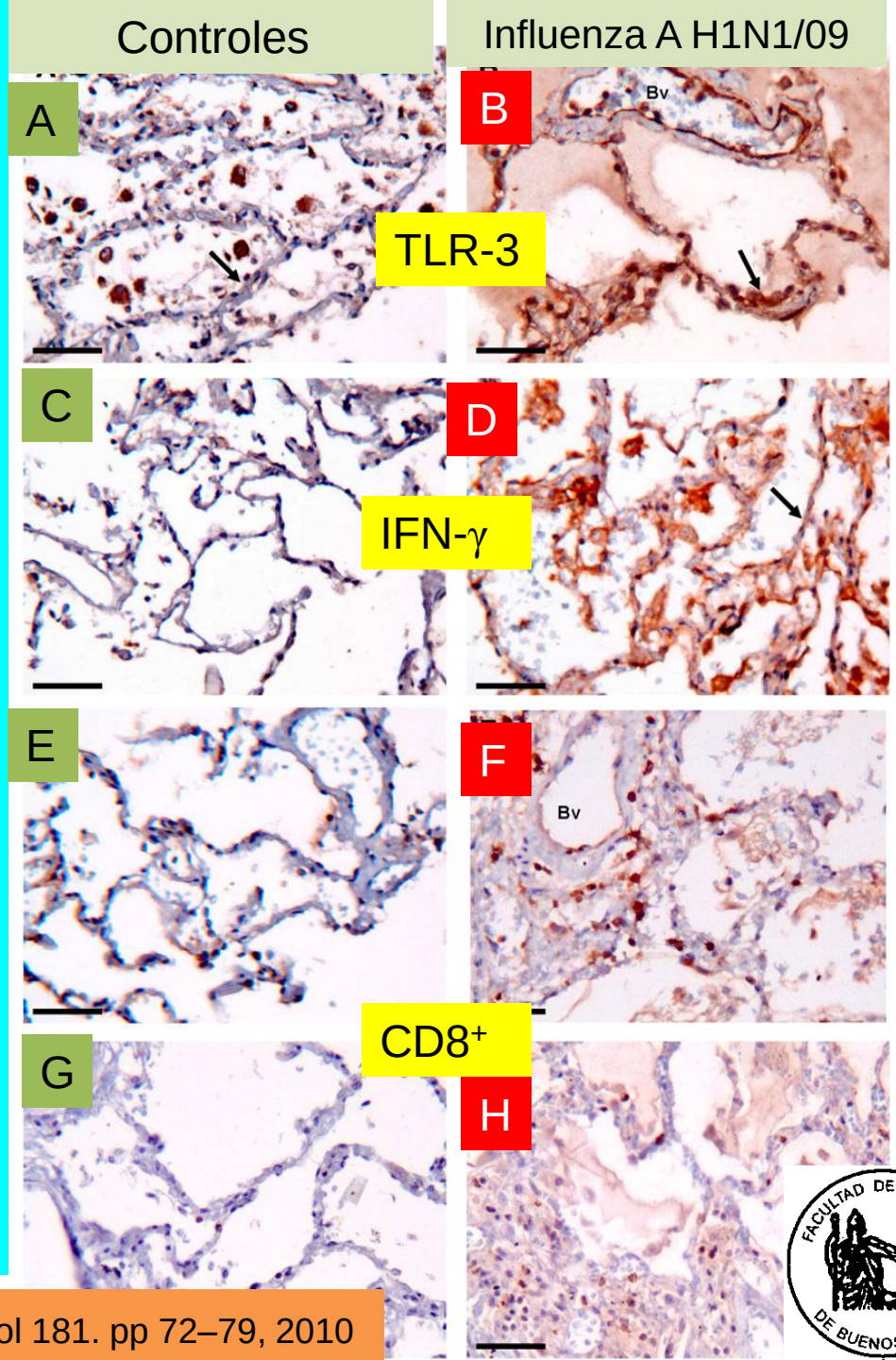


DEFECTUOSA ELIMINACIÓN VIRAL

DISMINUCIÓN O AUSENCIA DE DAÑO CELULAR

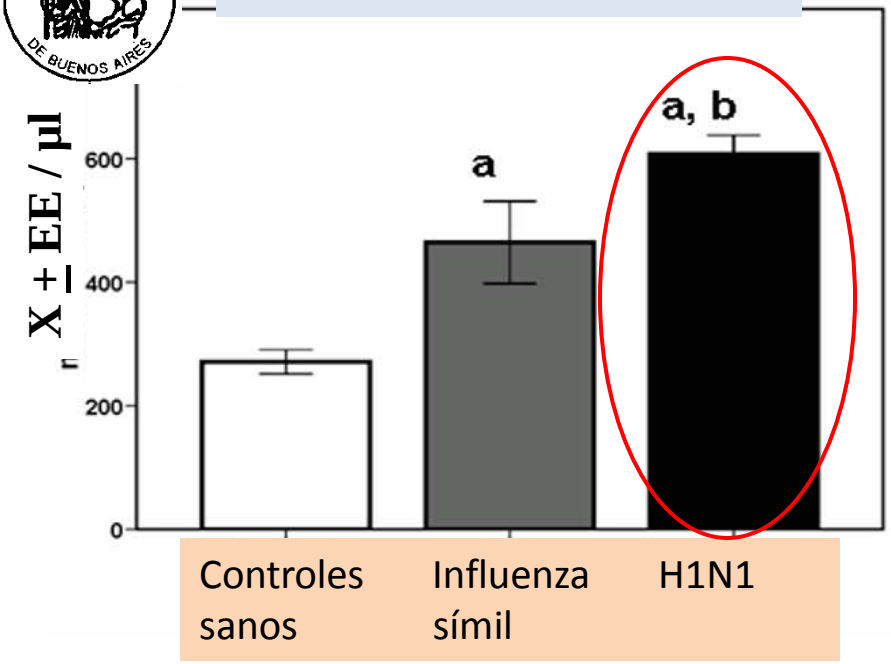
1. **A:** Expresión de TLR-3 en macrófagos, células epiteliales alveolares y endoteliales. (Bv: vaso sanguíneo). **B:** Significativa expresión de TLR-3 en macrófagos (flecha), células del epitelio alveolar y capilares.
2. **C.** Débil expresión de interferón-γ en macrófagos alveolares. **D.** Significativa expresión de interferón-γ en macrófagos y células epiteliales (flecha).

3. **E y G**. Escasas células $CD8^+$ y granzima B^+, respectivamente, en las paredes alveolares.
4. **F y H**. Aumento del número de células $CD8^+$ y granzima B^+, con tendencia al agrupamiento alrededor de los pequeños vasos (Bv). La barra indica 50 µ.

