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Type A

(8 RNA segments)

9 Kb

(*polimerasis complex*)

(PB1, PB2, PA) (Efficient growth)

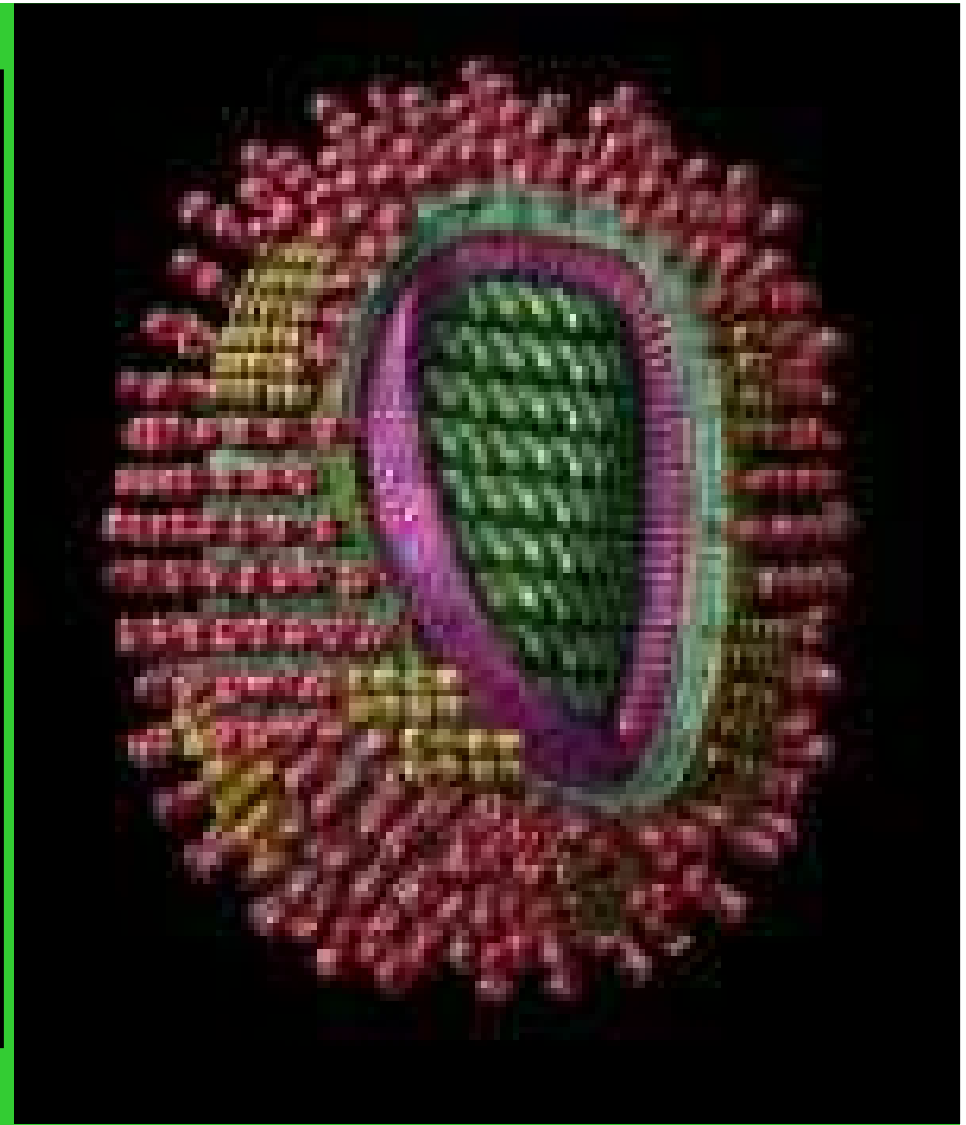
4° - HA (Haemoagglutinin) (Infectivity)

5° - NP(Nucleoprotein)

6° - NA (Neuroaminidase)

7° - M1 , M2 (MatrixProt.)

8° - NS .1, NS 2 (Pathogenicity)



Type A Mammals and Birds (At the time, 16 HA and 9 NA are known)

Type B Human
Mammals

Type C Human

Dhori/Thogoto (Tick borne)

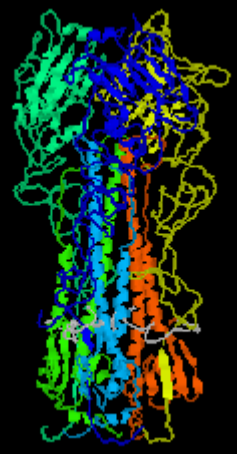
Refresh genes Time = Reproductive cycle /
Mutation rate
Human mutation
of single gene (1000 bases)
might take thousands of generations

Antigenic drift / Shift
Recombination

Macro Evolution

RNA virus exhibit the highest
mutation rate of any group of
organism
one per genome per replication
(around $10^{-3} / 10^{-5}$)

Micro Evolution

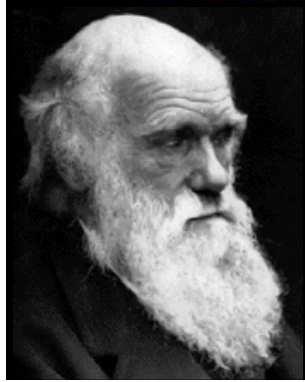
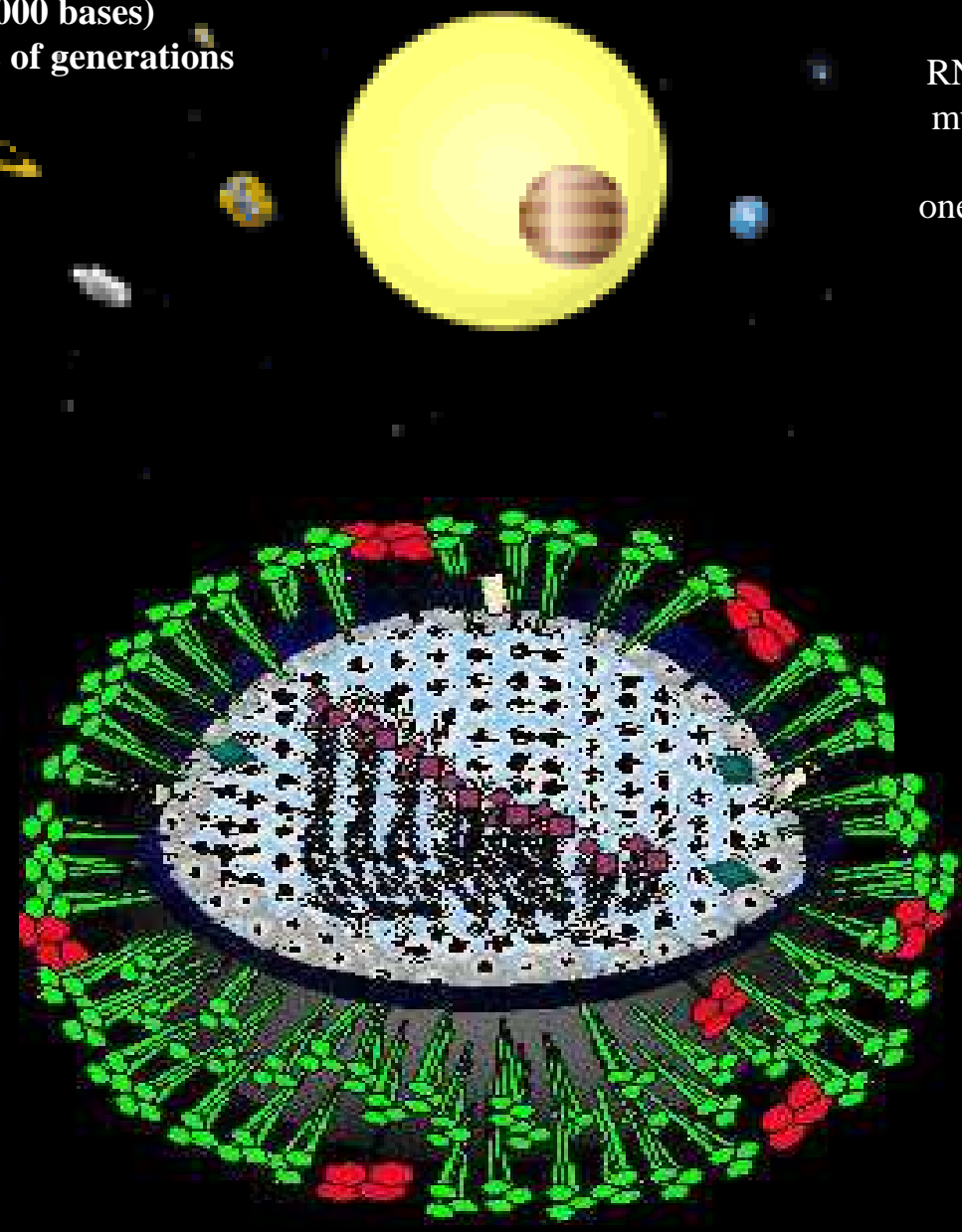


Population size

10^{12} viral particles in an organism

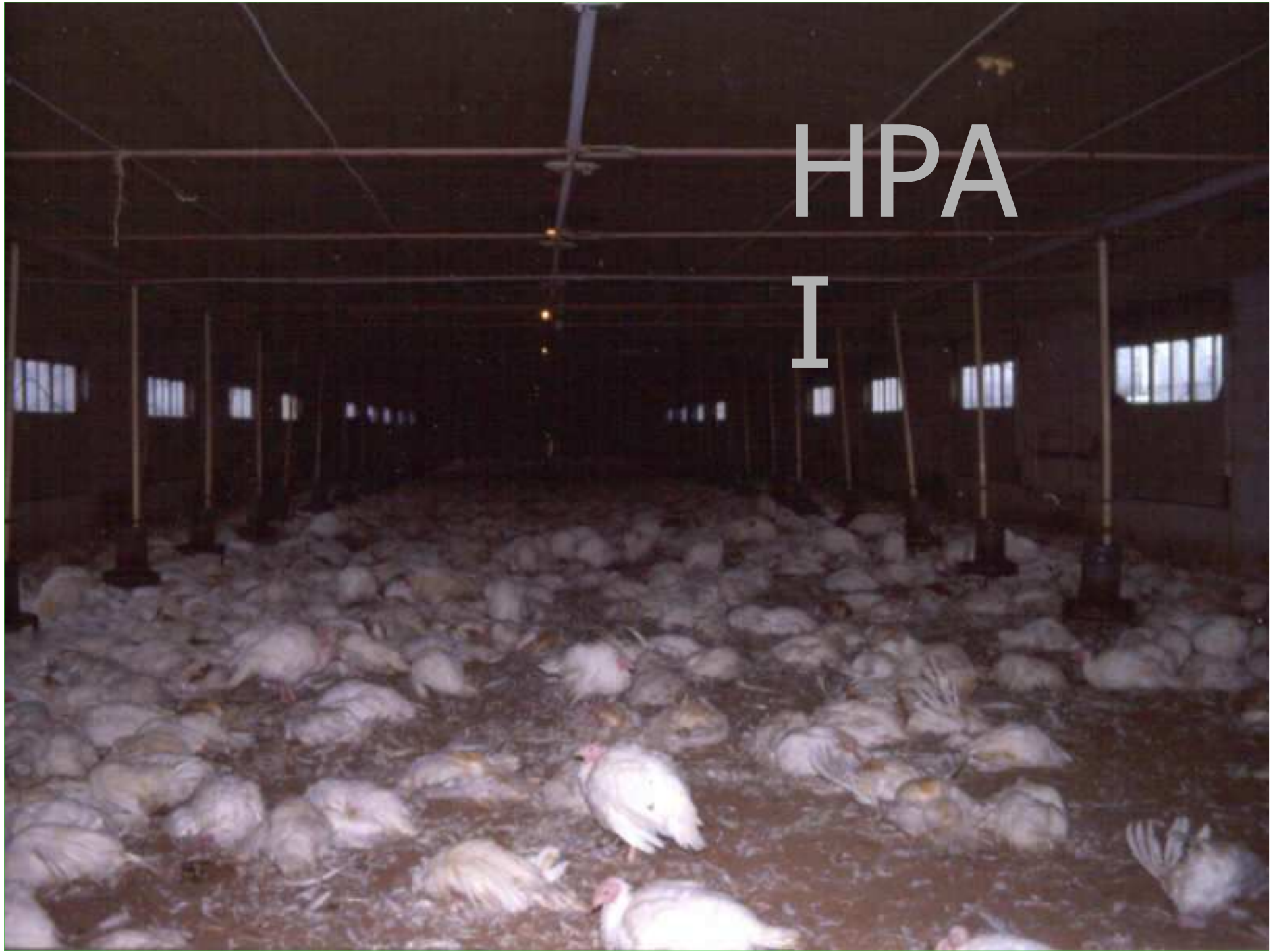
Population growth

one virus can produce 100,000
viral copies
in 10 hours



HPA

I



Avian viruses
H1 → H16, N1 → N9

- H7 N7 U. K. Dutch
- H5 N1 Hong-Kong 1997
- H9 N2 "Chinese"
- H5 N1 Hong-Kong 2003
- H5 N1 Bruxel 2004



mixing



- H1N1
- H2N2
- H3N2



Horse viruses
H3N8 / H7N7

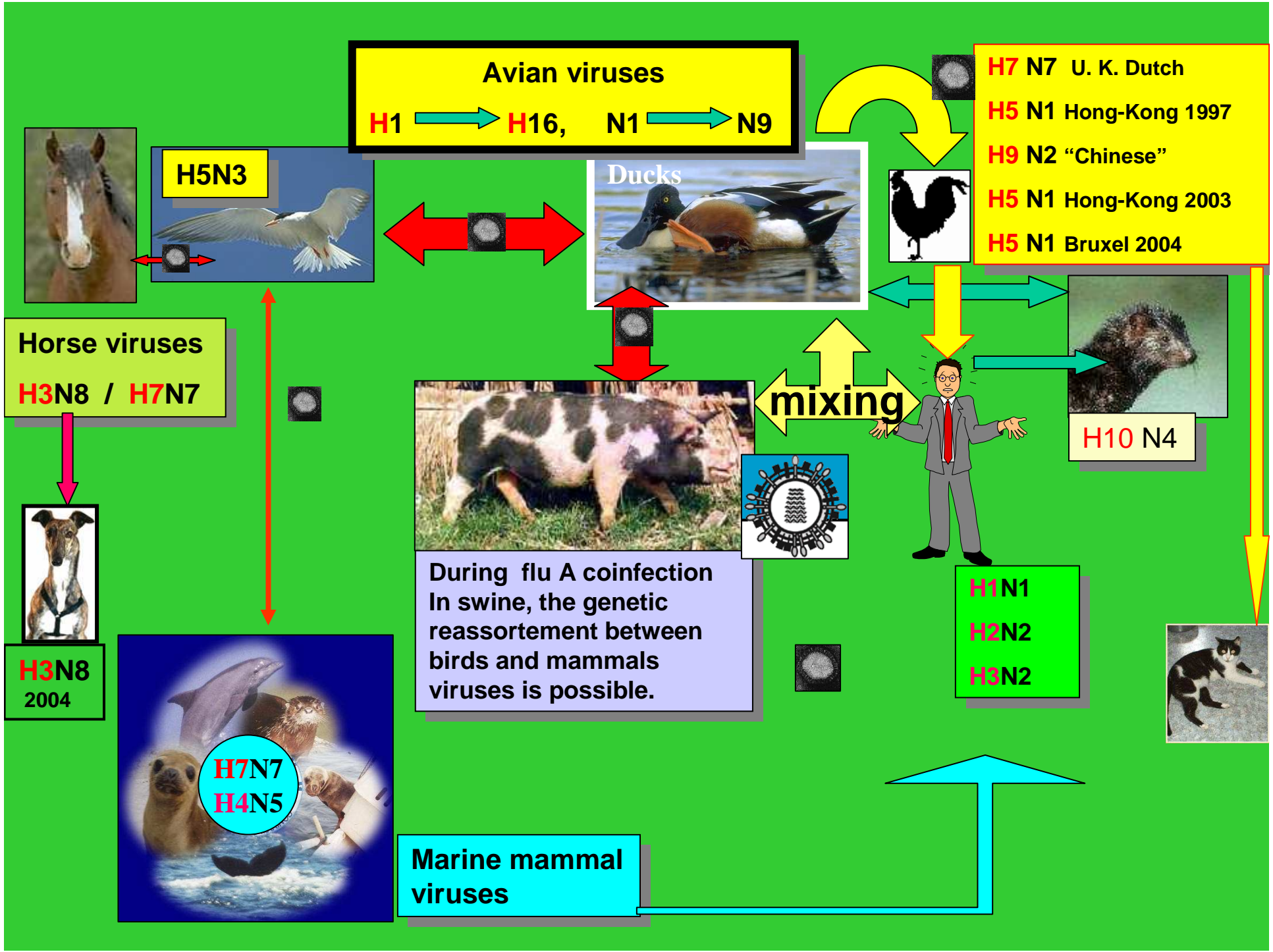


H3N8
2004



Marine mammal viruses

During flu A coinfection
In swine, the genetic
reassortment between
birds and mammals
viruses is possible.