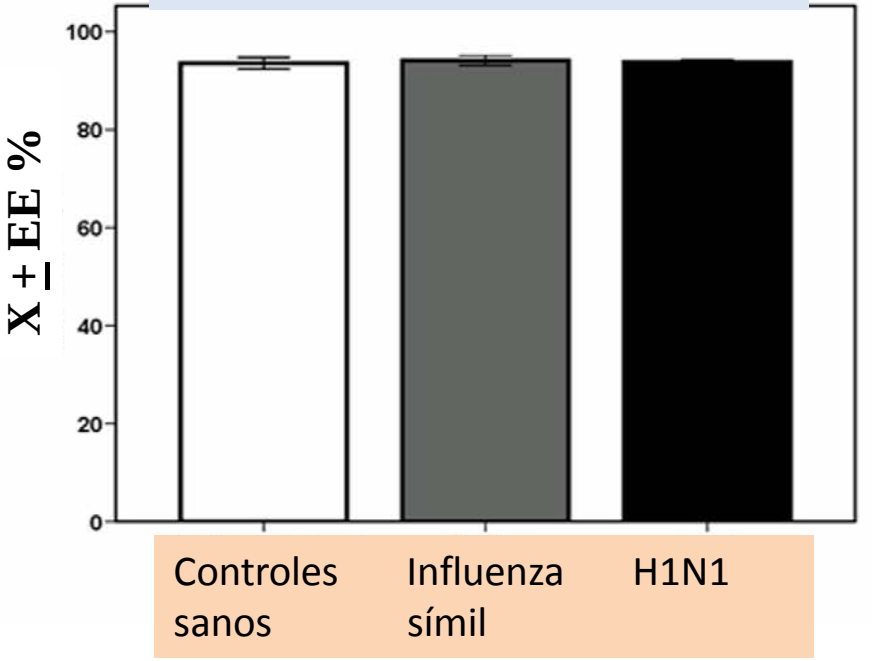


Fuente: Mauad *et al*, Am J Respir Crit Care Med Vol 181. pp 72–79, 2010

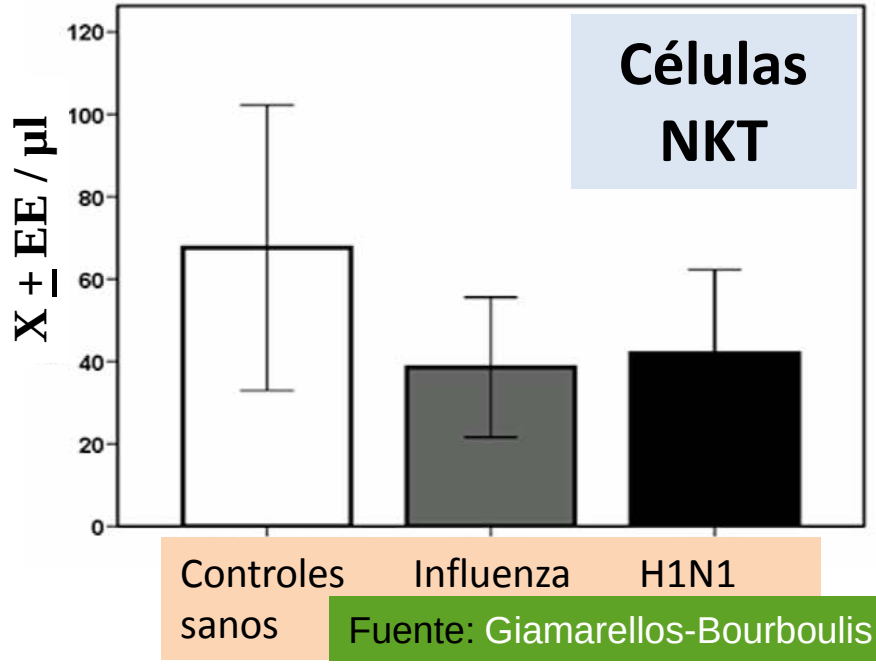
Monocitos



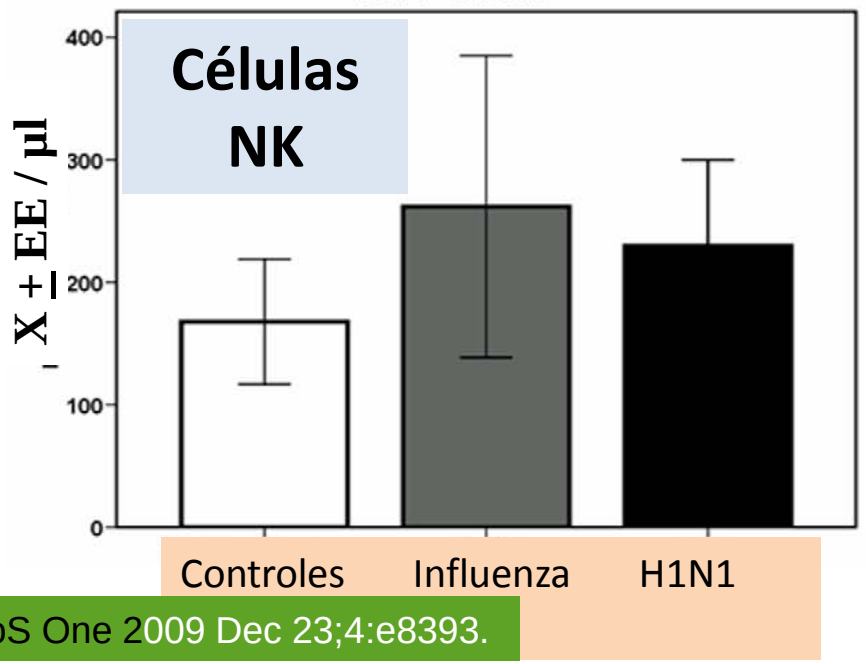
HLA-DR en monocitos



Células NKT

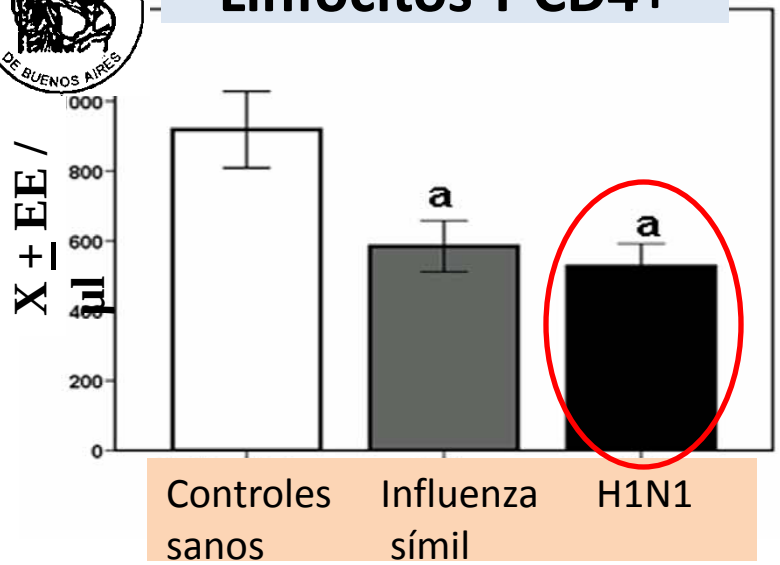


Células NK

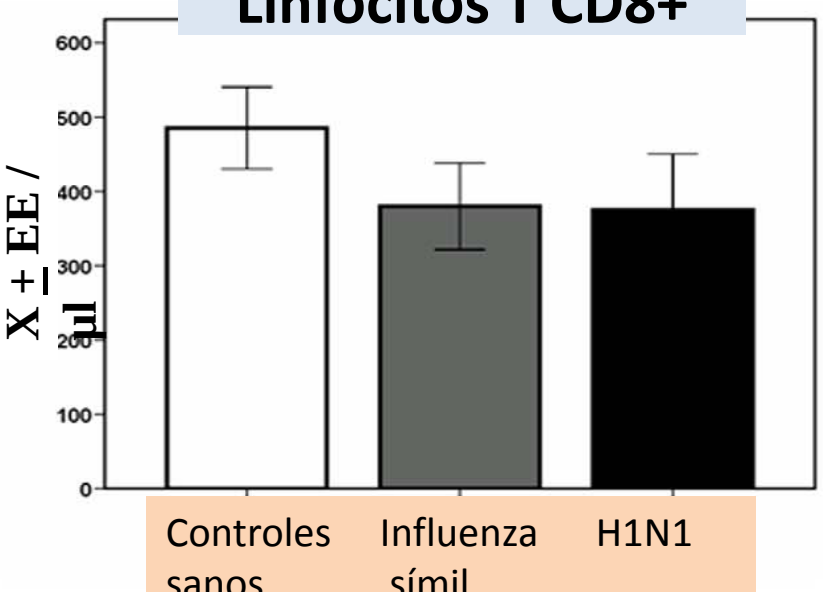


Fuente: Giamarellos-Bourboulis *et al*; PLoS One 2009 Dec 23;4:e8393.

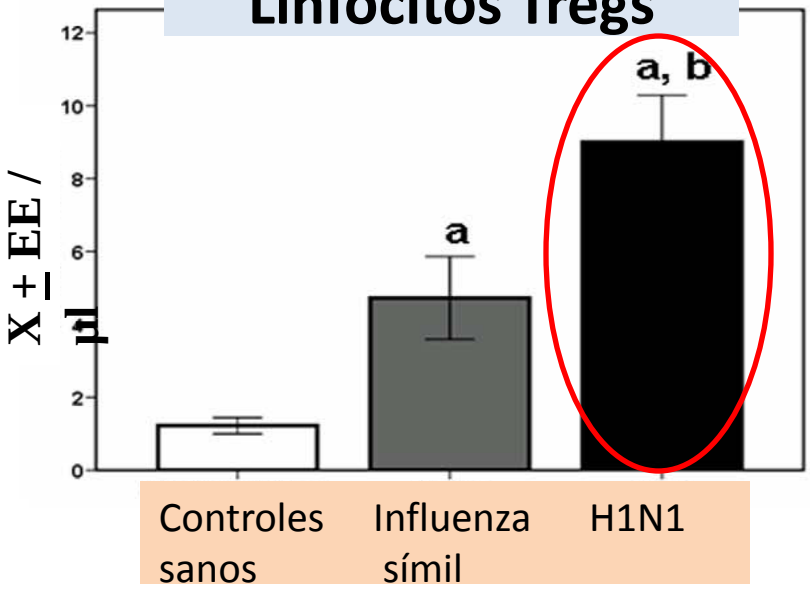
Linfocitos T CD4+



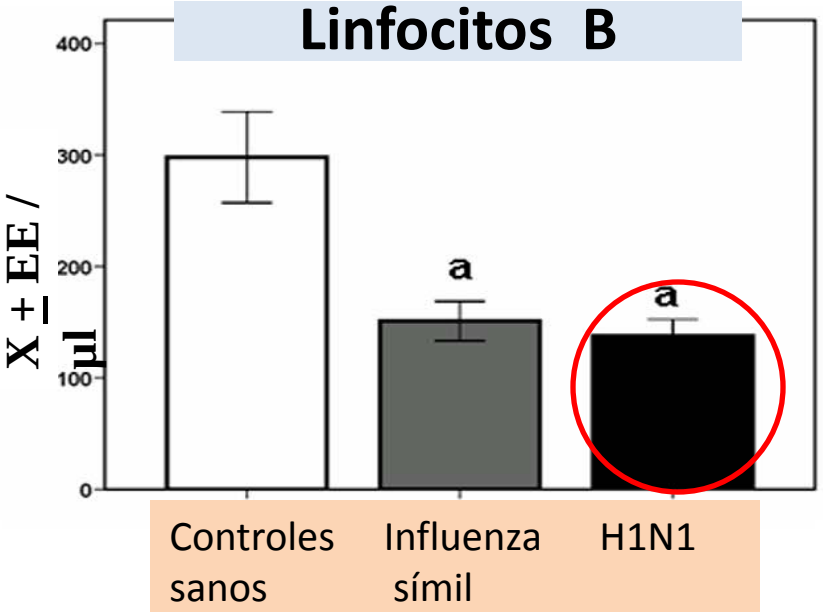
Linfocitos T CD8+



Linfocitos Tregs



Linfocitos B



Fuente: Giamarellos-Bourboulis *et al*; PLoS One 2009 Dec 23;4:e8393.

La unión de la Hemaglutinina (HA) de Influenza A (H1N1/2009) al receptor celular ocurre con menor afinidad que la observada con la HA del virus pandémico de 1918.

Potenciales implicancias:

✓La menor afinidad de la unión al receptor podría reducir la inflamación y la patología consiguiente.

✓El virus Influenza A (H1N1/2009) podría causar daño tisular en un territorio pulmonar más amplio pero con una menor gravedad que el virus de la gripe española de 1918 (H1N1) y el virus altamente patogénico H5N1 aviar.

PB2 residue 271 plays a key role in enhanced polymerase activity of influenza A viruses in mammalian host cells.

The genomic comparison of influenza A virus isolates has identified highly conserved residues in influenza proteins that are specific to either human or avian viruses, including 10 residues in PB2. We characterized the activity of avian polymerase complexes containing avian-to-human mutations at these conserved PB2 residues and found that, in addition to the E627K mutation, the PB2 mutation T271A enhances polymerase activity in human cells. We confirmed the effects of the T271A mutation using recombinant WSN viruses containing avian NP and polymerase genes with wild-type (WT) or mutant PB2. The 271A virus showed enhanced growth compared to that of the WT in mammalian cells in vitro. The 271A mutant did not increase viral pathogenicity significantly in mice compared to that of the 627K mutant, but it did enhance the lung virus titer. Also, cell infiltration was more evident in lungs of 271A-infected mice than in those of the WT. Interestingly, the avian-derived PB2 of the 2009 pandemic H1N1 influenza virus has 271A. The characterization of the polymerase activity of A/California/04/2009 (H1N1) and corresponding PB2 mutants indicates that the high polymerase activity of the pandemic strain in mammalian cells is, in part, dependent on 271A. Our results clearly indicate the contribution of PB2 amino acid 271 to enhanced polymerase activity and viral growth in mammalian hosts

Bussey K *et al.* J Virol. 2010 May;84(9):4395-406.

Factores virales en la infección por Influenza A (H1N1/2009) pandémica

- **Patogenicidad: ++ → +++**

✓ *Probable existencia de factores aún desconocidos.*

✓ No se detectaron (inicialmente) diferencias genéticas entre las cepas que infectaron a individuos con curso leve ó grave de la enfermedad.

Ciertos factores de virulencia de otros virus Influenza no serían funcionales en Influenza A (H1N1/2009):
PB1-F2 (pro-apoptótica en macrófagos): truncada por señal STOP en codón 12
NS1 (anti-IFN): truncada

- **Transmisibilidad: eficiente en humanos.**

✓ Resultados controversiales en hurones infectados con cepas aisladas en México y EE.UU. *vs* otra de Holanda: ¿hay eficiente transmisión mediante aerosoles?

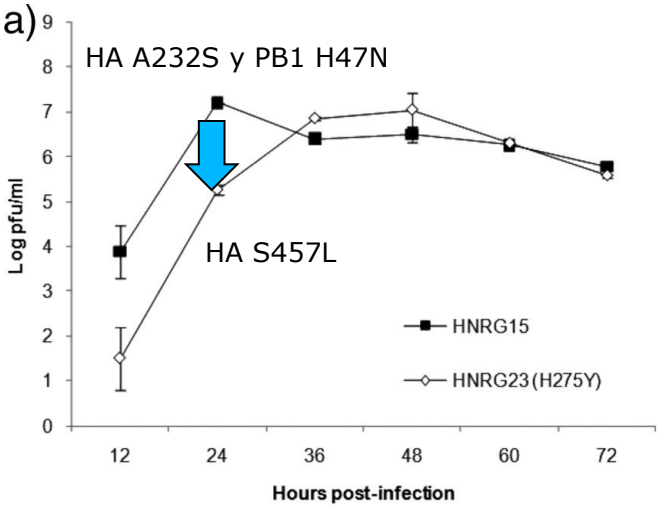
¡¡¡Hurones infectados con la cepa holandesa no estornudaban, pero los infectados con las cepas americanas sí lo hacían!!!

¿Por qué?

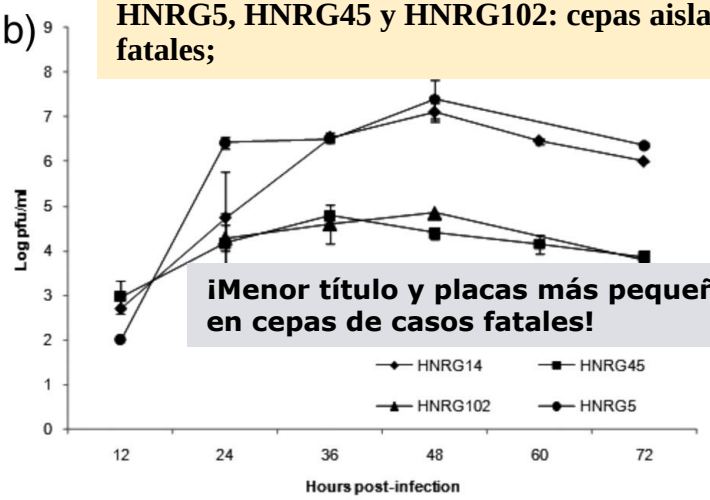
Cinética de la replicación viral de cepas de Influenza pandémica de Buenos Aires

Título viral en MDCK

El mismo paciente con una población *wt* y luego resistente al oseltamivir

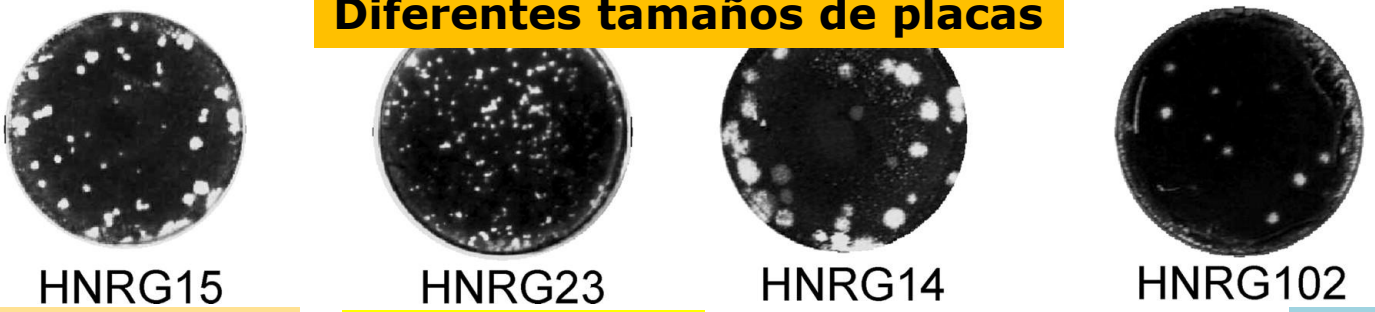


HNRG14: cepa aislada de un caso leve;
HNRG5, HNRG45 y HNRG102: cepas aisladas de casos fatales;



¡Menor título y placas más pequeñas en cepas de casos fatales!

Diferentes tamaños de placas



Cepa salvaje o *Wild type (wt)*

Cepa mutante H275Y resistente al oseltamivir

Cepa de un caso leve

Cepa de un caso fatal

Journal of Virology

Barrero, P. R. *et al.* 2011. J. Virol. 85(2):1058-1066

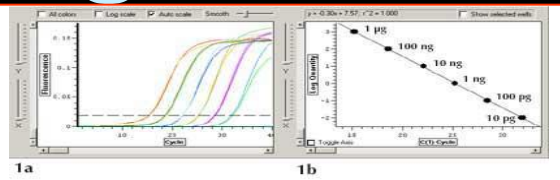
Severe pandemic 2009 H1N1 influenza disease due to pathogenic immune complexes.

Pandemic influenza viruses often cause severe disease in middle-aged adults without preexisting comorbidities. The mechanism of illness associated with severe disease in this age group is not well understood. Here we find preexisting serum antibodies that cross-react with, but do not protect against, 2009 H1N1 influenza virus in middle-aged adults. Nonprotective antibody is associated with immune complex-mediated disease after infection. We detected high titers of serum antibody of low avidity for H1-2009 antigen, and low-avidity pulmonary immune complexes against the same protein, in severely ill individuals. Moreover, C4d deposition--a marker of complement activation mediated by immune complexes--was present in lung sections of fatal cases. Archived lung sections from middle-aged adults with confirmed fatal influenza 1957 H2N2 infection revealed a similar mechanism of illness. These observations provide a previously unknown biological mechanism for the unusual age distribution of severe cases during influenza pandemics.

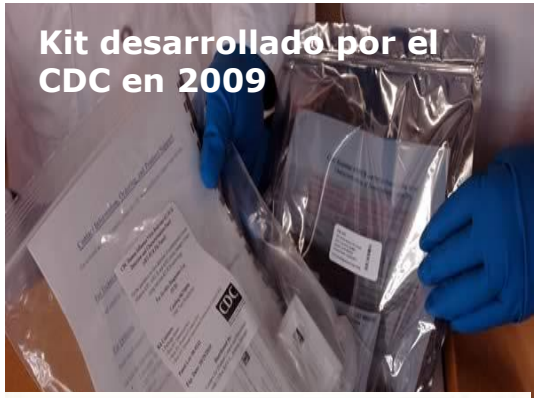
Monsalvo AC, *et al* .
Nat Med. 2011 Feb;17(2):195-9

El diagnóstico virológico

Métodos directos:



- Muestra clínica: Hisopado nasal bilateral y faríngeo.
- Detección genómica por RT-PCR o RT-PCR en tiempo real (*confirmación: Cultivo viral y secuenciamiento nucleotídico de amplicones de RT-PCR*)



Los <u>tests rápidos para detección de antígeno N</u> de Influenza tienen una sensibilidad del 10-70% (<u>falsos negativos</u> cuando hay bajo título viral en la muestra clínica) .

<u>Métodos indirectos</u>: La serología puede establecer un diagnóstico retrospectivo, con fines epidemiológicos o de investigación.

Síntesis: Pandemia de gripe A
Patogénesis y diagnóstico

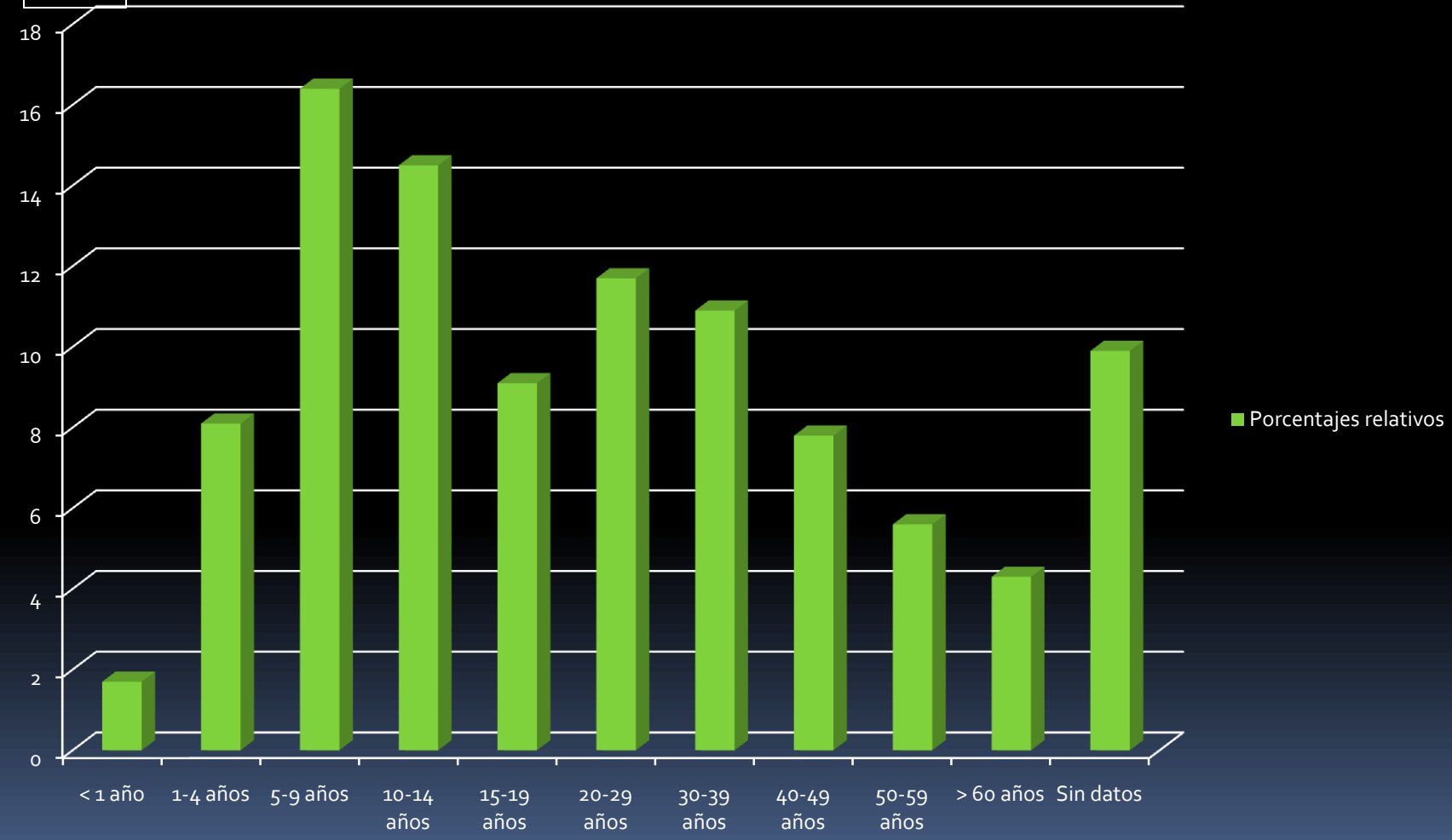
- Virus *Influenza* A (H1N1/2009) emergente con triple reasociación genómica.
- Marcadores moleculares virales conocidos: no explican los casos graves observados.
- La HA del virus es semejante a la de 1918.
- Pacientes con gripe A (H1N1/2009) exhiben monocitosis y linfopenia de LT $CD4^+$ y LB, con ↑ de Tregs
  (*nº pacientes = limitado*)
- El diagnóstico virológico se realiza mediante RT-PCR en tiempo real a partir de Hisopado N-F.