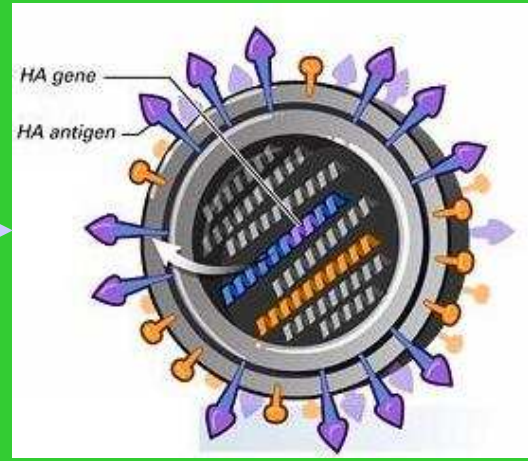


Un master di metamorfosi

Variabilità antigenica

Virus Influenzali

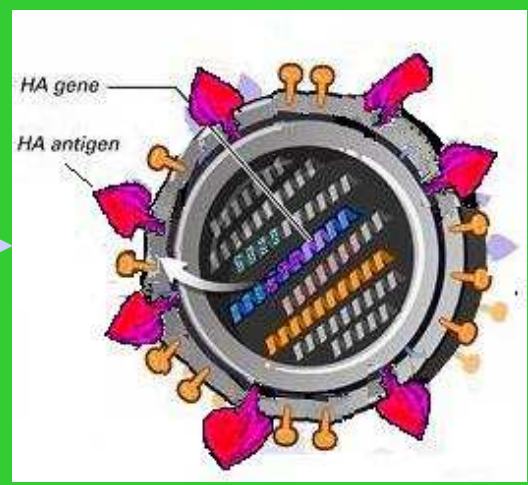
Antigenic Drift



Tipo A, B

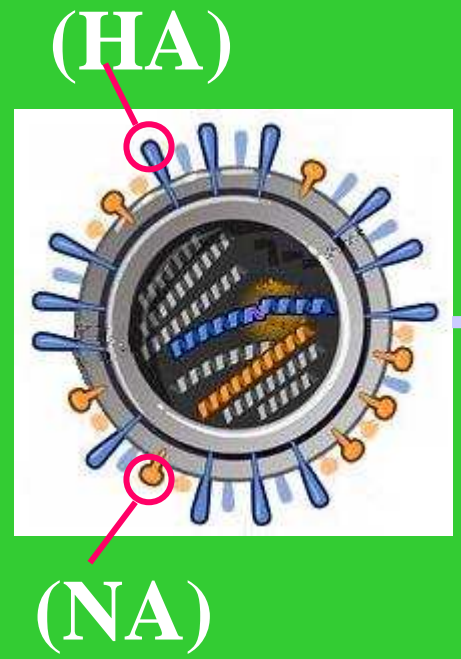
epidemico

Antigenic Shift



Tipo A

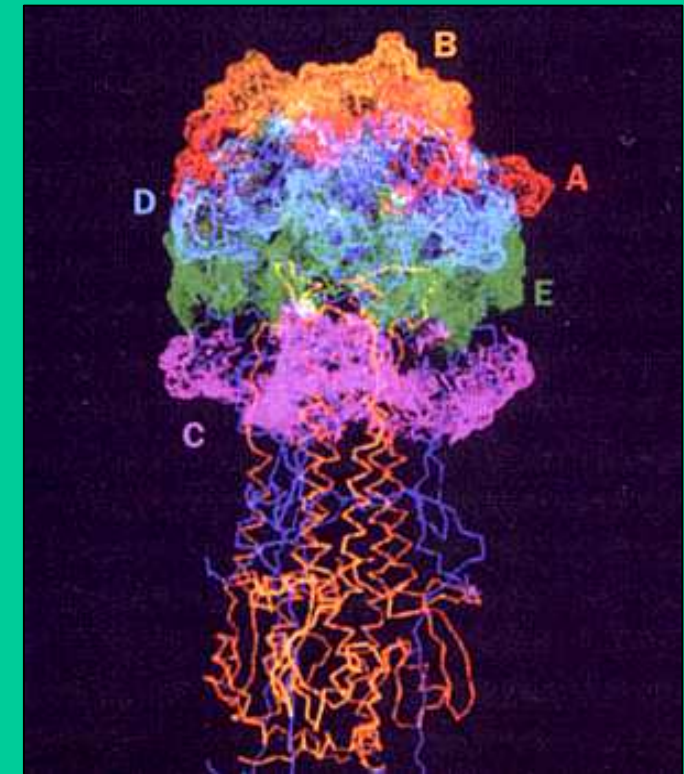
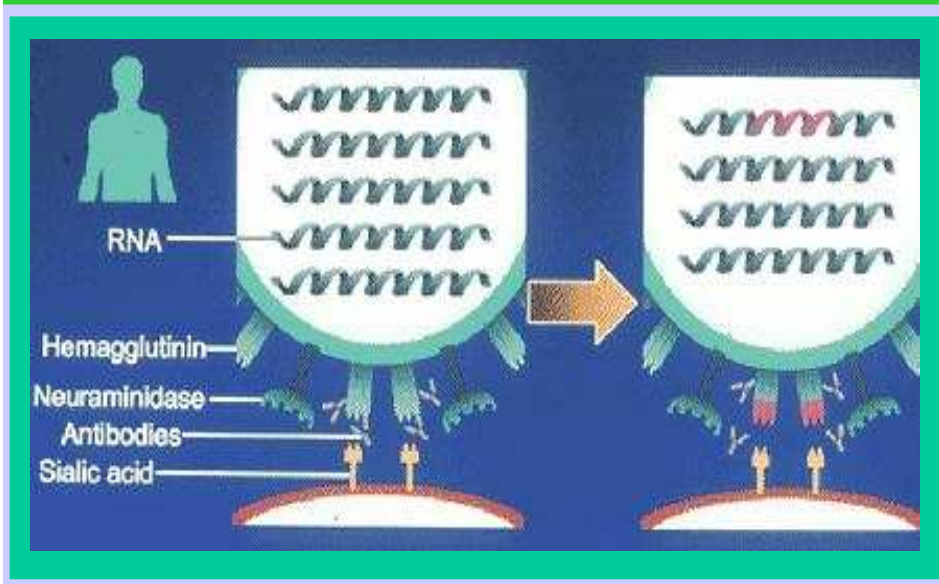
pandemico



Flu A & Flu B: *antigenic drift*

Mutazioni casuali

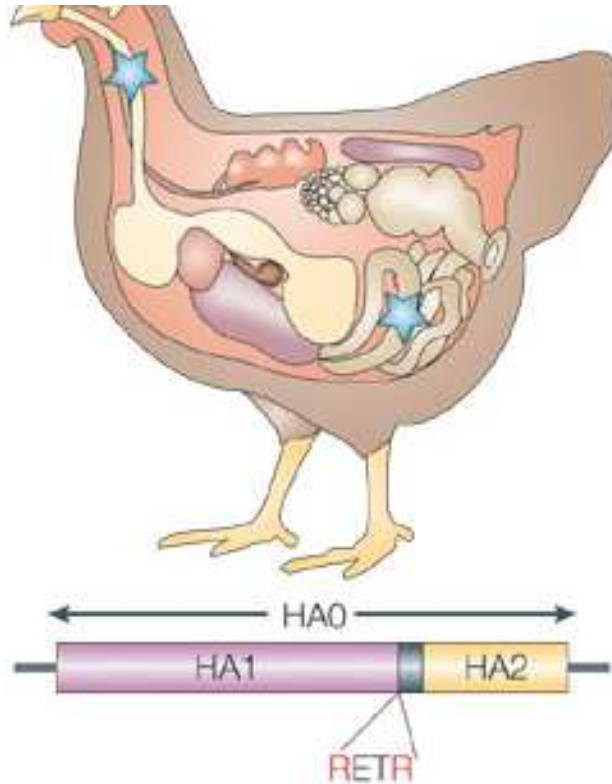
cambiano HA/NA



Sequenza di aminoacidi basici (R / K) nel sito di clivaggio dell'emoagglutinina (HA0 - HA1/HA2)

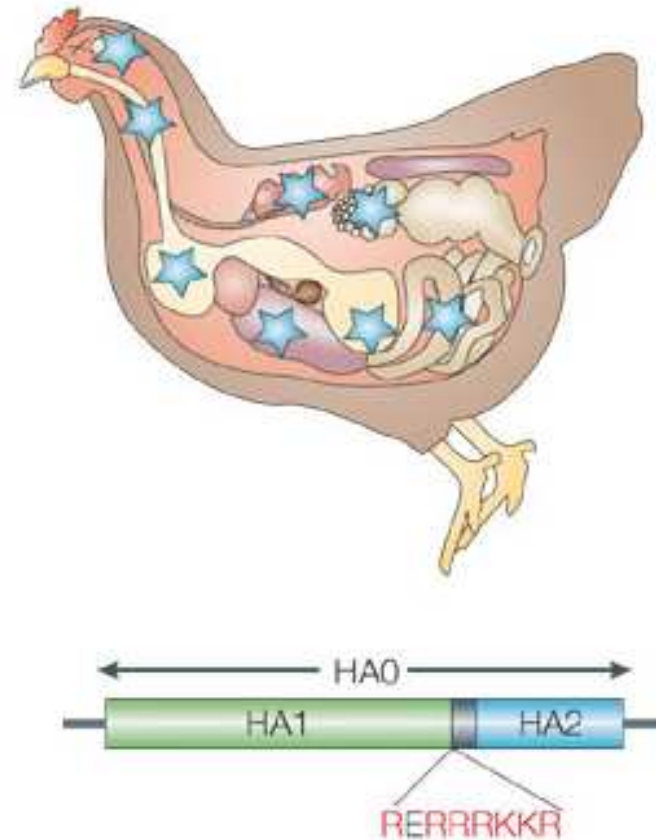
LPAI

Proteasi localizzate negli organi respiratori e nell'intestino



HPAI

Proteasi ubiquitarie (**Furina like**)

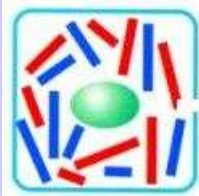
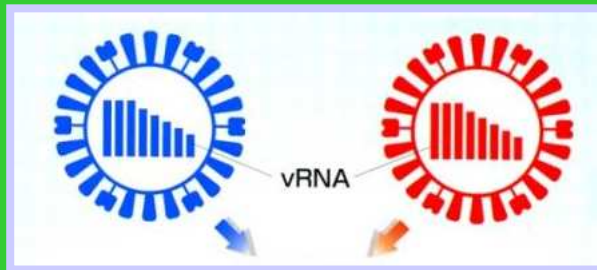