Situación epidemiológica en Argentina

- Total de casos de Enfermedad tipo influenza (ETI): 1.660.285
- Tasa semana 34: 5,8/ 10.000 habitantes
- Tasa acumulada: 264,1 / 10.000 habitantes
- Casos confirmados a la Semana 35: 8.851
- Casos hospitalizados por ETI: 9.840
- Defunciones: 514

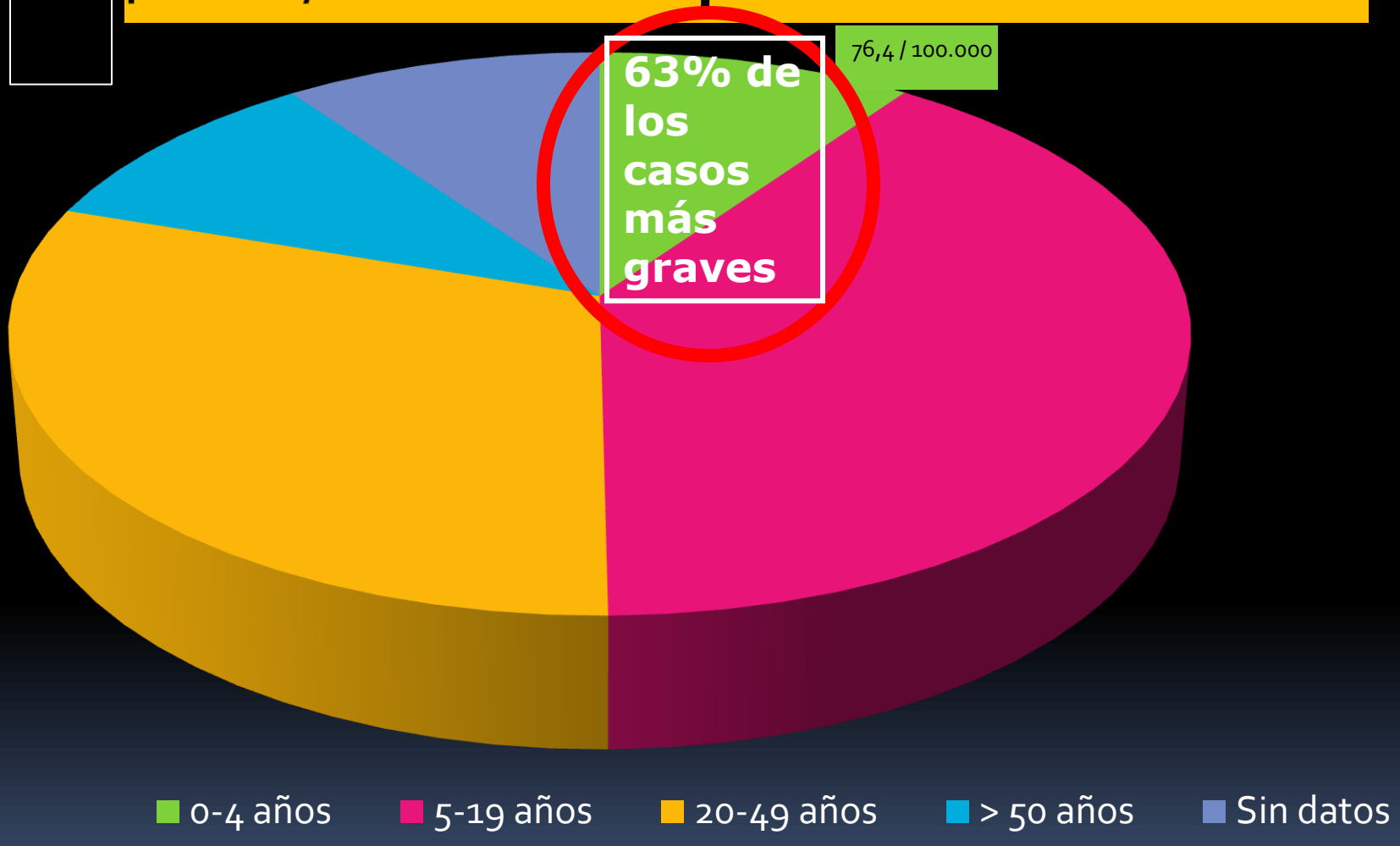
Fuente: Ministerio de Salud. 12 de Septiembre 2009.

Distribución porcentual de casos confirmados de Influenza A (H1N1) en Argentina según el grupo etario



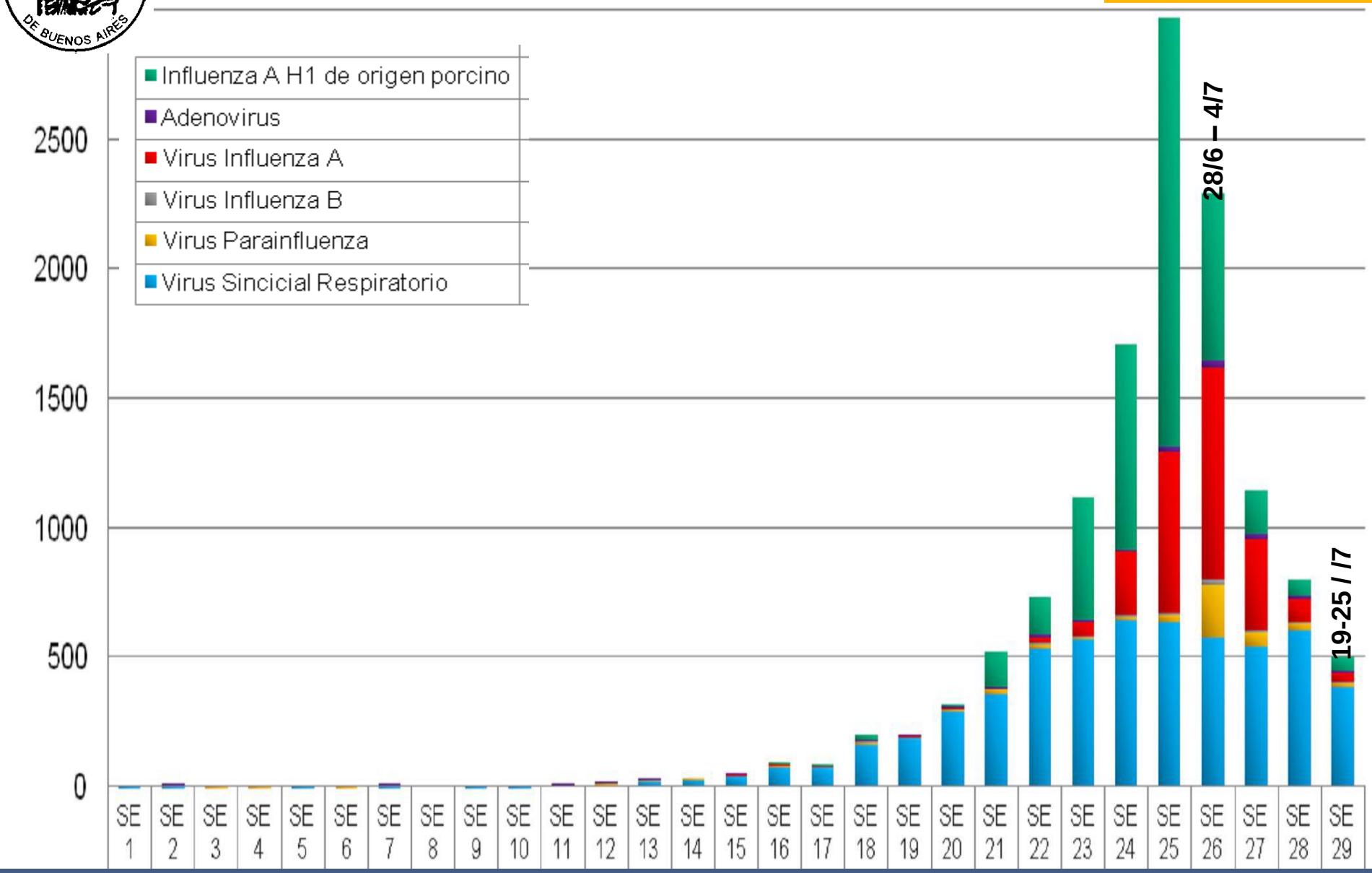
Fuente: Ministerio de Salud

41/100,000 Porcentajes relativ os



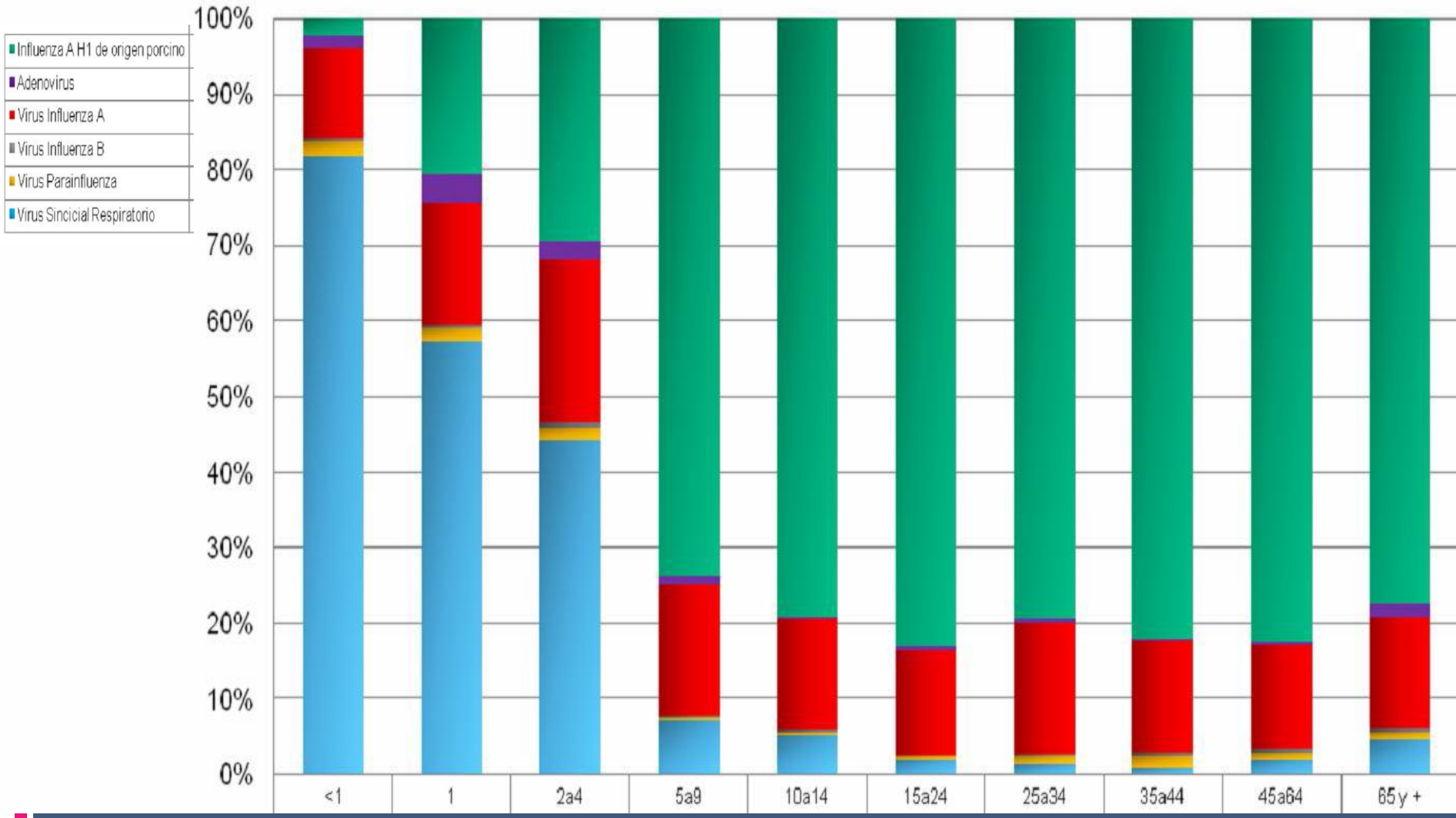
Fuente: Ministerio de Salud

Pico de defunciones



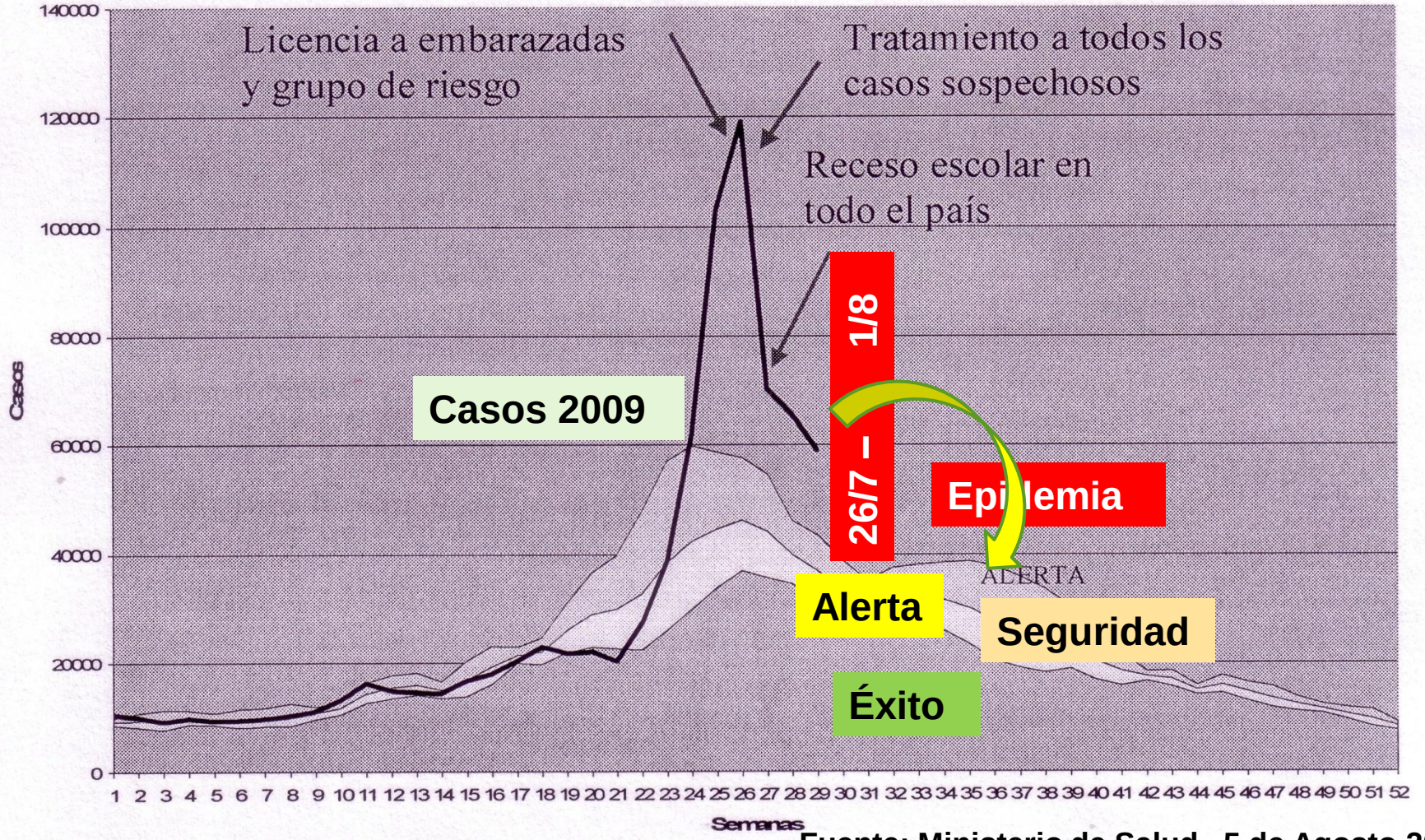
Fuente: Ministerio de Salud, 5 -8-2009

Distribución relativa de virus respiratorios en Argentina 2009 según grupo etario



Fuente: Ministerio de Salud

Devenir de la pandemia de *Influenza* A (H1N1/09)en Argentina



Fuente: Ministerio de Salud,. 5 de Agosto 2009

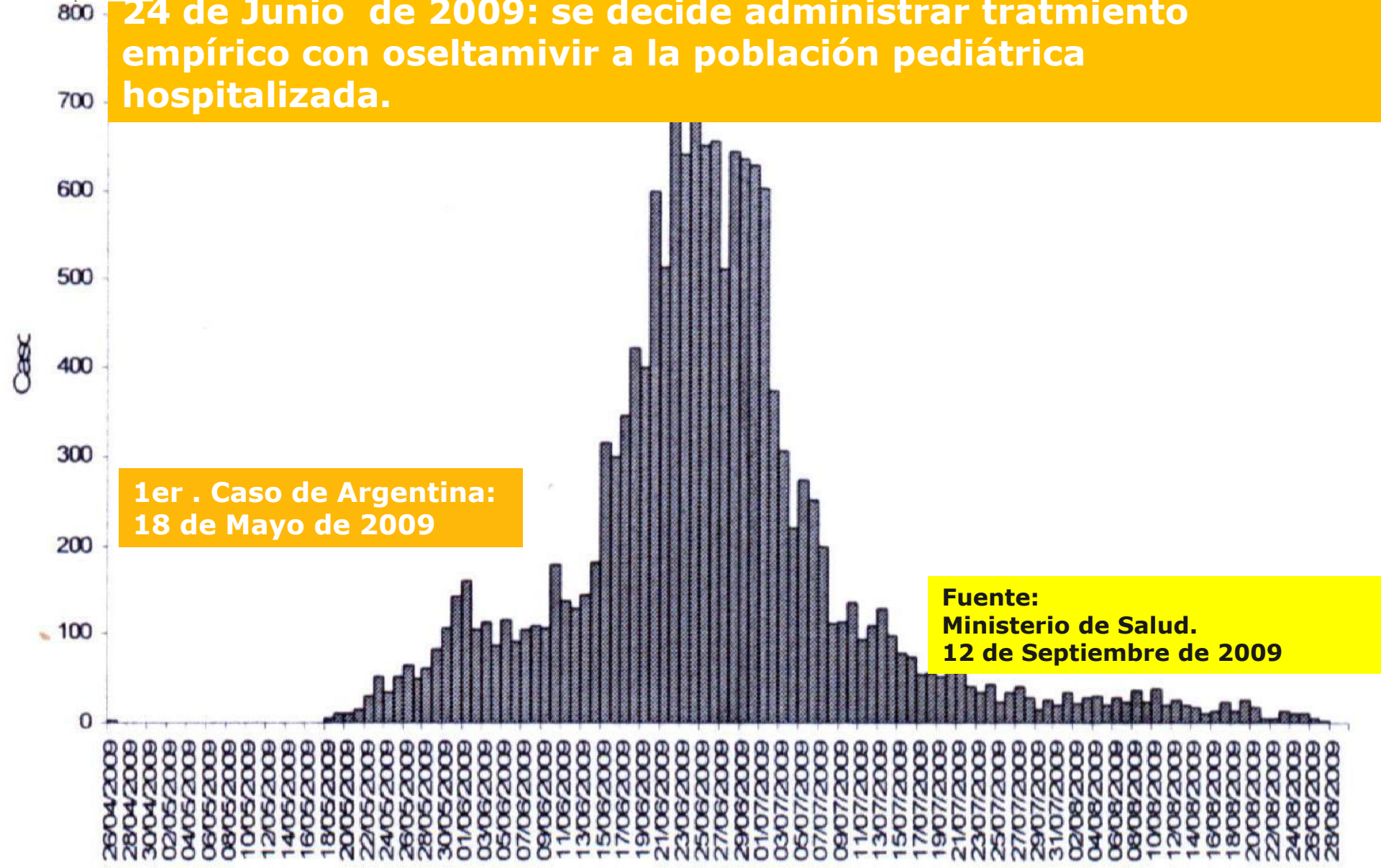
Indicadores de la pandemia en Argentina

- ✓Tendencia: *decreciente*
- ✓Intensidad de la actividad ETI: *elevada*
- ✓Dispersión geográfica: *generalizada*
- ✓Sistema de salud: *afectado moderadamente*

Fuente: Ministerio de Salud, 5 -8-2009

Gráfico 1: Distribución de casos confirmados y en estudio según fecha de inicio de síntomas. Argentina 2009. n= 15.455