Influenza A: *antigenic shift*

Modello1

Flu A
Aviaria

Flu A
Umana



riassortimento



Modello2

Flu A
Aviaria



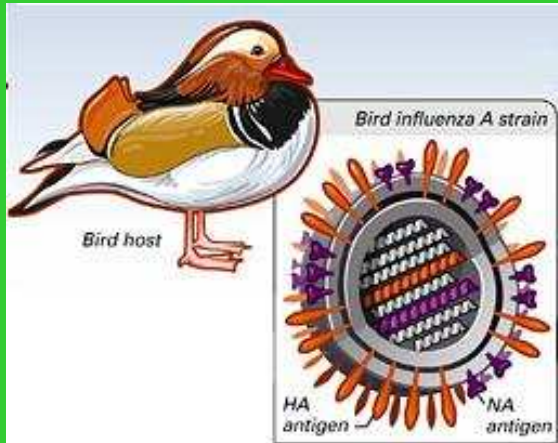
Salto di specie



UOMO

antigenic shift

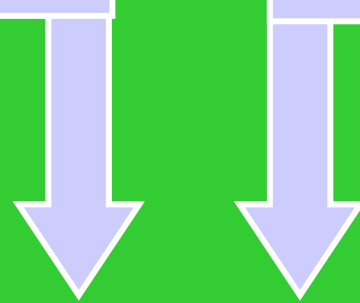
MODELLO 1: riassortimento




Virus dell'influenza aviario



Virus dell'influenza umano



MIXING VESSEL → 

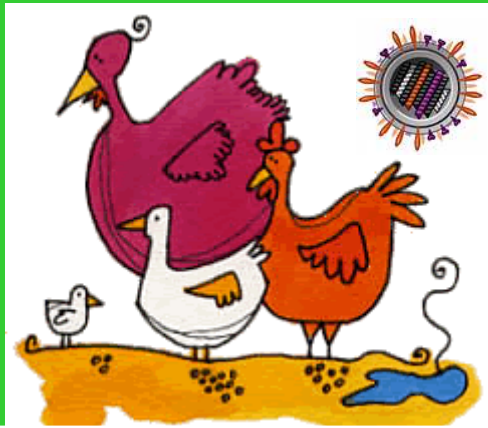
Recettori $\alpha 2,3$ $\alpha 2,6$



Virus riassortante

antigenic shift

MODELLO 2: salto di specie

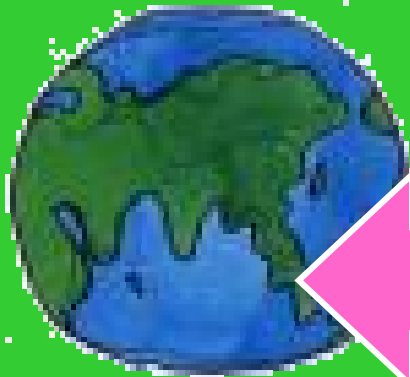


Virus dell'influenza aviario

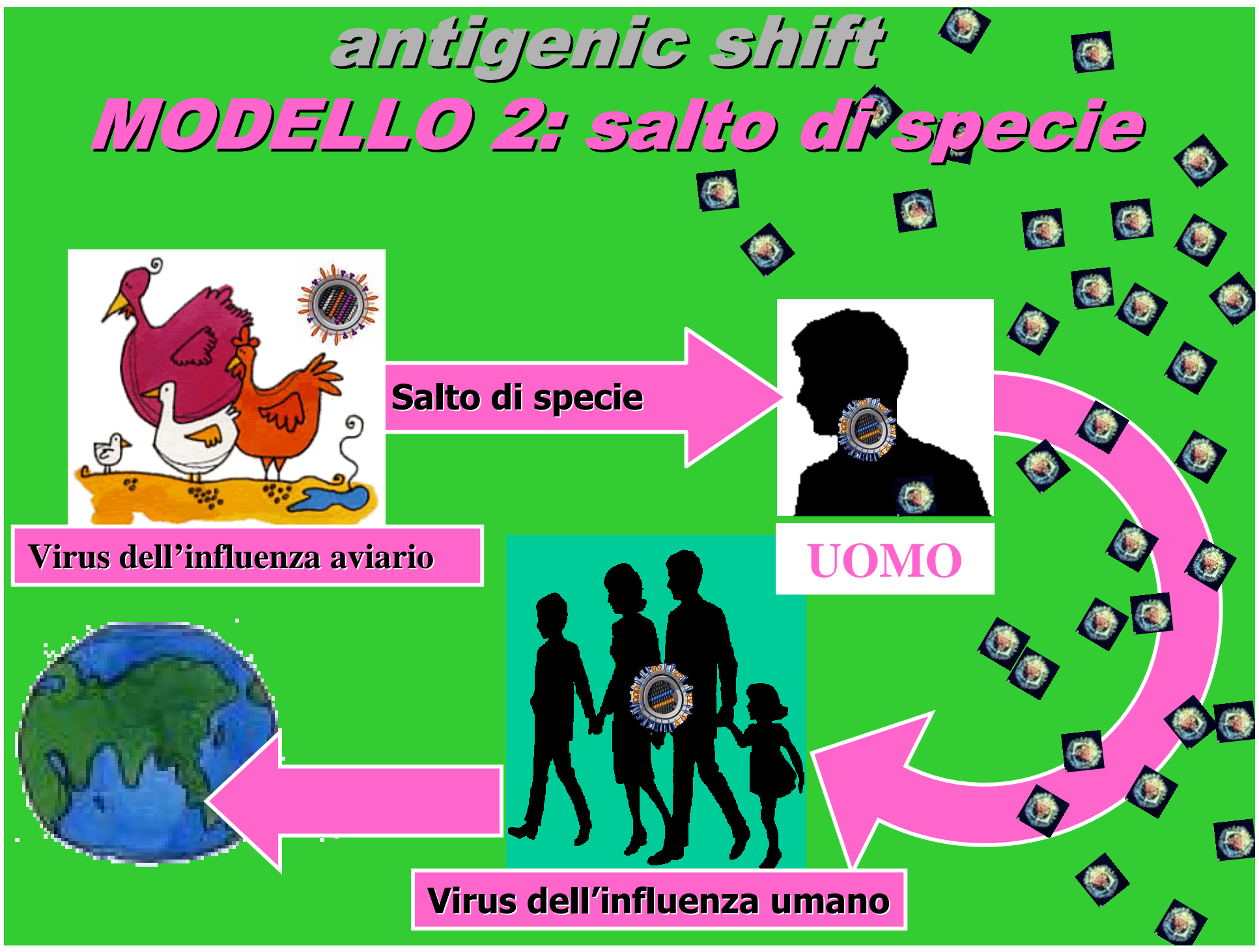
Salto di specie



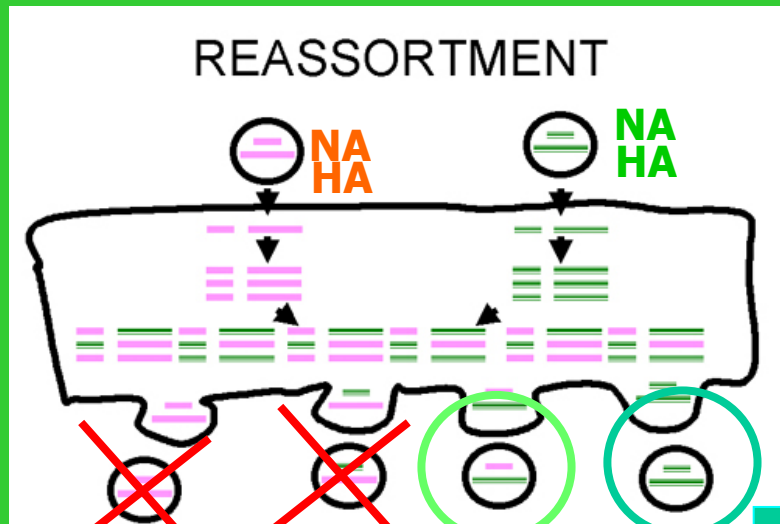
UOMO



Virus dell'influenza umano

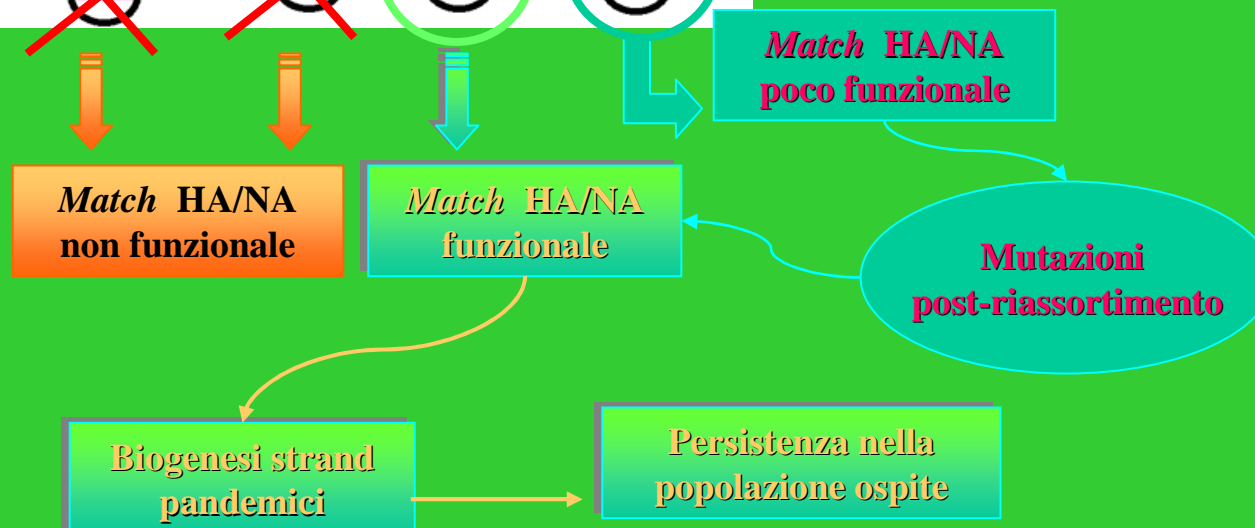


Biogenesi di ceppi pandemici *match* HA-NA



**SET GENICI ISOLATI
IN NATURA:**

UOMO: H1H1, H2N2, H3N2,
SUINO: H3N2, H1H1

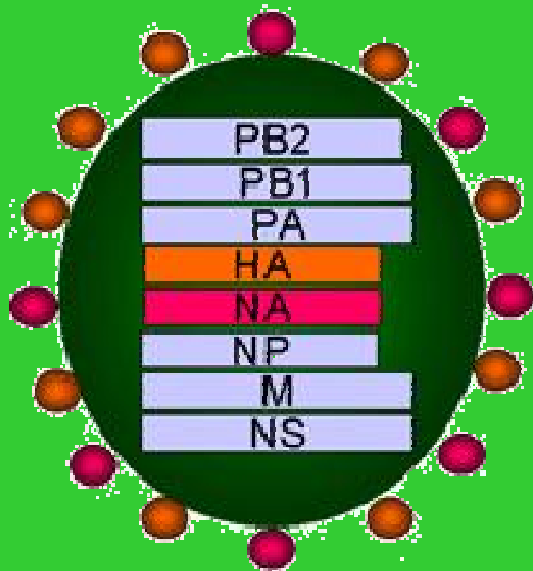


Biogenesi di ceppi pandemici

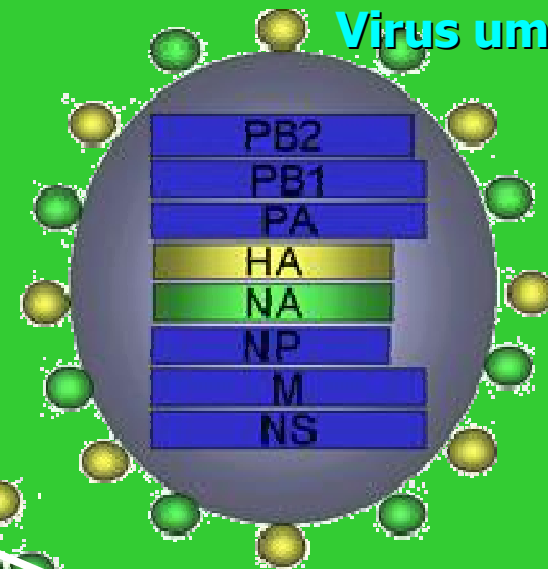
Capacità replicativa

**Complesso polimerasico:
PB1, PB2, PA**

Virus aviario



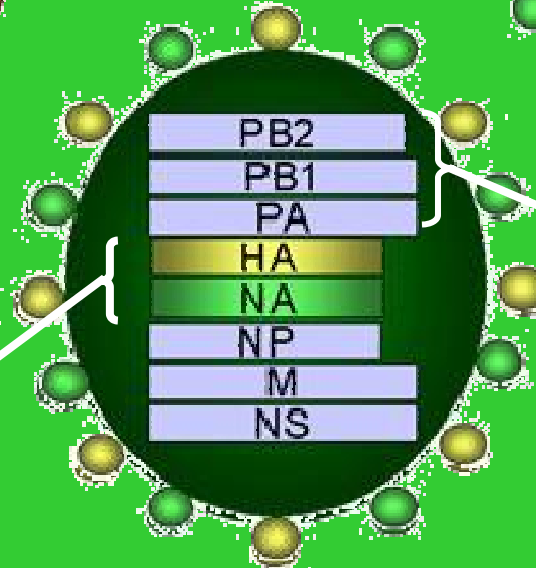
Virus umano



X

Virus riassortante

Specificità d'ospite



**Efficienza di
replicazione**

Pandemie Influenzali

1918	(Spanish Flu)	H1N1
1957	(Asian Flu)	H2N2
1968	(Hong Kong Flu)	H3N2
1977	(The USA)	H1N1

Dal 1977 H3N2 e H1N1 co-circolano

The 1918 flu virus is resurrected

JK Taubenberger

Nature 6 ottobre 2005

Caratterizzazione molecolare dei geni di PA-PB1-PB2

È un virus "avian-like"

differiscono solo di pochi aminoacidi dalle sequenze *consensus* aviarie:

7 PB1

7 PA

5 PB2

aa
627

Importante per
l'adattamento alle
cellule di mammifero

Alta virulenza:

differenza di **10 aa** per il complesso polimerasico tra virus
aviari e umani

H1N1

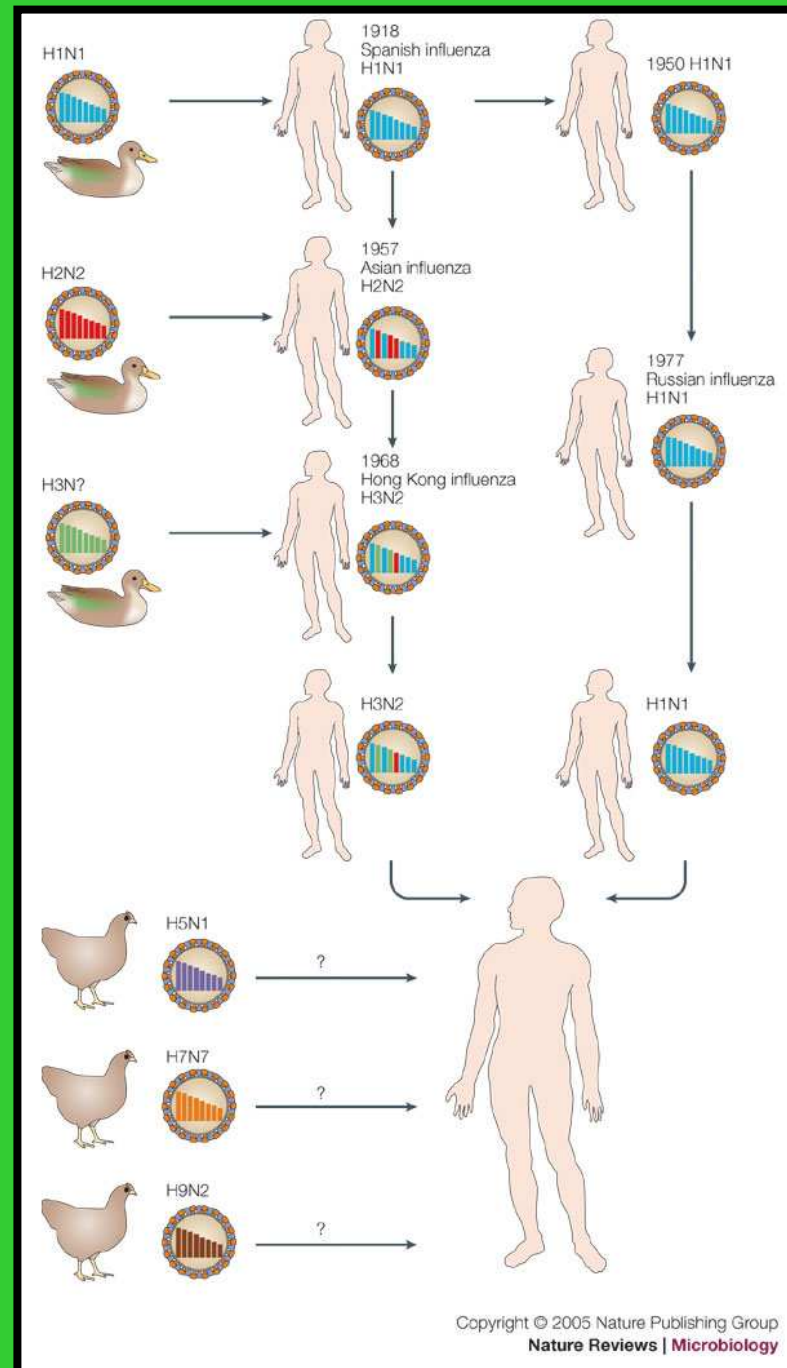
H5N1

H7N7

Codoni codificanti il peptide F2 di PB1
(induzione della morte cellulare)



Pandemie Influenzali: Passate e Future?



The New York Times

L'influenza mette le ali

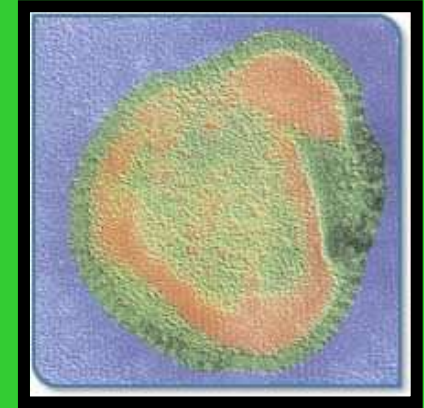


Infezioni umane da virus aviari

1997	H5N1	HK: 18 casi, 6 morti
1999	H9N2	HK, Cina: 7 casi
Feb 2003	H5N1	HK: 2 casi, 1 morto
Feb 2003	H7N7	NLD: 83 congiuntiviti, 1 morto
Dec 2003	H9N2	HK: 1 caso
Mar 2004	H7N3	Canada: 2 casi
Jan 2004- Oct 2005	H5N1	(118 casi, 61 morti)

Reservoir : Why Ducks?

Species where the virus can replicate and maintain itself indefinitely in the time



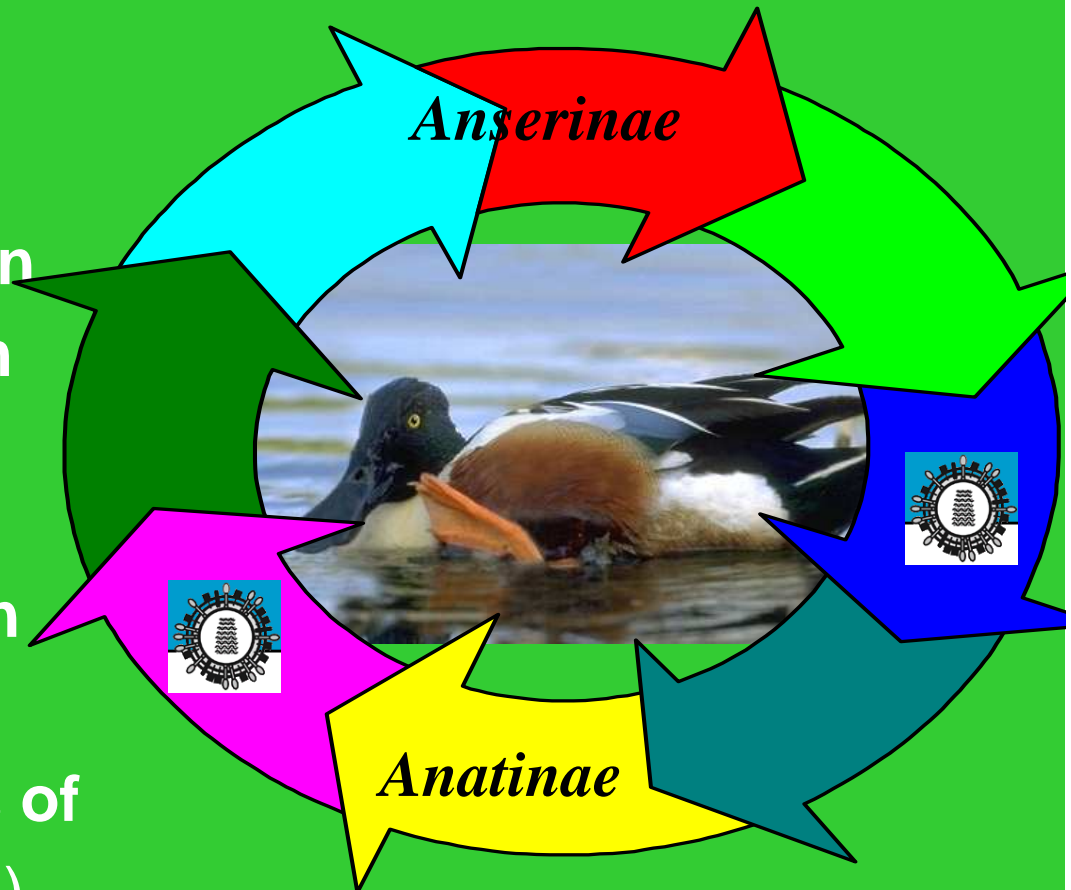
Water related species

Migratory behaviour

Cyclic Interaction between populations coming from different areas

Intestinal virus replication

Virus shedding by means of faeces (from 2 to 4 weeks)



The Duck populations wintering in Western Palearctic ranges between 13 and 15 million of birds

The Mallard duck has an European population near 5 million of birds,
75000/100.000 of these are wintering in Italy

The Teal winters in Mediterranean areas with 2,5 million of birds (51.000 in Italy)

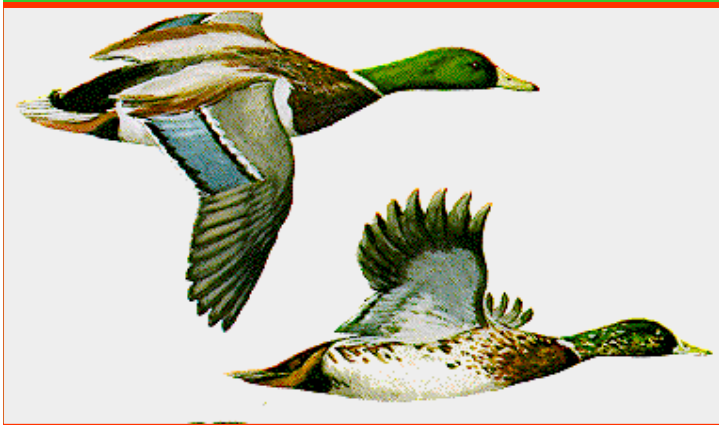
The Wigeon winters in the same region with 1,5 million (71.000 in Italy)

The Pintail has 1,3 million of birds (200.000 winter in the Mediterranean areas)

The Shoveler European population consists of 1 million of birds, 100.000 of these
winter in Mediterranean areas and 20.000 in Italy

The Pochard winters in the Mediterranean areas with 750.000 birds (43.000 in Italy)

The Tufted duck winters in Italy with 8.500 birds





Duck migrations in Europe from N/E to S/W

Mouling areas : Aggregation of many different ducks and viruses populations (during - June, Jul y)



REPRODUCTIVE AREAS

**Post-reproductive- first step-
May, June Moul migration**

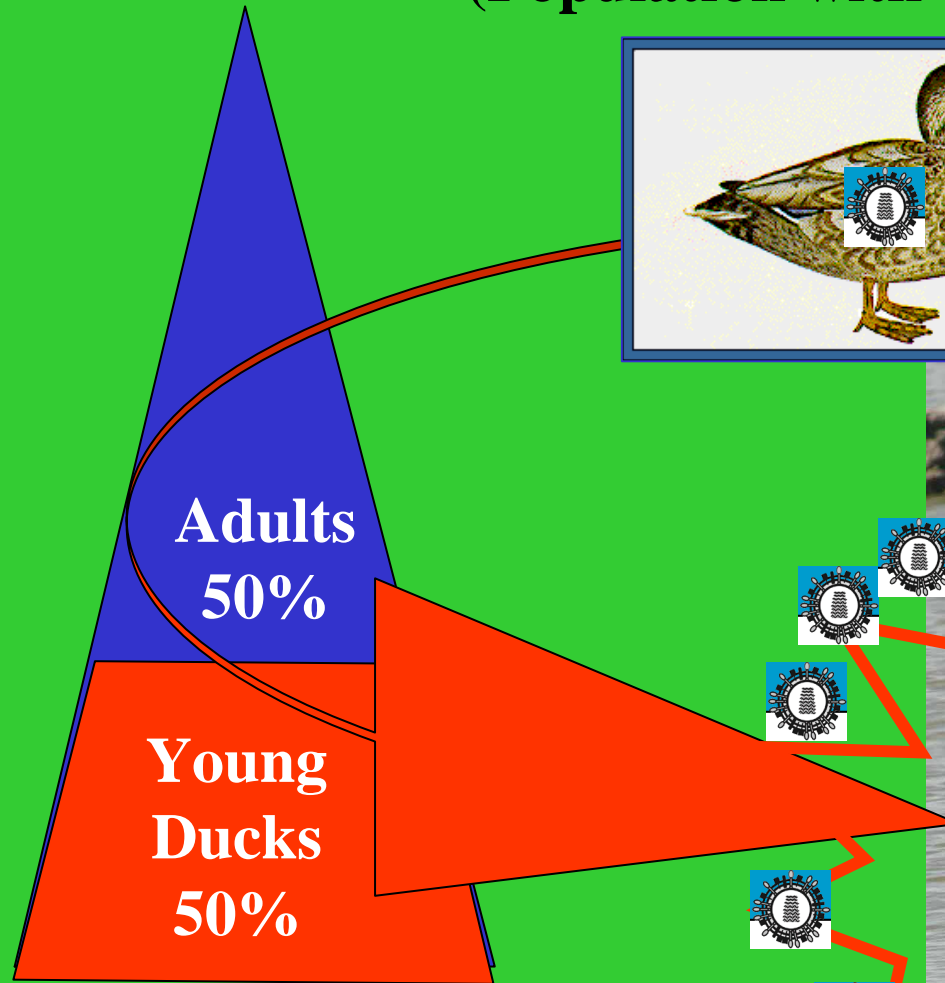
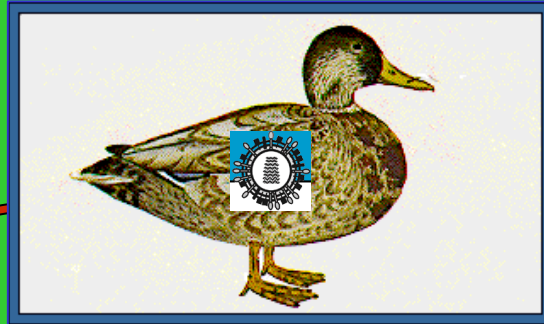
REPRODUCTIVE AREAS