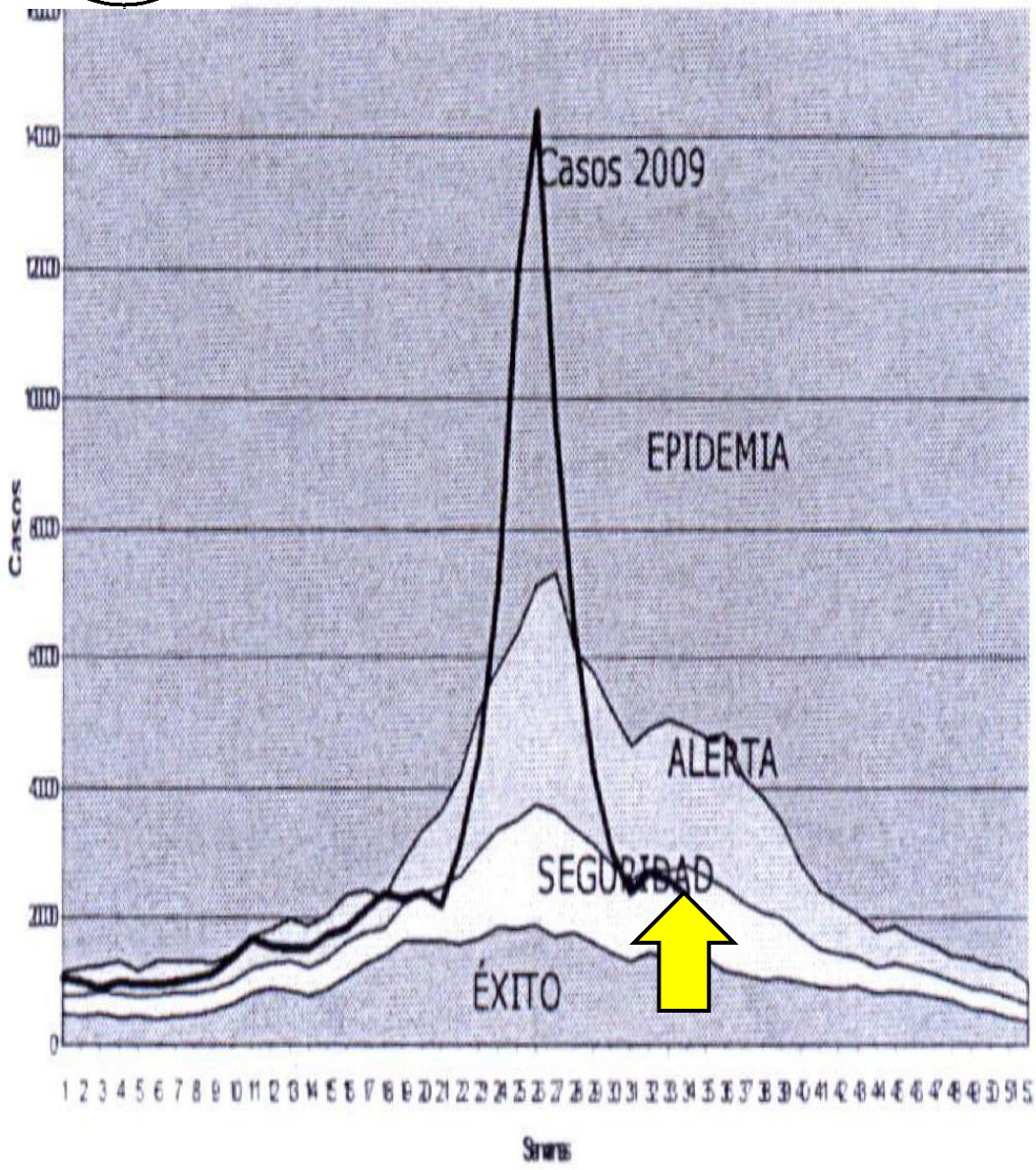
24 de Junio de 2009: se decide administrar tratmiento empírico con oseltamivir a la población pediátrica hospitalizada.



1er . Caso de Argentina: 18 de Mayo de 2009

Fuente:
Ministerio de Salud.
12 de Septiembre de 2009

Corredor Endémico Semanal de Enfermedades Tipo Influenza. Argentina 2009.

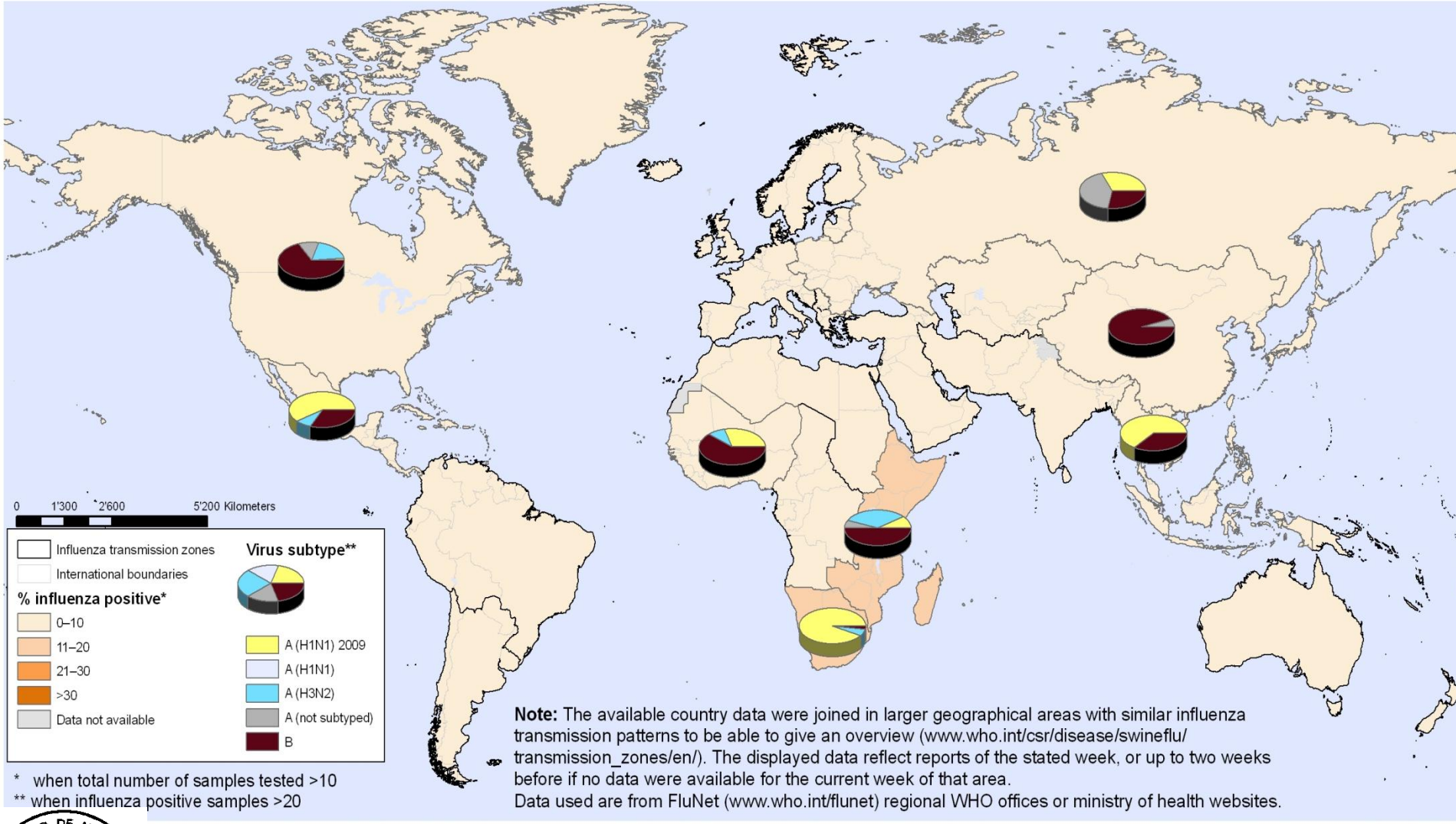


La vigilancia ETI registra un aumento de casos de Enfermedad Tipo Influenza notificados a partir de la semana 22. Como muestra el canal endémico (gráfico 8) , el número de casos supera el canal de alerta a partir de la semana 24, alcanzando una tasa máxima de 36,6 casos por 10.000 habitantes en la semana 26 (28 de junio al 4 de julio). A partir de la semana 27 se aprecia **un descenso evidente** en el número de casos. Sin embargo, la **intensidad de la actividad de enfermedad tipo influenza para la SE 34 es aún moderada** (5,8 por 10.000 hab.).

Fuente:
Ministerio de Salud.
12 de Septiembre de 2009

Percentage of respiratory specimens that tested positive for influenza
By influenza transmission zones

Status as of week 20
15-21 May 2011



* when total number of samples tested >10
** when influenza positive samples >20

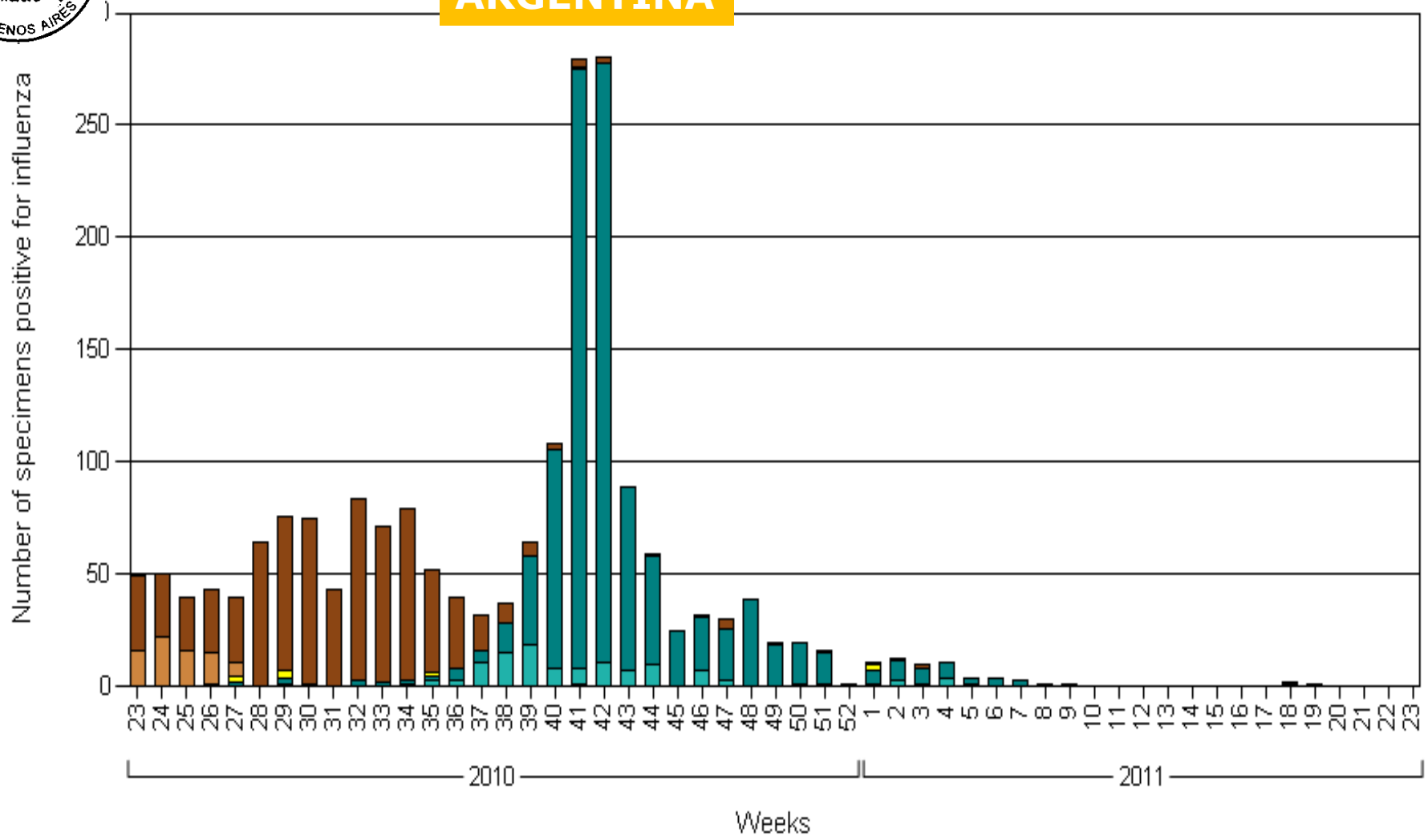
Note: The available country data were joined in larger geographical areas with similar influenza transmission patterns to be able to give an overview (www.who.int/csr/disease/swineflu/transmission_zones/en/). The displayed data reflect reports of the stated week, or up to two weeks before if no data were available for the current week of that area.
Data used are from FluNet (www.who.int/flunet) regional WHO offices or ministry of health websites.