**Wintering migrations - second step-
from August to October**

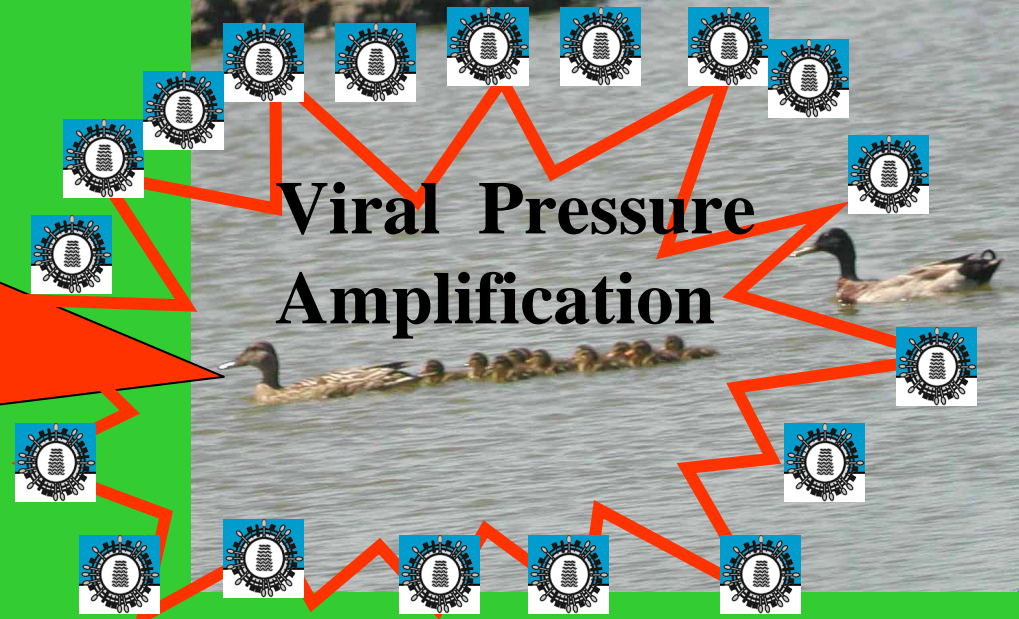
Wintering areas



Endemic Subtypes *e.g.* HA1 Summer /Autumn Period (Population with immunity)



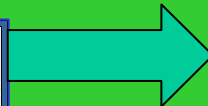
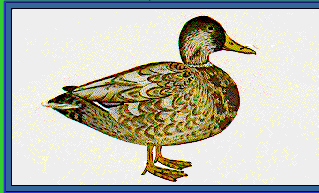
*Ducks population
Structure*



“New Subtypes”

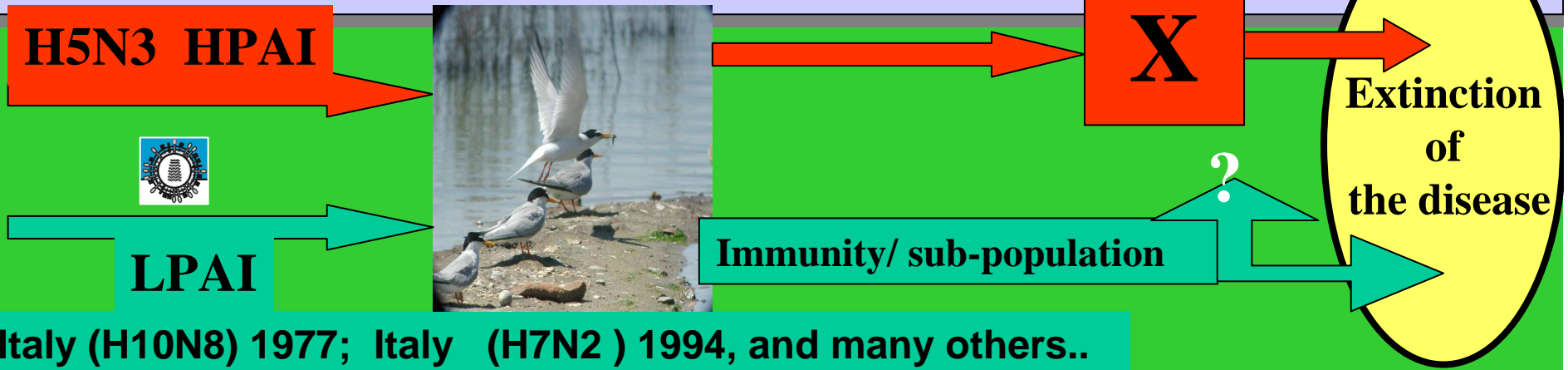
Population without immunity

e.g H7
(Italy, 2001)



Epiphenomenon species:

Host species where the virus can spread and replicate but in this species the virus is not able to maintain itself indefinitely in the time



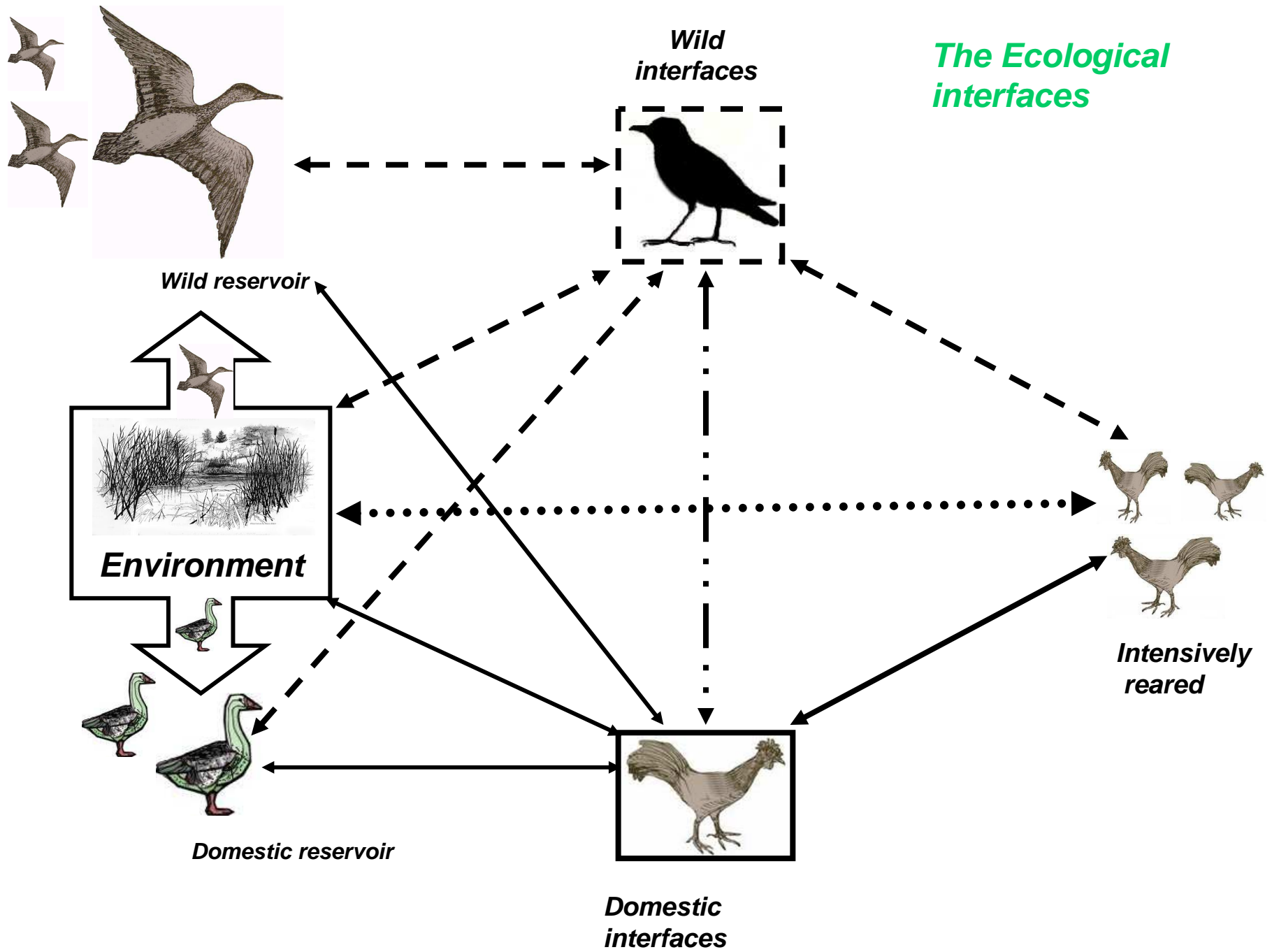
[Italy (H10N8) 1977; Italy (H7N2) 1994, and many others..

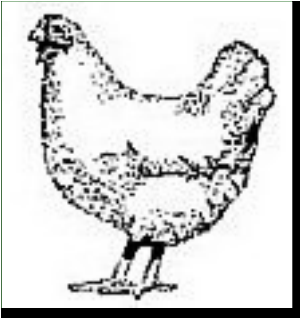


Epiphenomenon role during migration

- flock size
- migratory in long/ short distance
- time necessary to migrate
- migration routes and stages





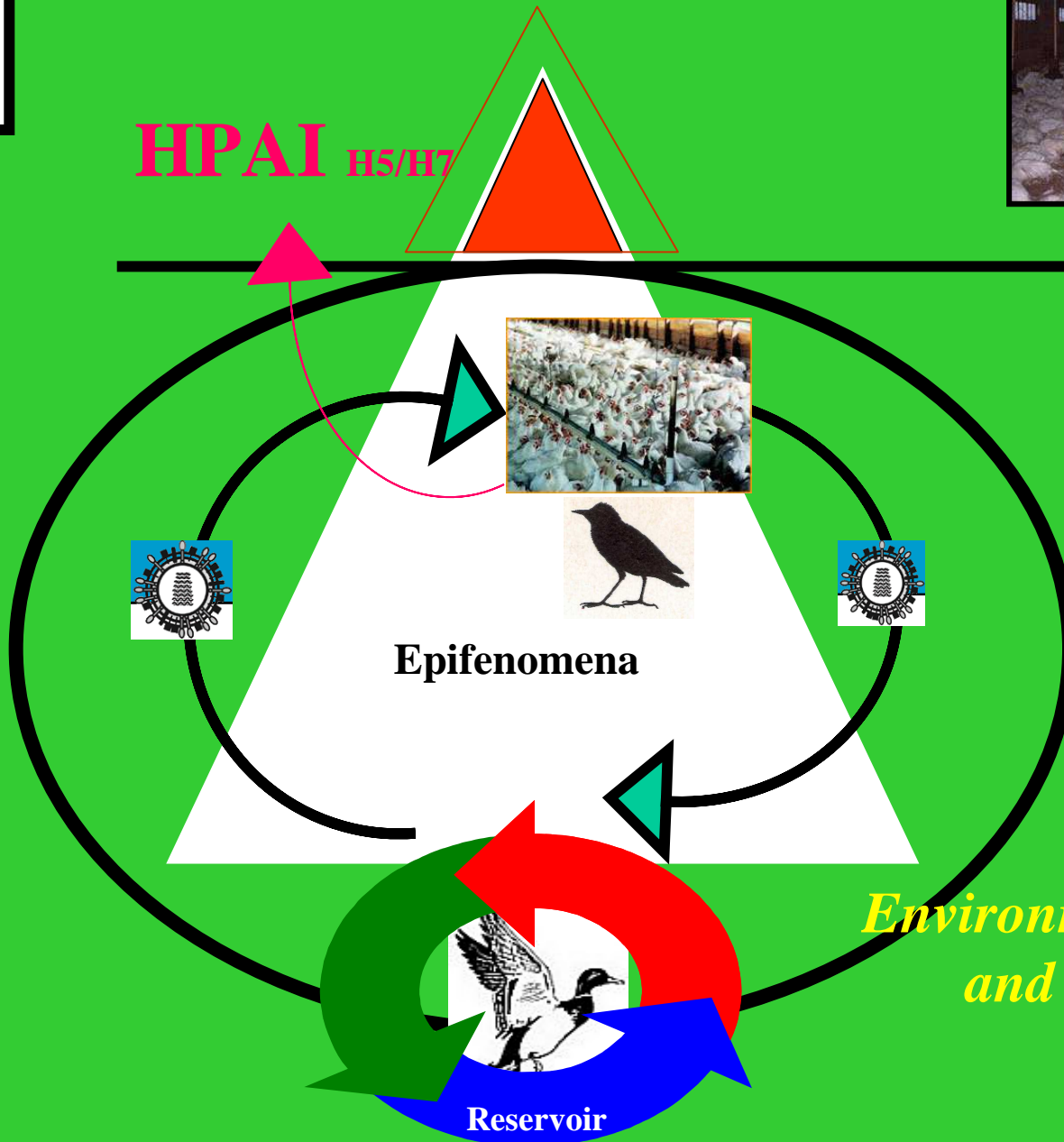


The chicken's role (strains LPAI/HPAI)



HPAI _{H5/H7}

threshold



Epifemomena

*Environmental factors
and immunity*

Reservoir

The host (duck) and the virus have developed a coevolution, in this condition the virus live an evolutionary freezing

With low frequency of viral changes
(*Convergent evolution*)



Few viral variants

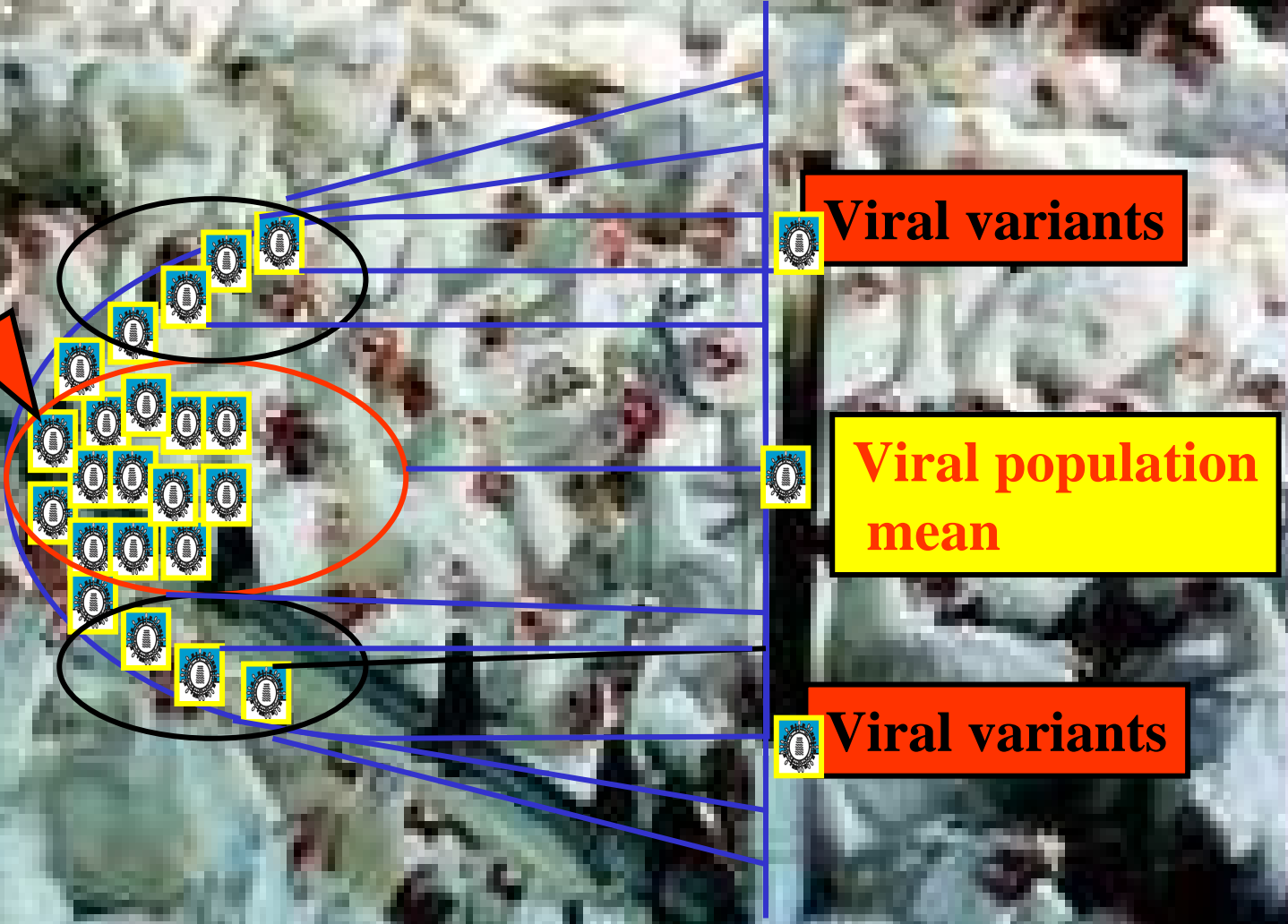
mean

Few viral variants

Viral population



Non adapted host-The Comet Theory
(Divergent Evolution)

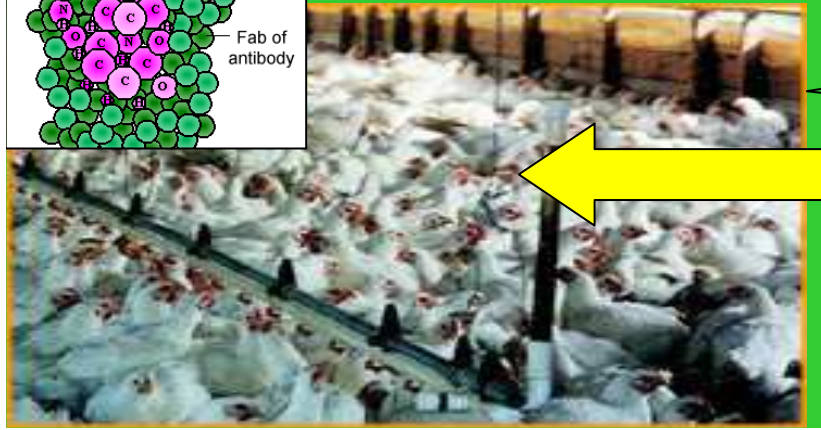
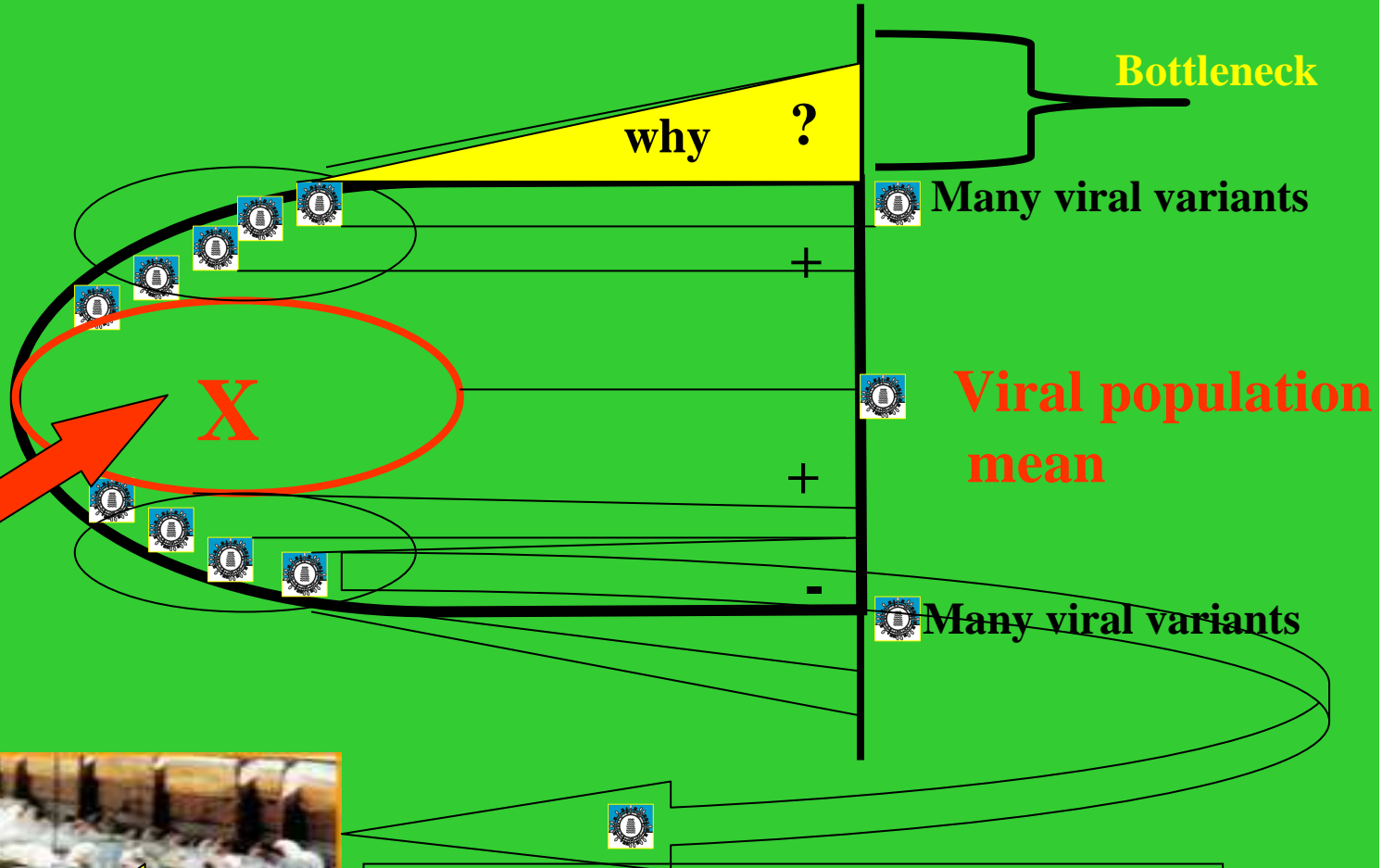
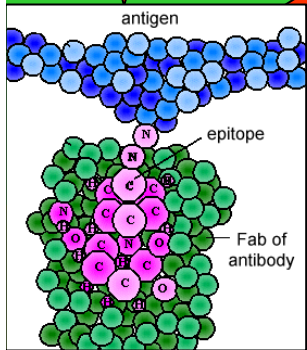


The Comet Theory

Divergent Evolution



Molecular mimicry



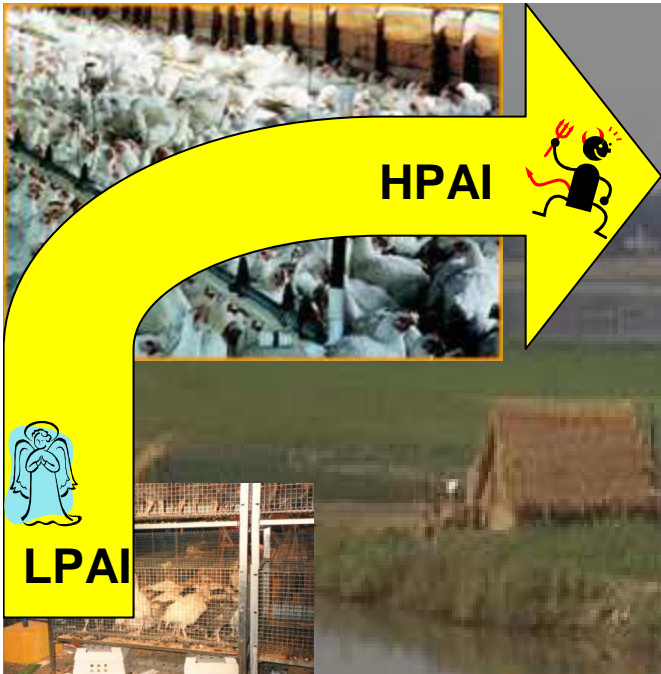
High frequency of viral changes
host and virus without coevolution
host with high population turn over

During Poultry infection, the possible evolution from LPAI to HPAI may occur

HPAI usually kill the domestic and wild reservoirs only in the first outbreak period;

HPAI kills domestic non reservoir birds (i.e. chickens, Turkeys). and some wild epiphenomenon species.

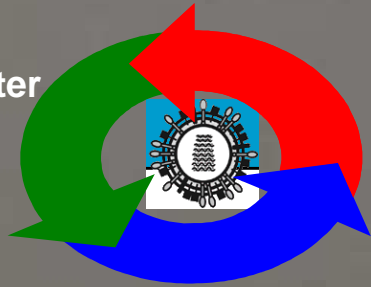
subsequently the virus could reduce its patogenicity for the reservoir and the spread of the infection by migrations might be possible.



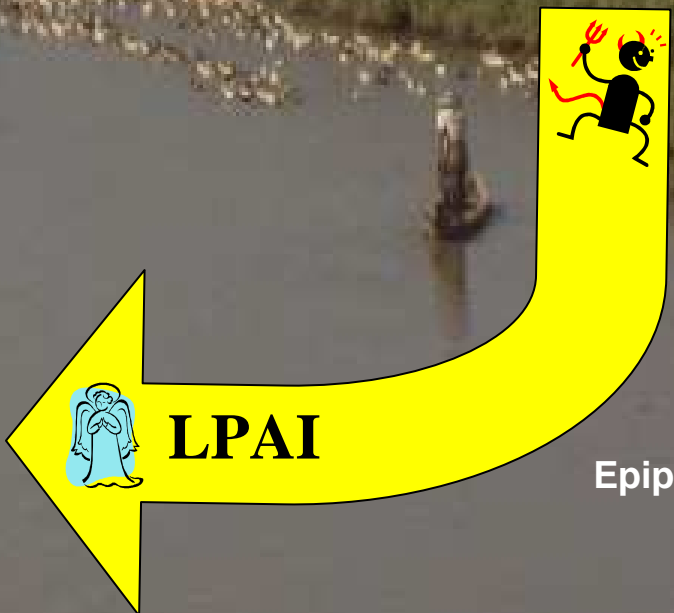
Ecological interfacies

Domestic reservoir LPAI

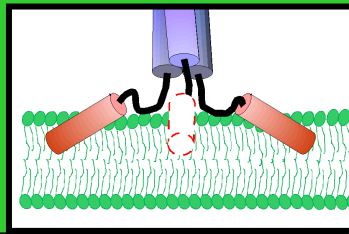
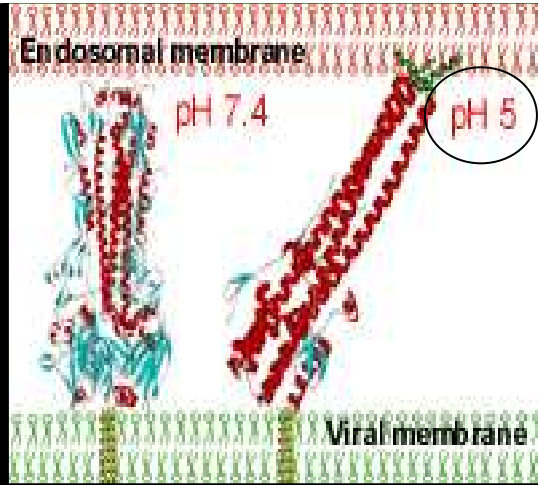
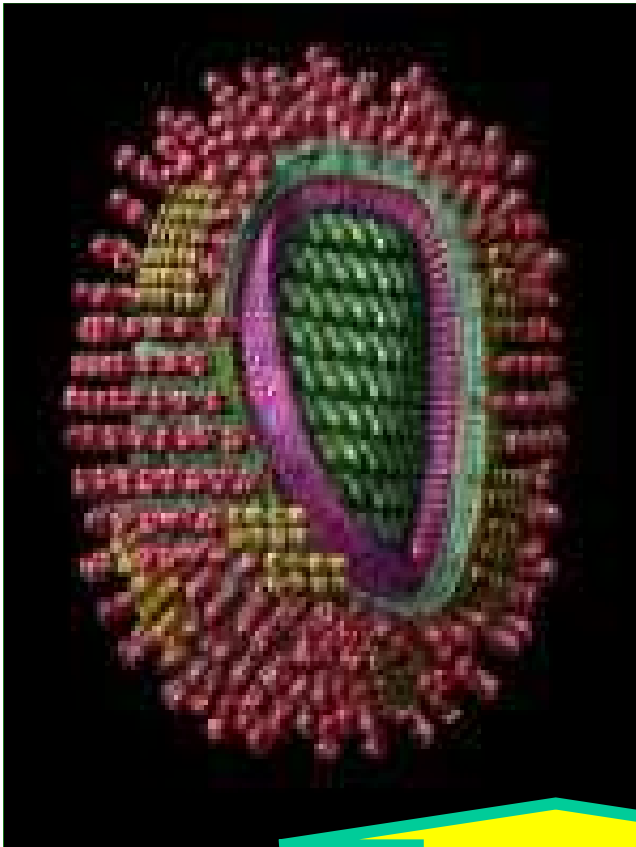
Contaminated water



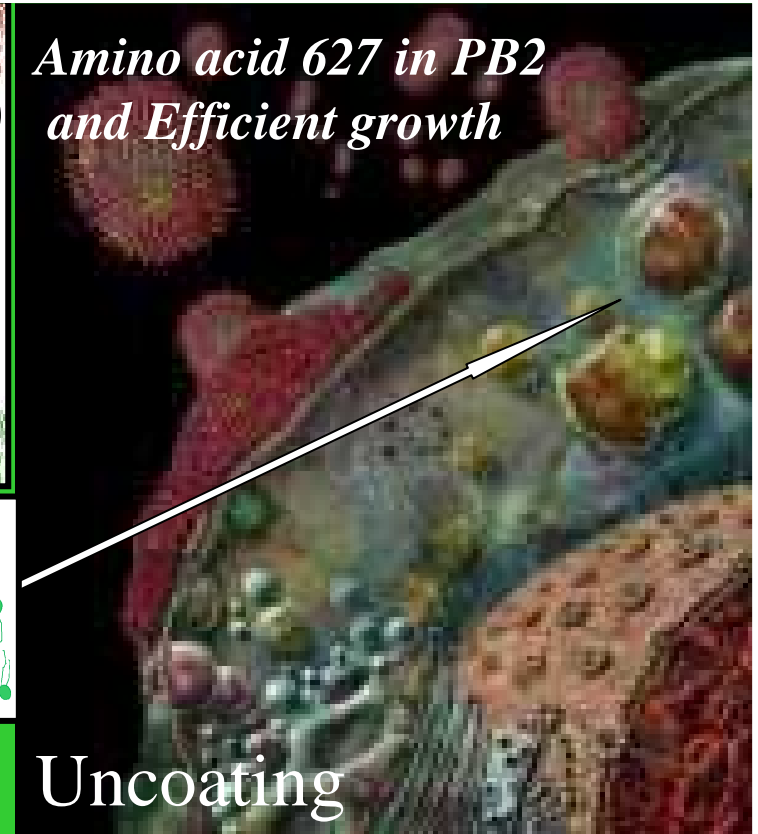
Wild reservoir LPAI



Epiphenomenon species



Infectivity

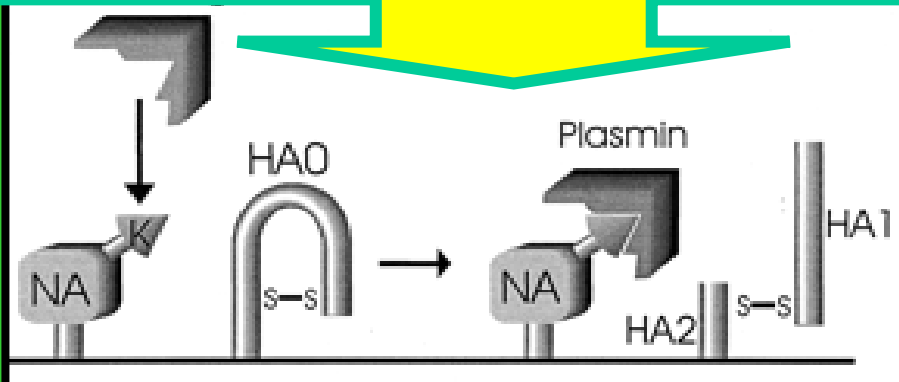
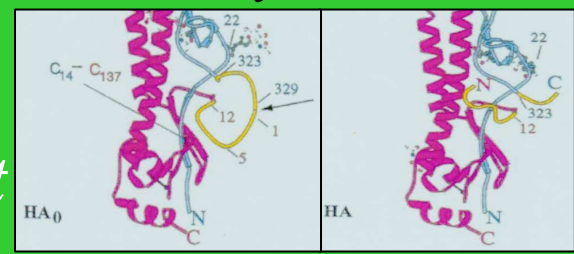


Uncoating

Amino acid 627 in PB2 and Efficient growth

The host spectrum

Proteolytic activation



Attachment

Structure of the cleavage site and pathogenicity

Tabelle di sensibilità e specie colpite: interpretazione del ruolo

-spill over

- fattori di presenza : presenza incostante o meno sul territorio

- momento biologico delle specie (- + giovani sensibili)


- assenza di informazioni sullo stato immunitario della popolazione



Order: Anseriformes

<i>Cygnus atratus</i>	Black swan		+			+	Ellis et.al. 2004
<i>Cygnus cygnus</i>	Whooper Swan	+				+	20050826.2527
<i>C.melanocoryphus</i>	Black-necked swan		+			+	Ellis et al. 2004
<i>Cygnus olor</i>	Mute swan	+				+	Update on Avian Influenza #35
<i>Dendrocygna viduata</i>	White-faced whistling-duck		+			+	Ellis et.al. 2004
<i>Branta sandvicensis</i>	Hawaiian goose		+			+	Ellis et.al. 2004
<i>Netta peposaca</i>	duck		+			+	Ellis et.al. 2004
<i>Netta rufina</i>	pochard		+			+	Ellis et.al. 2004
<i>Tadorna ferruginea</i>	Ruddy shelduck	+				+	Information Vol.18-no.21



Order: Charadriiformes							
Larus atricilla	Laughing gull				+	-	Perkins and Swayne, 2003
Larus brunnicephalus	Brown-headed gull	+	+			+	Chen et.al. 2005;and Liu et.al. 2005
Larus ichthyæus	Great black-headed gull	+	+			+	Chen et.al. 2005;and Liu et.al. 2005
Larus ridibundus	Black-headed gull	+				+	Ellis et.al. 2004
Tringa ochropus	Green sandpiper	+				-	OIE Mission to Russia 2005
Order: Ciconiiformes							
Anastomus oscitans	Asian open-billed stork	+				+	ProMED 20041214.3303
Ardea cinerea	Grey heron	+				+	Ellis et.al. 2004
Ardea herodias (?)	Great blue heron	+				+	ProMED 20051130.3460, 20051201.3463 (HPAI H5)
Ardeola bacchus	Chinese pond heron	+				+	OIE 2005 Disease Information Vol. 18-no2
Egretta garzetta	Little egret	+				+	Ellis et.al. 2004