Data Source: World Health Organization
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

Number of specimens positive for influenza by subtypes

ARGENTINA



El diagnóstico virológico

Métodos directos:

- **Muestra clínica: Hisopado nasofaríngeo y orofaríngeo.**
- **Detección genómica por RT-PCR o RT-PCR en tiempo real**

ó

- **Cultivo**

Los tests rápidos para detección de antígeno N de Influenza tienen una sensibilidad del 10-70% (falsos negativos cuando hay bajo título viral en la muestra clínica) .

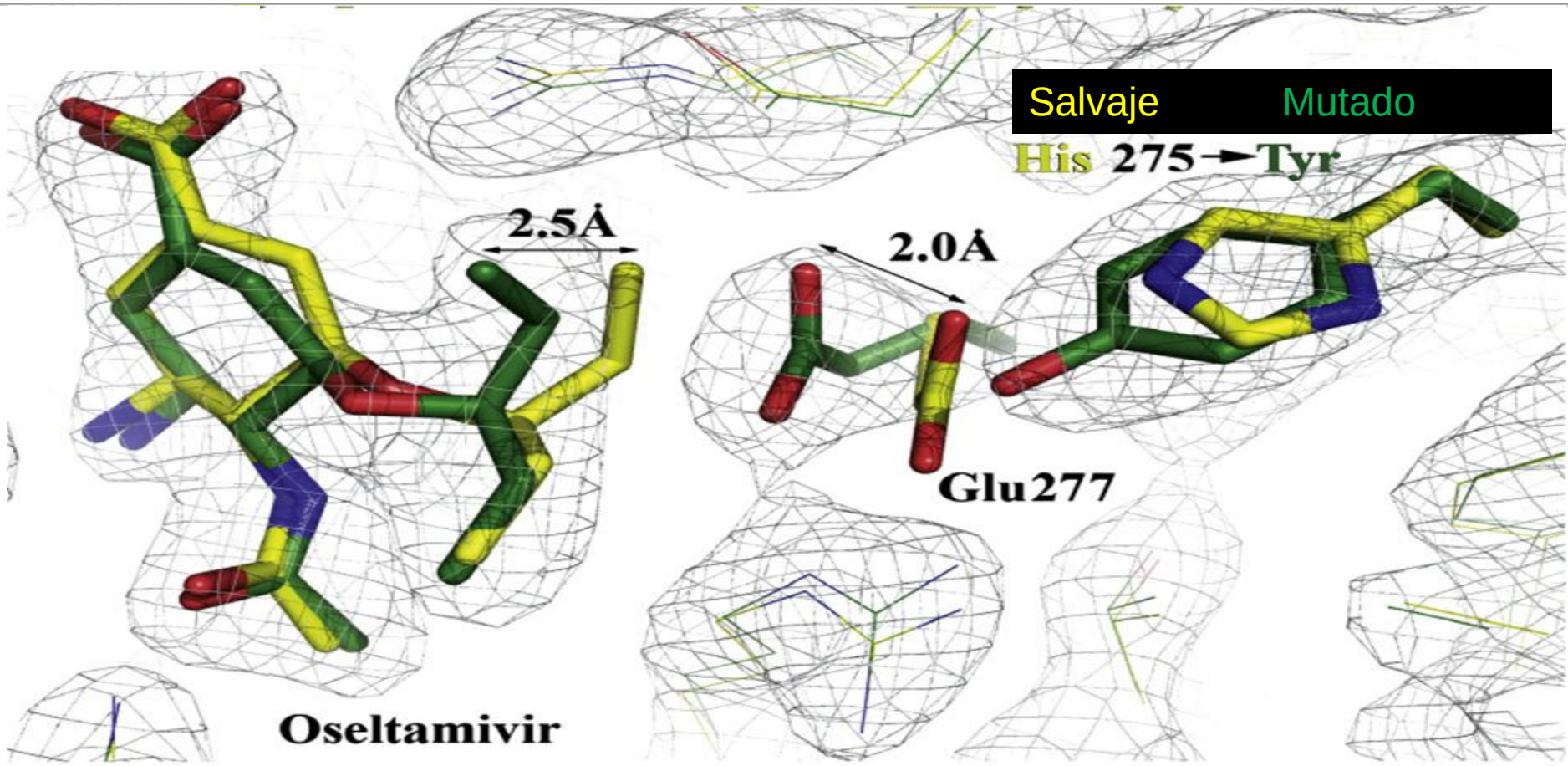
Métodos indirectos: La serología puede establecer un diagnóstico retrospectivo, con f fines epidemiológicos o de investigación.

Tratamiento

Inhibidores de la neuraminidasa:

- ✓**Oseltamivir**
- ✓**Zanamivir**

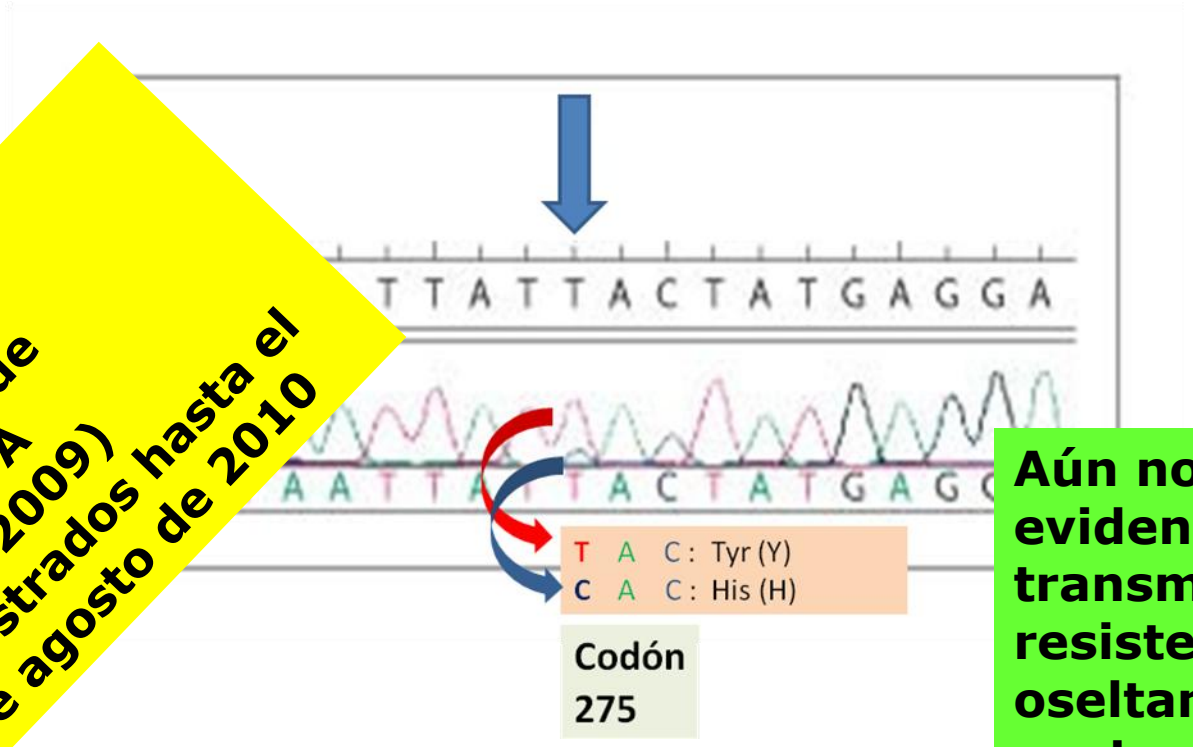
Estructural tridimensional cristalográfica Neuraminidasa de cepas de Influenza A H1N1 /2009 con resistencia al oseltamivir



Fuente: *Collins PJ et al. Vaccine 27: 6317–6323; Dec 2009.*

Se detectaron casos de resistencia al oseltamivir

H275Y (reemplazo de histidina por tirosina) codificada en el *gen de la neuraminidasa* del virus *Influenza* se asocia a la resistencia a dicho antiviral



302 casos de resistencia al oseltamivir de Influenza A (H1N1/2009) demostrados hasta el 4 de agosto de 2010

Aún no hay evidencias de la transmisión de la resistencia al oseltamivir a los contactos.

Emergence of intratreatment resistance to oseltamivir in pandemic influenza A H1N1 2009 virus.

METHODS:
Complementary DNAs, including the 275 codon, were obtained by reverse transcriptase PCR using viral RNAs extracted from nasopharyngeal or tracheal aspirates. Conventional sequencing and pyrosequencing were performed on each sample. In order to measure the virus susceptibility to oseltamivir, 50% inhibitory concentration determinations were performed by chemiluminescence.

RESULTS:
Sequential samples of two paediatric patients under oseltamivir treatment were analysed. Pretreatment samples were composed of 100% oseltamivir-sensitive variants. In case 1, the oseltamivir-resistant variant was found 8 days after the beginning of treatment. In case 2, the viral population became resistant on the second day of treatment, with 83% of the viral population bearing the mutation and this reached 100% on the seventh day.

CONCLUSIONS:
We describe the intratreatment emergence of oseltamivir resistance in two paediatric patients. Pyrosequencing allowed us to detect variant mixtures, showing the transition of the viral population from sensitive to resistant.

FACULTAD DE MEDICINA DE BUENOS AIRES

Hospital de Niños Ricardo Gutiérrez

Valinoto LA, *et al. Antivir Ther* 2010;15(6):923-7.

Profilaxis activa

EE.UU. : la FDA *(Food and Drug Administration)* aprobó el 21/09/2009 la utilización de 4 vacunas.
3 son inactivadas (administración inyectable) y
1 es atenuada (para administrar por vía intranasal).

China: está inmunizando con una vacuna inactivada propia.

Response after One Dose of a Monovalent Influenza A (H1N1) 2009 Vaccine —Preliminary Report

Background **A novel influenza A (H1N1) 2009 virus is responsible for the firstinfluenza pandemic in 41 year. A safe and effective vaccine is urgently needed.**
A randomized, observer-blind, parallel-group trial evaluating two doses of an inactivated, split-virus 2009 H1N1 vaccine in healthy adults between the ages of 18 and 64 years is ongoing at a single site in Australia.

Methods **This preliminary report evaluates the immunogenicity and safety of the vaccine 21 days after the first of two scheduled doses. A total of 240 subjects, equally divided into two age groups (<50 years and 50 years), were enrolled and underwent randomization to receive either 15 µg or 30 µg of hemagglutinin antigen by intramuscular injection. We measured antibody titers using hemagglutination-inhibition and microneutralization assays at baseline and 21 days after vaccination. The coprimary immunogenicity end points were the proportion of subjects with antibody titers of 1:40 or more on hemagglutination-inhibition assay, the proportion of subjects with either seroconversion or a significant increase in antibody titer,**
and the factor increase in the geometric mean titer.