Order: Columbiformes

Columba livia	Feral pigeon	+			+	+	Ellis et.al. 2004
Streptopelia tranquebarica	Red-collared dove	+				?	ProMED 20041214.3303

Order: Falconiformes



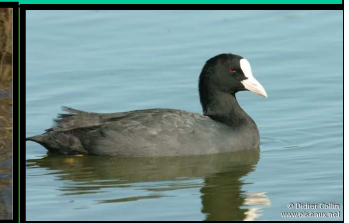
Falco peregrinus	Peregrine falcon	+				+	OIE 2004 Disease Information Vol.17-no.5; Hong Kong Final Report 7/30/03
Ichthyophaga ichthyaetus	Grey-headed fish-eagle		+			+	FAO AIDE report #16
Spilornis cheela?	Serpent eagle		+			+	FAO AIDE report #16
Spizaetus nipalensis	Crested hawk-eagle	+				-	van Borm et.al., 2005

Order: Galliformes							
Alectoris chukar	Chukar partridge				+	+	Perkins and Swayne, 2003
Colinus virginianus	Bobwhite quail				+	+	Perkins and Swayne, 2003
Corunx coturnix japonicus	Japanese quail				+	+	Perkins and Swayne, 2003
Gallus domesticus	Domestic chicken		+			+	Subbarao et.al. 1998
Meleagris gallopavo	Turkey				+	+	Perkins and Swayne, 2003
Numida meleagris	Pearl guineafowl				+	+	Perkins and Swayne, 2003
colchicus	pheasant				+	+	Perkins and Swayne, 2004



Order: Gruiformes

chloropus	Common moorhen	+				+	(HPAI H5)
Fulica atra	Green Brown (red-legged)	+				-	OIE Mission to Russia 2005
Rallina fasciata?	crake	+				+	Xinhua News 1/11/06



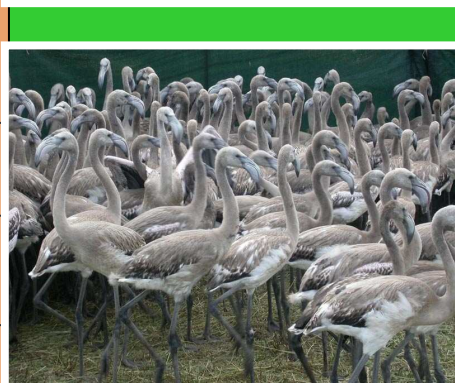
Order: Struthioniformes

novaehollandiae	Emu					+	-	2003
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Order: Passeriformes							
Carpodacus mexicanus	House finch		+			+	Perkins and Swayne, 2003
Copsychus saularis?	Oriental magpie robin	+				+	Oriental Magpie Robin tests H5N1 positive
Corvus macrorhynchos	Jungle crow	+				+	Mase et.al., 2005, Report of the Highly Pathogenic Avian Influenza Infection Route Elucidation Team, June 30, 2004.
Dicrurus macrocercus	Black drongo	+				?	ProMED 20041214.3303
Gracula religiosa	Hill mynah		+			+	ProMED 20051021.3075; 20051022.3085.
Leiothrix lutea	Red-billed leiothrix		+			+	ProMED 20051021.3075; 20051022.3085.
Lonchura punctulata	Scaly-breasted munia	+				?	ProMED 20041213.3303
Oriolus chinensis chinensis	Black-naped oriole		+			+	ProMED 20051021.3075; 20051022.3085.
Passer domesticus	House sparrow		+			-	Perkins and Swayne, 2003
Passer montanus	Eurasian tree-sparrow	+				+	Ellis et.al. 2004
Pica pica sericea	Korean magpie	+				+	Kwon et al. 2005
Sturnus vulgaris	European starling				+	-	Perkins and Swayne, 2003
Taeniopygia guttata	Zebra finch				+	+	Perkins and Swayne, 2003



Order: Pelecaniformes								
Phalacrocorax carbo	Great cormorant		+			+	OIE 2005, Disease Information Vol.18-no.21	
Phalacrocorax niger	Little cormorant		+			?	ProMED 20041214.3303	
Order: Phoenicopteriformes								
Phoenicopterus ruber	Greater flamingo			+		+	Ellis et.al. 2004	
Order: Psittaciformes								
Melopsittacus undulatus	Budgerigar					+	+	Perkins and Swayne, 2003
Order: Strigiformes								
Bubo nipalensis	Spot-bellied eagle-owl			+			+	FAO AIDE report #16
Ketupa ketupu	Buffy fish-owl			+			+	FAO AIDE report #16
Ketupa zeylonensis	Brown fish-owl			+			+	FAO AIDE report #16
Strix uralensis	Spotted wood-owl			+			+	FAO AIDE report #16



PROBLEMI NEL TROVARE GLI ANIMALI MALATI

Gli animali malati e morti sono rapidamente rimossi dai predatori e dagli scavenger, e perciò non disponibili per le indagini

Come quantificare i casi?

Soggetti ammalati: entità dei sintomi
“collaborazione” della specie mezzi diagnostici
“abilità” del veterinario nel **Trovare gli animali**

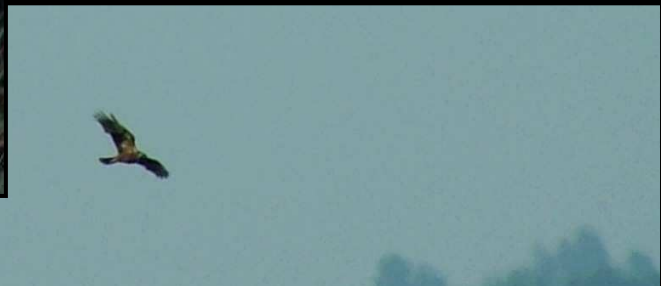
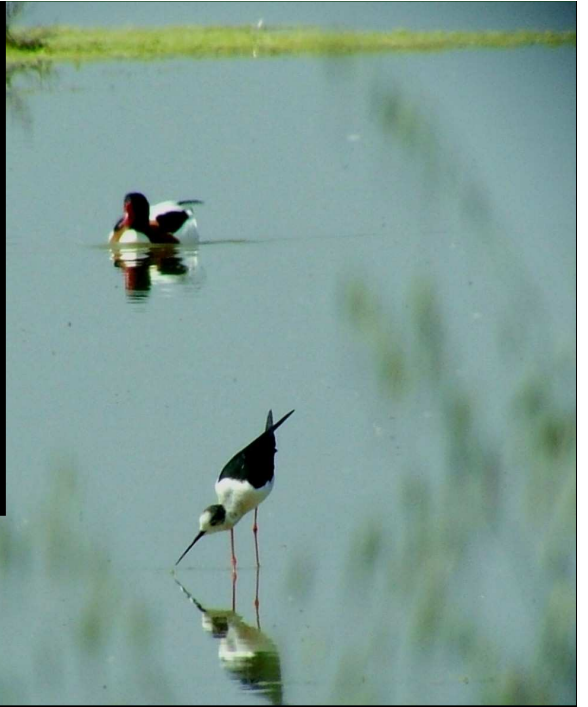
Ricerche di carcasse:
marcatura delle carcasse

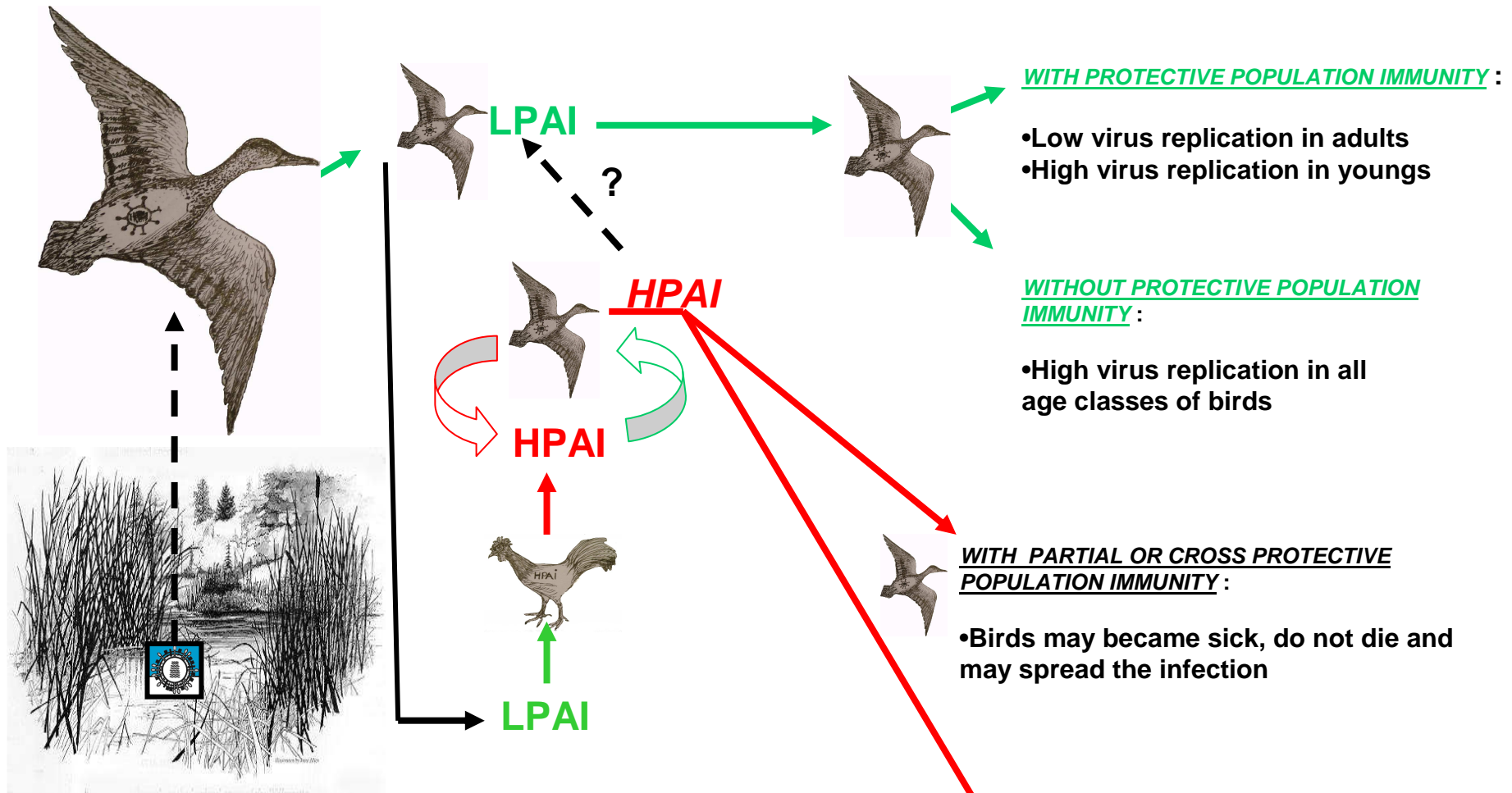
Anatidi 0% (0/50)
 12% (6/50)

Taglia Anatidi
 Passeriformi

Eccezioni
costituite da alcune epidemie







FACTORS CODITIONING THE VIRAL SPREAD

- Incubation period
- Virus shedding
- Age of the bird
- Persistence of the virus in the environment
- Dimension of the flock
- Migration activities
- Congregation factors
- Season
- Others

In Late 2002 an outbreak of HPAI H5N1 cause dead among resident waterfowl and wild migratory birds in two Hong Kong nature parks. (Sturm, Ramirez *et al.* 2003)

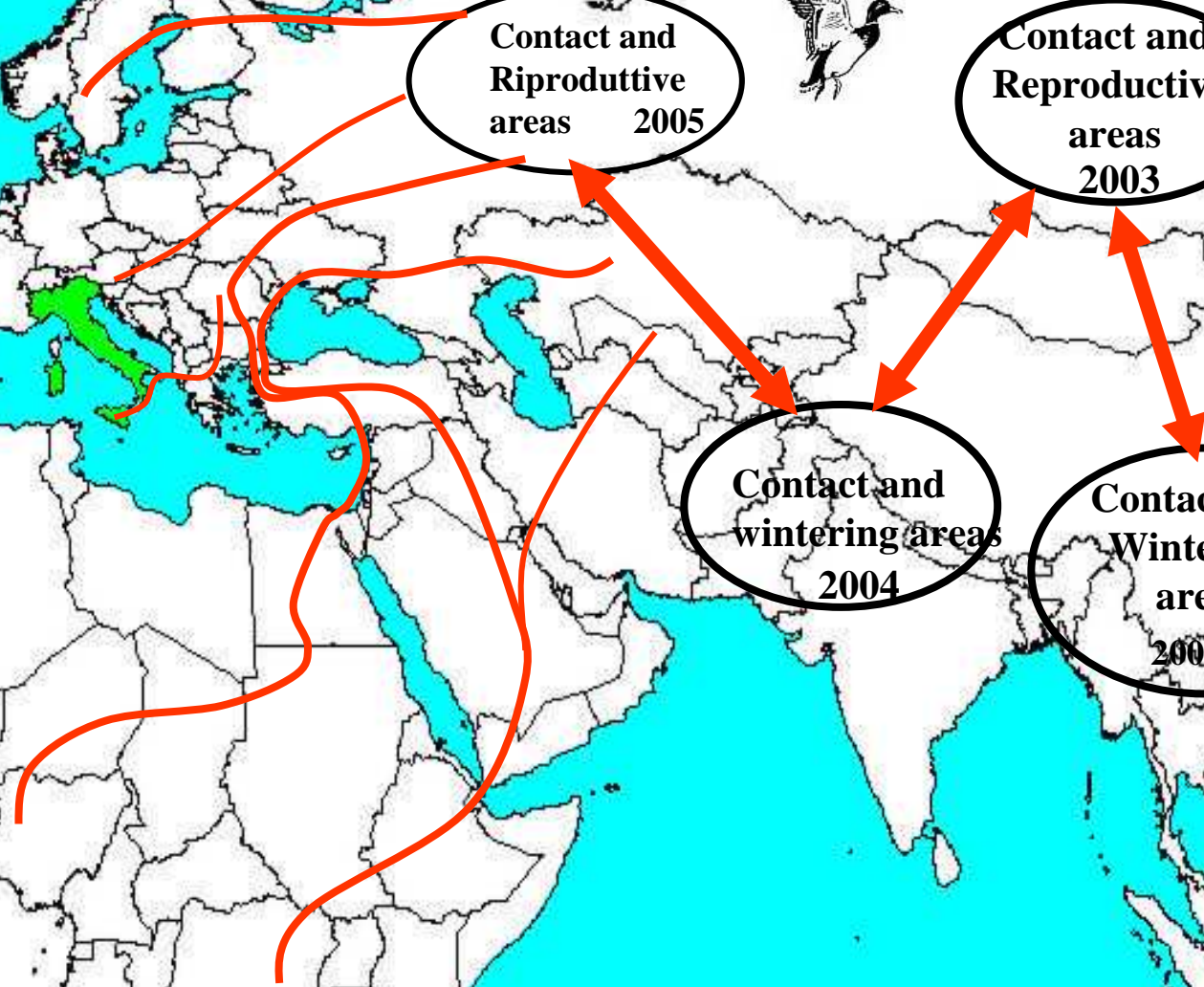


**Contact and
Riproduttive
areas 2005**

**Contact and
Reproductive
areas
2003**

**Contact and
wintering areas
2004**

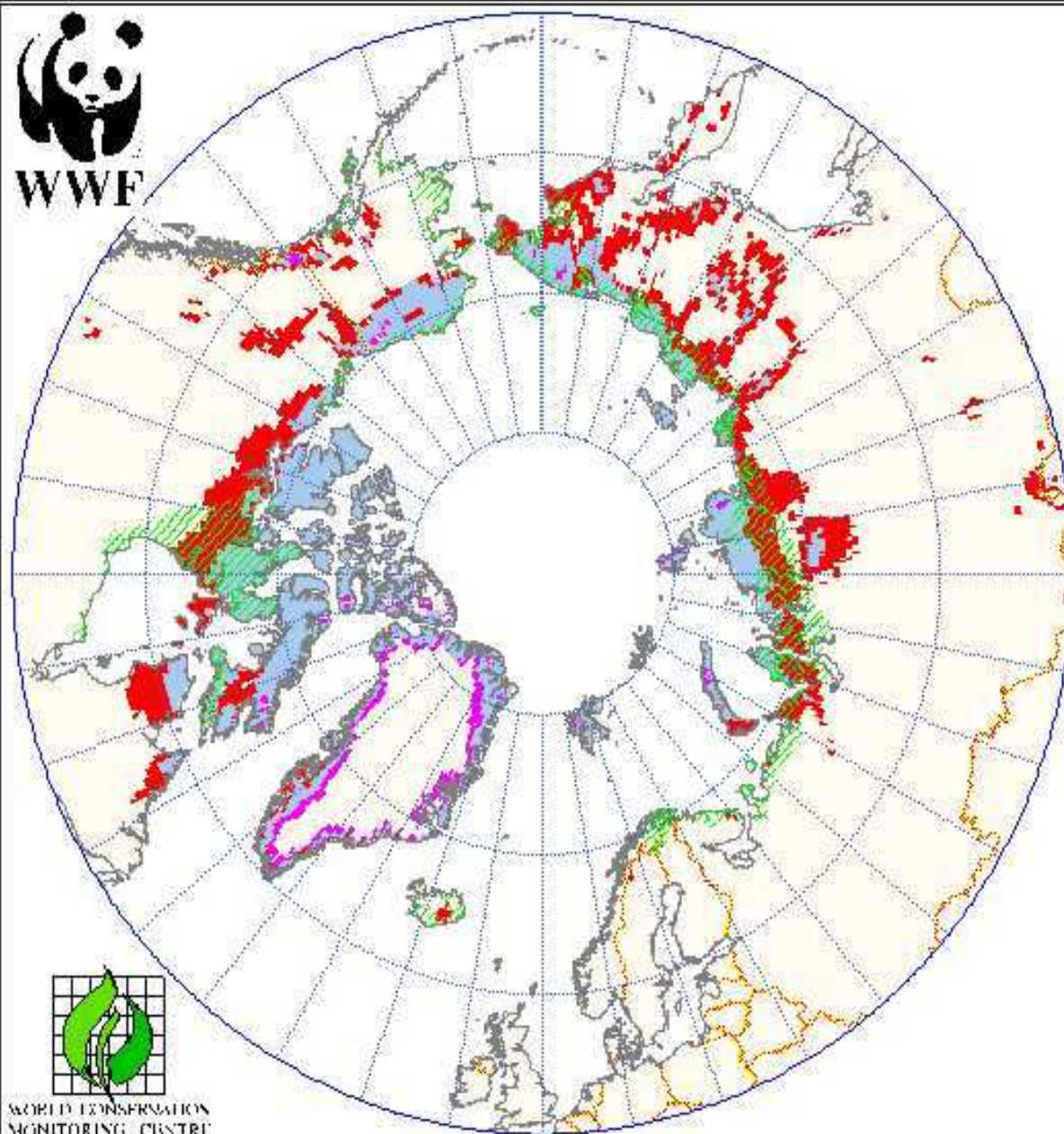
**Contact and
Wintering
areas
2002**



H5N1 movements between 2002-2005



WORLD CONSERVATION
MONITORING CENTRE



Climate Change and Biodiversity - Arctic Water Birds

Figure 21. Dunlin
(*Calidris alpina*)

LEGEND:

Bird distribution:

- Breeding Areas
- Breeding Areas, High Density

Change in Tundra Zone Over the Period of Doubling of CO₂ (60-100 years) Predicted as Moderate Warming by HADCM2SULC Climate Change Model

- Tundra, No Change
- Tundra Loss
- Expansion of Tundra
- Unclassified Land

0 1000 2000 3000 km

The material and the geographical designations in this report do not imply the expression of any opinion whatsoever on the part of WWF concerning the legal status of any country, territory, or area, or concerning the delimitation of its frontiers or boundaries.

Compiled by I. Lysenko and C. Zockler
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Date printed: 30 January 2000



STUDY AREA

**ORBETELLO LAGOON
TUSCANY - ITALY**

**VIROLOGICAL AND SEROLOGICAL
INFLUENZA SURVEILLANCE SYSTEM
(ISS - OMS National Influenza Center)
AMONG WILD WATERFOWL, SINCE 1992**



Image © 2006 DigitalGlobe

Pointer 42°28'04.56" N 11°12'11.16" E

Streaming ||||| 100%

Eye alt 11.58 mi

Monitoring the lagoon with sentinel ducks



Research in the reservoir species:

Fecal dropping



Duck trapping



cloacal swabs

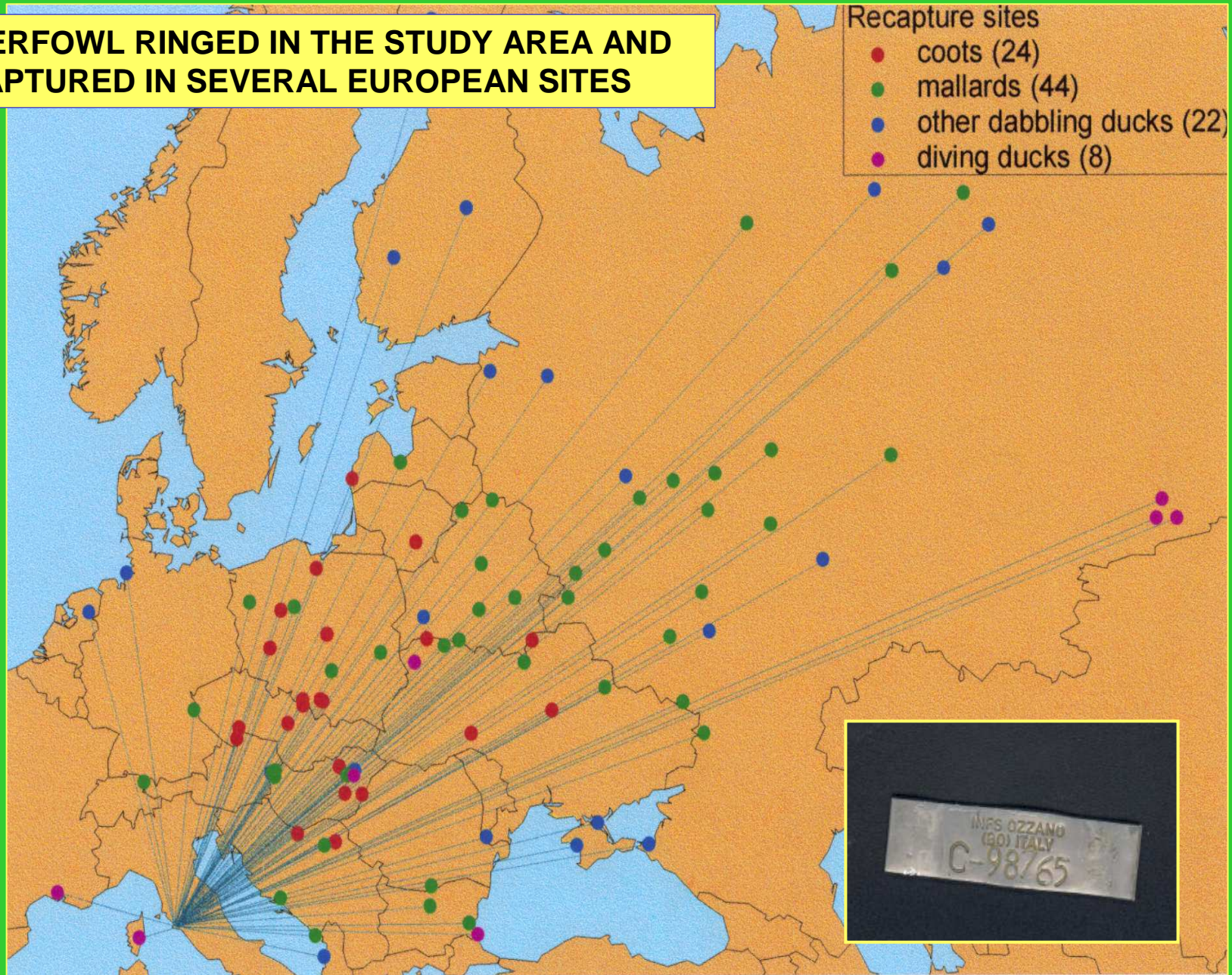


Blood sampling

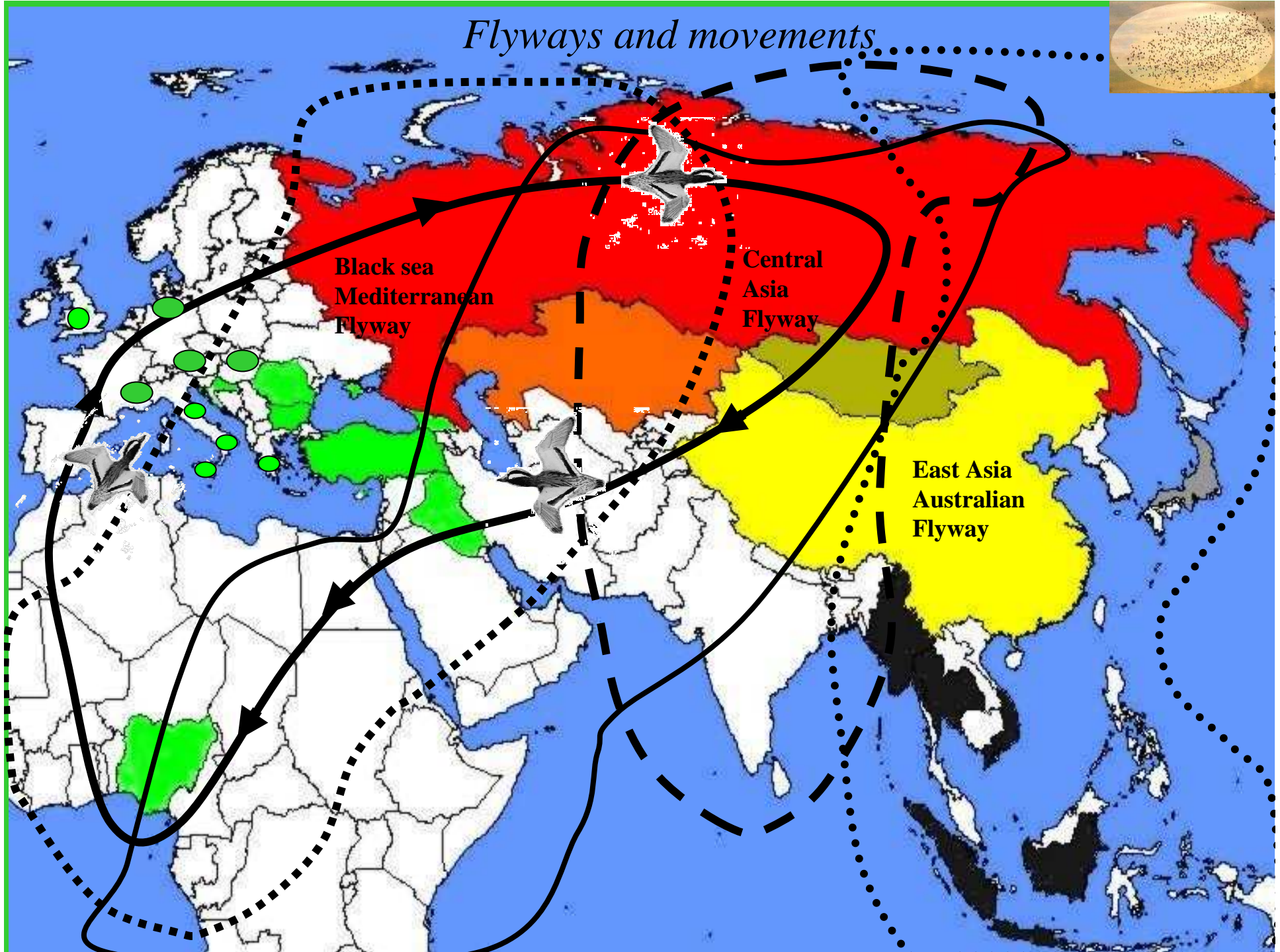


Ringling

**WATERFOWL RINGED IN THE STUDY AREA AND
RECAPTURED IN SEVERAL EUROPEAN SITES**



Flyways and movements



**Black sea
Mediterranean
Flyway**

**Central
Asia
Flyway**

**East Asia
Australian
Flyway**

1 - Mallard duck



2 - Other dabbling Duck



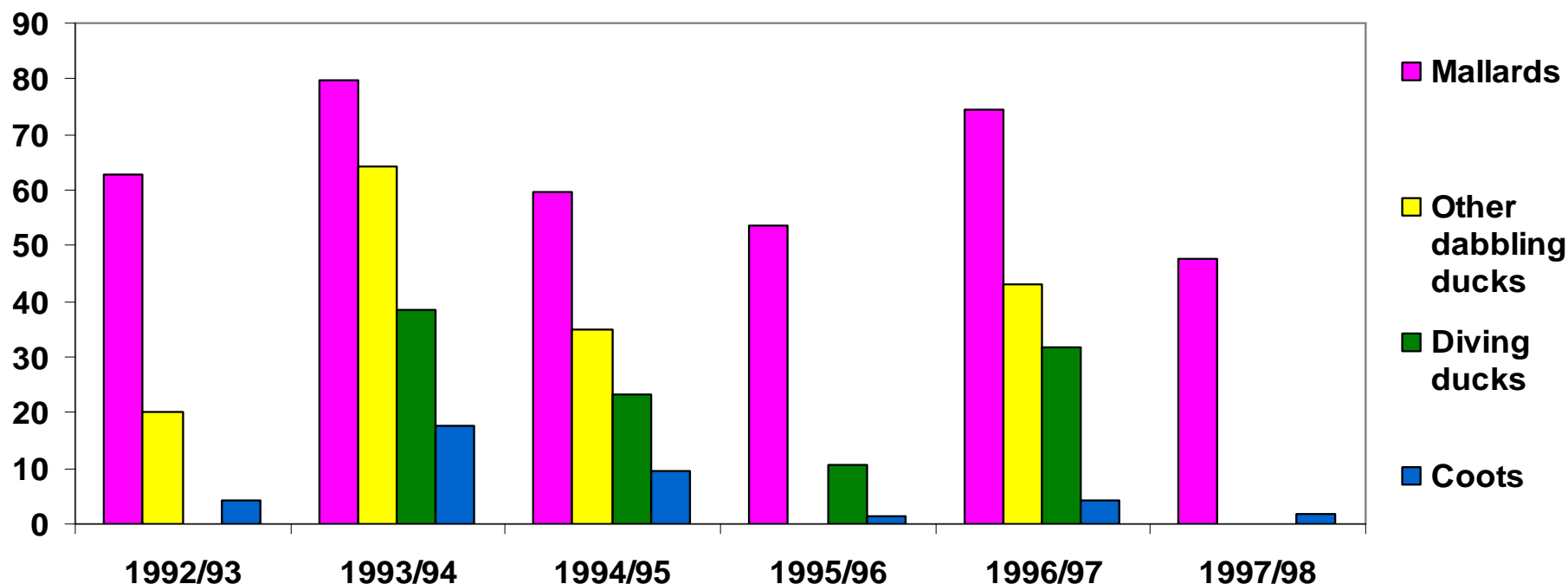
3- diving Duck



4 - Coots



SEROPREVALENCES TO INFLUENZA A VIRUSES IN WINTERING WATERFOWL (N° 1040 SAMPLED BIRDS)

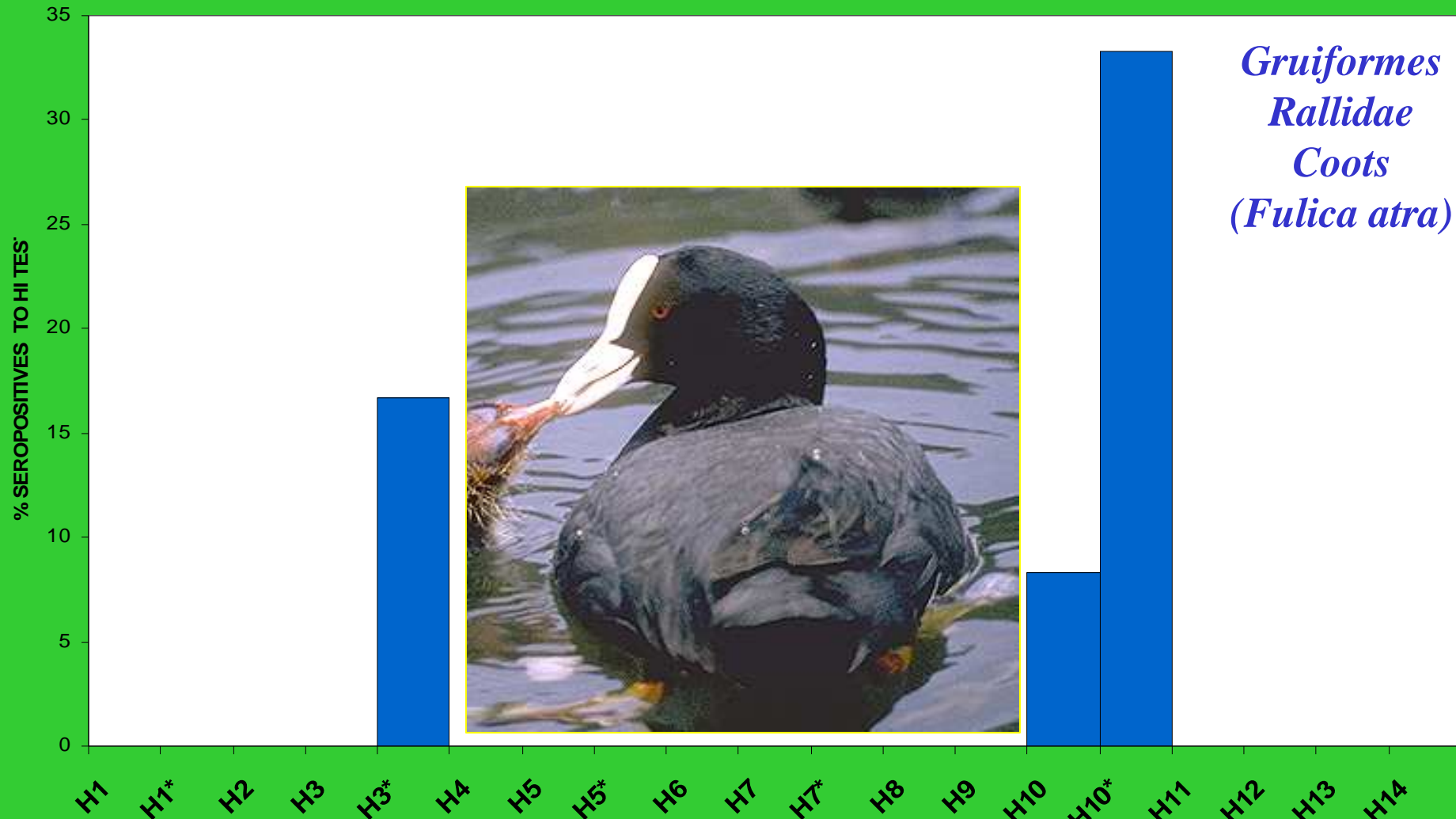


Results:

During all years, strong relations are shown in the seroprevalence of the duck groups.