Results **By day 21 after vaccination, antibody titers of 1:40 or more were observed in 116 of 120 subjects (96.7%) who received the 15-µg dose and in 112 of 120 subjects (93.3%) who received the 30-µg dose. No deaths, serious adverse events, or adverse events of special interest were reported. Local discomfort (e.g., injection-site tenderness or pain) was reported by 46.3% of subjects, and systemic symptoms (e.g., headache) by 45.0% of subjects. Nearly all events were mild to moderate in intensity.**

Conclusions **A single 15-µg dose of 2009 H1N1 vaccine was immunogenic in adults, with mild-to-moderate vaccine-associated reactions.**

Greemberg M.E. *et al* .
NEJM September 10, 2009

The NEW ENGLAND JOURNAL of MEDICINE

Trial of Influenza A (H1N1) 2009 Monovalent MF59-Adjuvanted Vaccine -- Preliminary Report.

BACKGROUND: The 2009 pandemic influenza A (H1N1) virus has emerged to cause the first pandemic of the 21st century. Development of effective vaccines is a public health priority. METHODS: We conducted a single-center study, involving 175 adults, 18 to 50 years of age, to test the monovalent influenza A/California/2009 (H1N1) surface-antigen vaccine, in both MF59-adjuvanted and nonadjuvanted forms. Subjects were randomly assigned to receive two intramuscular injections of vaccine containing 7.5 mug of hemagglutinin on day 0 in each arm or one injection on day 0 and the other on day 7, 14, or 21; or two 3.75-mug doses of MF59-adjuvanted vaccine, or 7.5 or 15 mug of nonadjuvanted vaccine, administered 21 days apart. Antibody responses were measured by means of hemagglutination-inhibition assay and a microneutralization assay on days 0, 14, 21, and 42 after injection of the first dose.

RESULTS: Results of an interim analysis of the responses to the 7.5-mug dose of MF59-adjuvanted vaccine by days 14 and 21 are presented (data from four of the seven groups studied, for a total of 100 subjects). The most frequent local and systemic reactions were pain at the injection site and muscle aches, noted in 70% and 42% of subjects, respectively. Two subjects reported fever, with a temperature of 38 degrees C or higher, after the first dosing. Antibody titers, expressed as geometric means, were generally higher at day 14 among subjects who had received two 7.5-mug doses of the MF59-adjuvanted vaccine than among those who had received only one by this time point (P=0.04 by the hemagglutination-inhibition assay and P<0.001 by the microneutralization assay). By 21 days after vaccination with the first dose of 7.5 mug of MF59-adjuvanted vaccine, the rates of seroconversion, as measured with the use of a hemagglutination-inhibition assay and a microneutralization assay, were 76% and 92% of subjects, respectively, who had received only one dose to date (with the second dose scheduled for day 21) and 88 to 92% and 92 to 96% of subjects, respectively, who had already received both doses (P=0.11 and P=0.64, respectively).

CONCLUSIONS: In preliminary analyses, the monovalent influenza A (H1N1) 2009 **MF59-adjuvanted vaccine generates antibody responses likely to be associated with protection within 14 days** after a single dose is administered.

Clark TW, *et al.* NEJM September 10, 2009

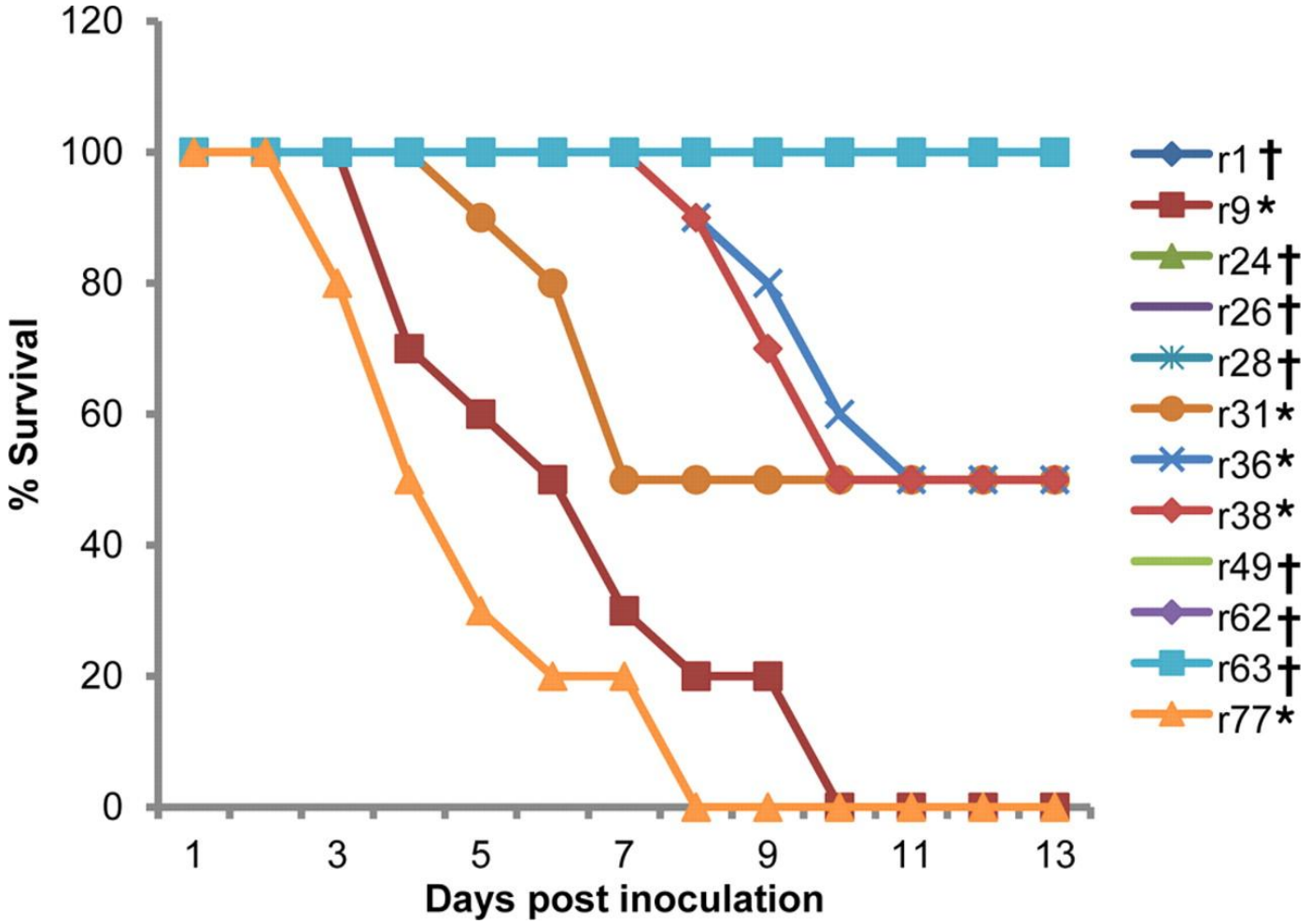
The NEW ENGLAND JOURNAL of MEDICINE

¿Es posible una reasociación entre cepas H5N1 y H1N1 pd 2009?

Gene mutations and reassortment are key mechanisms by which influenza A virus acquires virulence factors. To evaluate the role of the viral polymerase replication machinery in producing virulent pandemic (H1N1) 2009 influenza viruses, we generated various polymerase point mutants (PB2, 627K/701N; PB1, expression of PB1-F2 protein; and PA, 97I) and reassortant viruses with various sources of influenza viruses by reverse genetics. Although the **point mutations produced no significant change in pathogenicity, reassortment between the pandemic A/California/04/09 (CA04, H1N1) and current human and animal influenza viruses produced variants possessing a broad spectrum of pathogenicity in the mouse model. Although most polymerase reassortants had attenuated pathogenicity (including those containing seasonal human H3N2 and high-pathogenicity H5N1 virus segments) compared to that of the parental CA04 (H1N1) virus, some recombinants had significantly enhanced virulence. Unexpectedly, one of the five highly virulent reassortants contained a A/Swine/Korea/JNS06/04(H3N2)-like PB2 gene with no known virulence factors; the other four had mammalian-passaged avian-like genes encoding PB2 featuring 627K, PA featuring 97I, or both. Overall, the reassorted polymerase complexes were only moderately compatible for virus rescue, probably because of disrupted molecular interactions involving viral or host proteins. Although we observed close cooperation between PB2 and PB1 from similar virus origins, we found that PA appears to be crucial in maintaining viral gene functions in the context of the CA04 (H1N1) virus. These observations provide helpful insights into the pathogenic potential of reassortant influenza viruses composed of the pandemic (H1N1) 2009 influenza virus and prevailing human or animal influenza viruses that could emerge in the future.**

Song, M.-S. et al. 2011. J. Virol. 85(13):6275-6286

Sobrevida de ratones inoculados con virus con genoma reasociado experimentalmente que tienen igual o mayor patogenicidad que la cepa pandémica CA04 (H1N1)

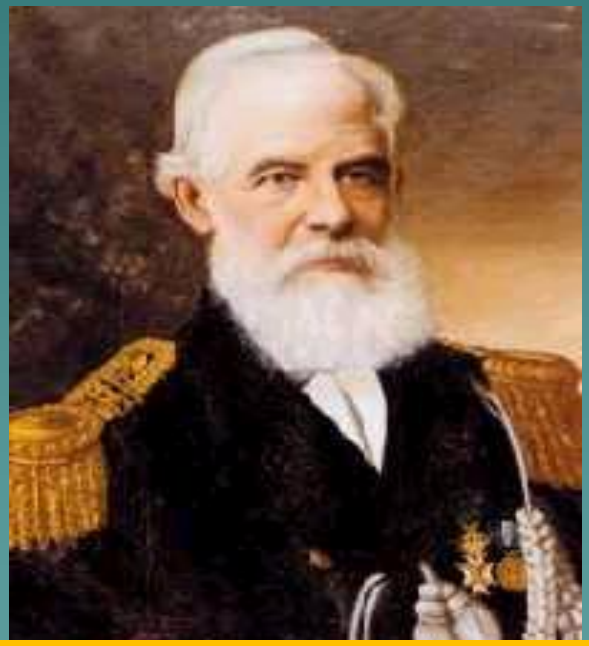


Song, M.-S. et al. 2011. J. Virol. 85(13):6275-6286

Formar(se) desde la Verdad, en el Bien, y por amor al prójimo

Francisco Xavier Muñiz

Carlo Urbani*



****F.J.M.: Médico jubilado, decidió dejar su quinta en La Reja, para atender pacientes de fiebre amarilla durante la epidemia que asoló Buenos Aires. Falleció -atendiendo pacientes- el 8 de abril de 1871.***
****C.U.: Médico de la OMS y de "Médicos sin Fronteras" que se contagió el SARS (síndrome agudo respiratorio grave) atendiendo pacientes, por lo que falleció el 29 de marzo de 2003. Dijo":"La salud y la dignidad son inseparables en el ser humano; es una obligación estar en contacto con las víctimas y garantizar sus derechos"***