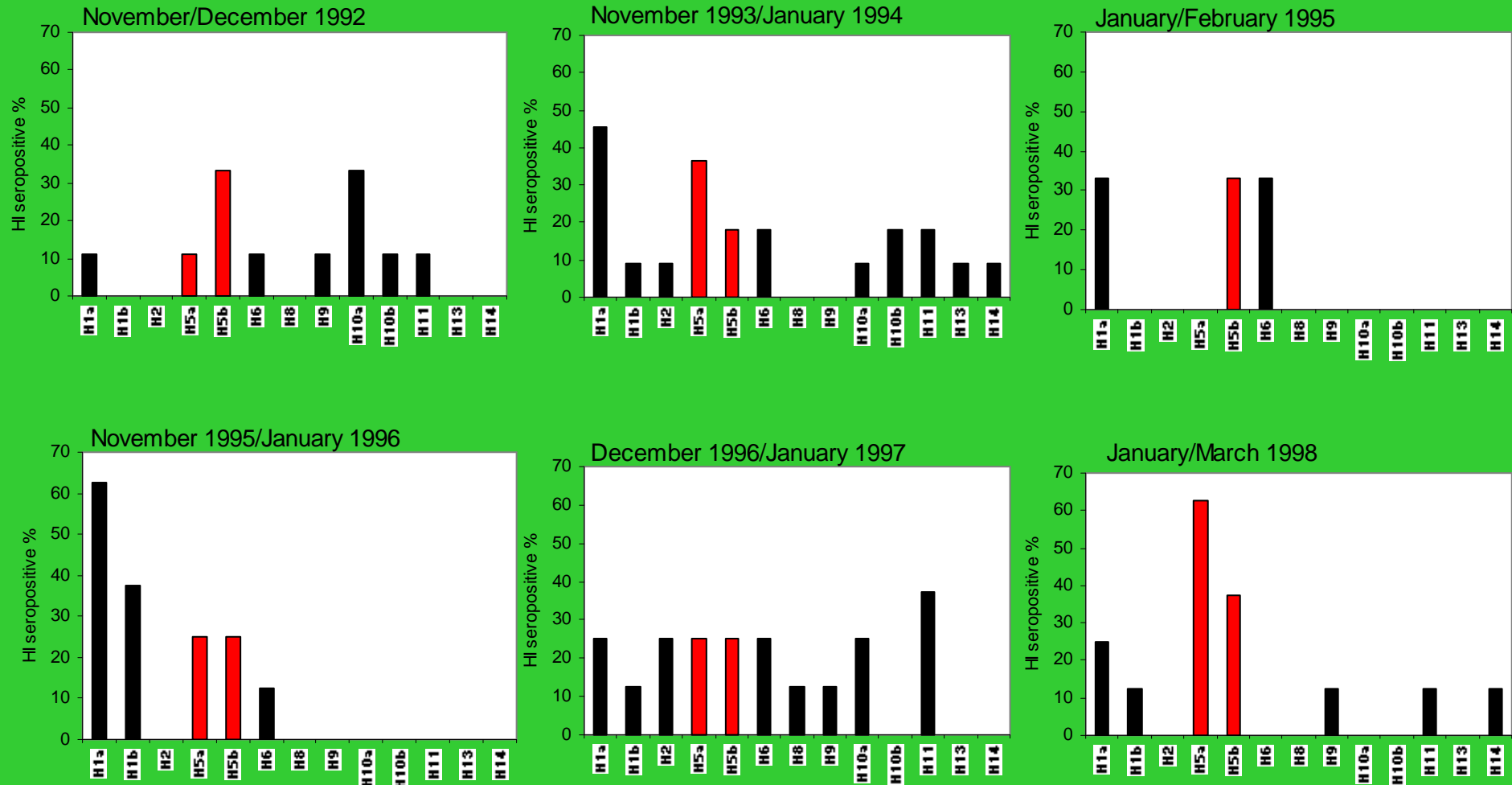
Seroprevalences are higher in adults than young Coots
No differences related to the age were shown in ducks

**SEROLOGICAL PERCENTAGE OF COOTS (*Fulica atra*) FOUND SEROPOSITIVE TO SUBTYPES OF INFLUENZA VIRUSES (1992-1998)
VALUES CALCULATED ON 24 COOTS NP-ELISA POSITIVE**



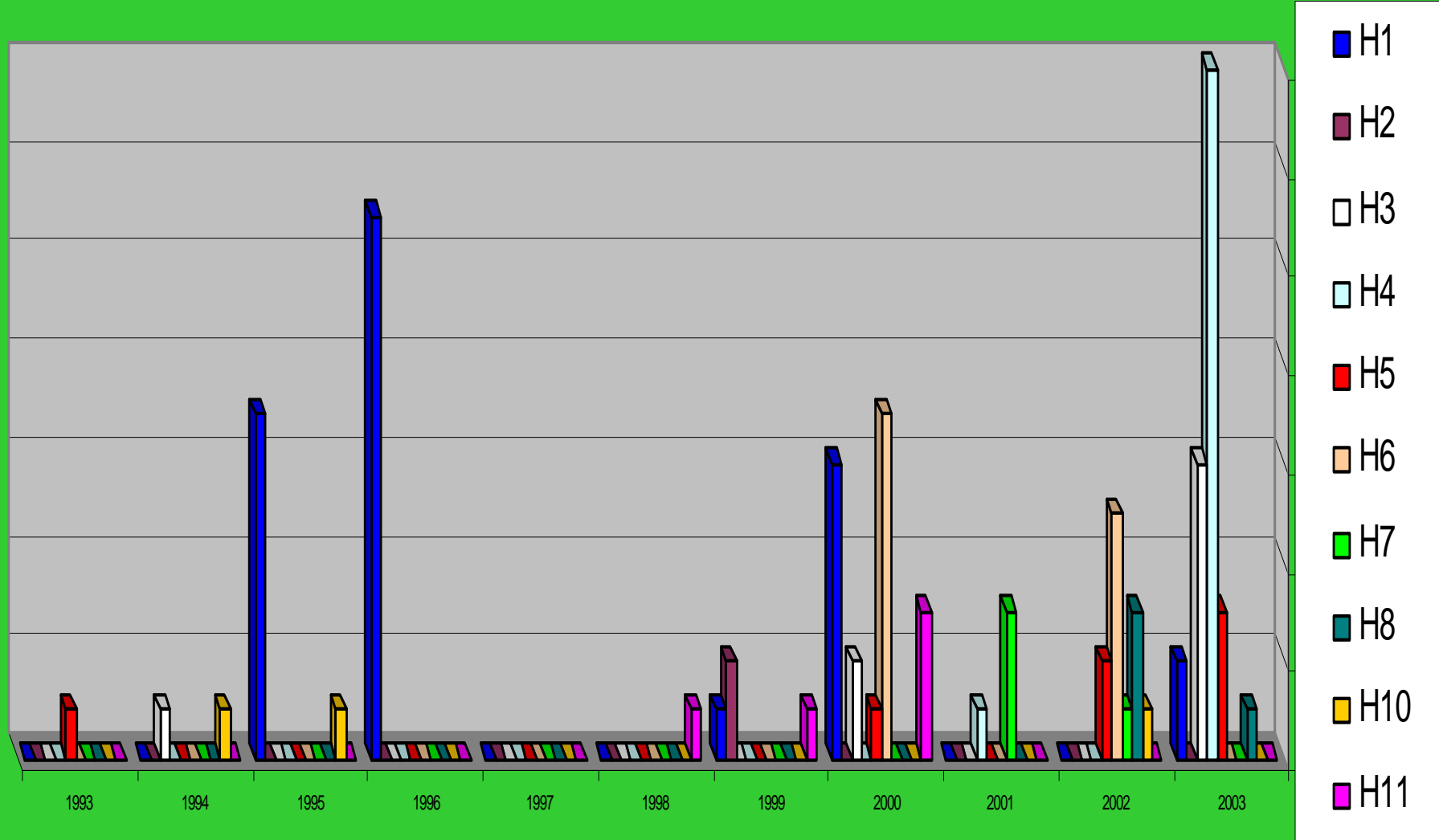
ANTIGENS: 14 REFERENCE STRAINS OF INFLUENZA VIRUSES HAVE BEEN USED (FROM H1 TO H14); 4 VIRUS (H1*, H3*, H5*, H10*) WAS ISOLATED FROM WINTERING WATERFOWL; 1 STRAIN WAS ISOLATED FROM TURKEY: A/TURKEY/ITALY/6423-1/99 (H7N1)

Percentuale di anatre HI-sieropositive, calcolate durante 6 periodi di svernamento (1992-1998) su animali NP-ELISA sieropositivi (N. 47) e con anticorpi verso almeno un sottotipo H (■ = H5)



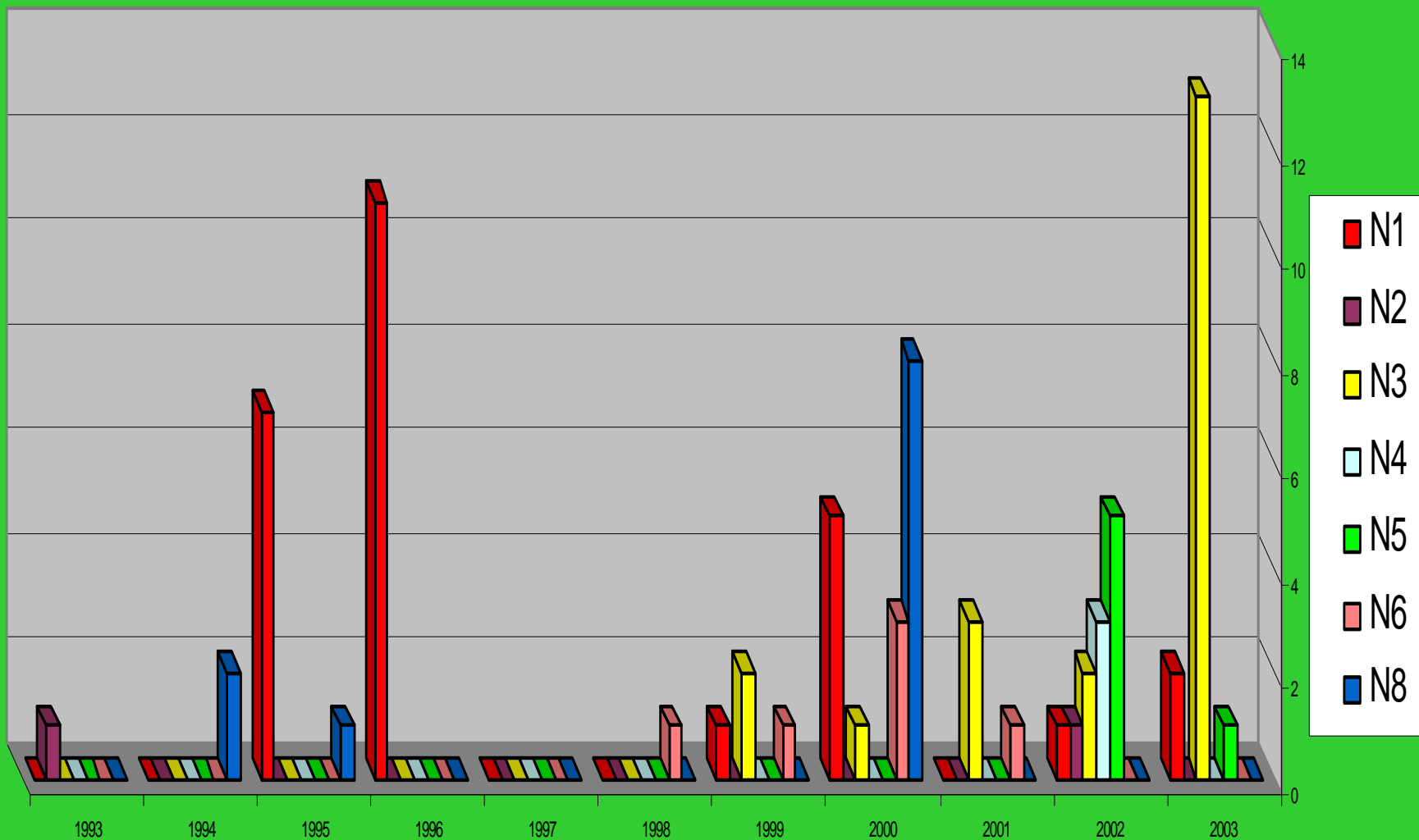


ANNUAL DISTRIBUTION OF HA INFLUENZA SUBTYPES ISOLATED FROM 2524 FERAL DUCKS AND 582 COOTS IN ITALY (1993-2003)

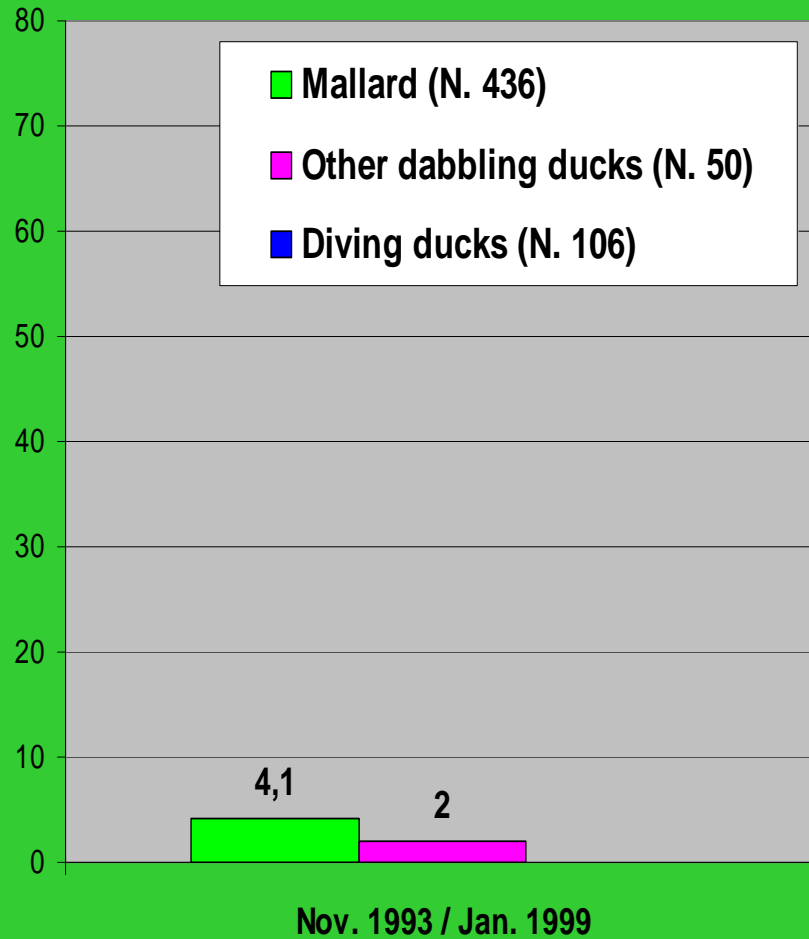


*H5N1 19/09/2005
Mirandola (MO)*

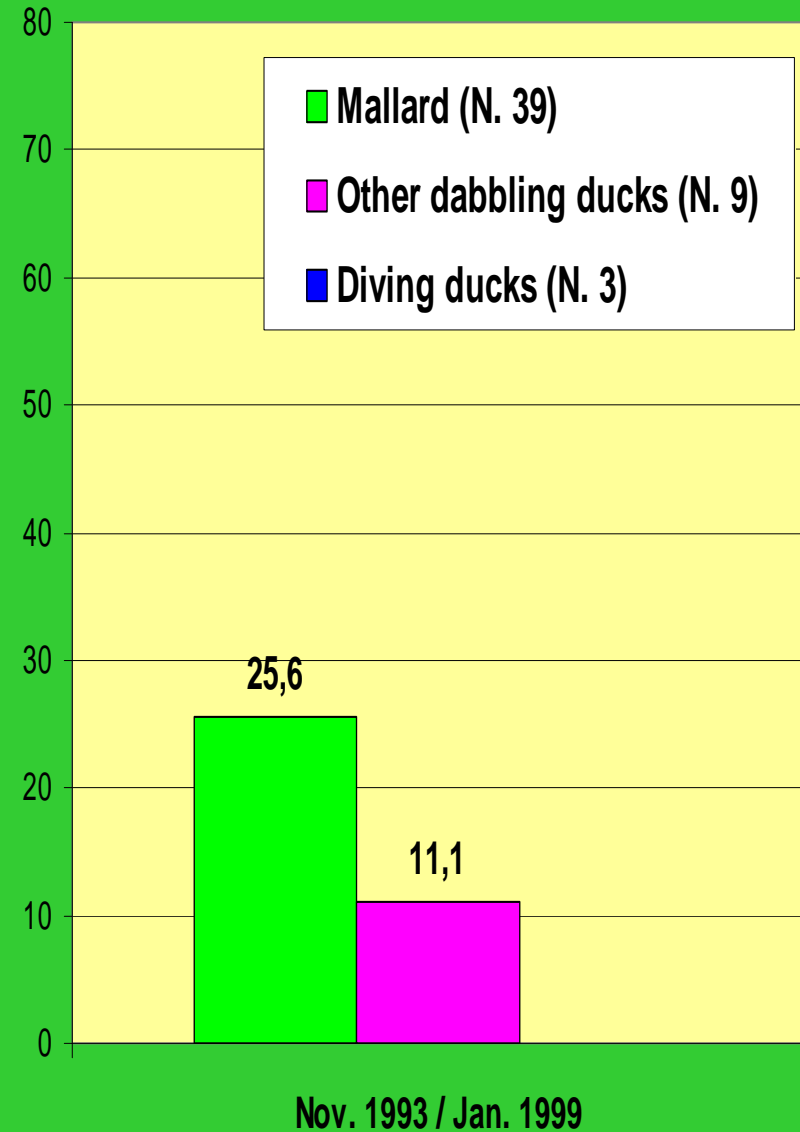
ANNUAL DISTRIBUTION OF NA INFLUENZA SUBTYPES ISOLATED FROM 2524 FERAL DUCKS AND 582 COOTS
IN ITALY (1993-2003)



VIRUS ISOLATION PREVALENCE % IN CAPTURED DUCKS



SEROCONVERSION % IN RECAPTURED DUCKS





Interspecies transmission of an H7N3 influenza virus from wild birds to intensively reared domestic poultry in Italy

Laura Campitelli,^{a,*} Elvira Mogavero,^a Maria Alessandra De Marco,^b Mauro Delogu,^c Simona Puzelli,^a Fabiola Frezza,^a Marzia Facchini,^a Chiara Chiapponi,^d Emanuela Foni,^d Paolo Cordioli,^e Richard Webby,^f Giuseppe Barigazzi,^d Robert G. Webster,^f and Isabella Donatelli^a

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^b Istituto Nazionale per la Fauna Selvatica "A. Ghini", Ozzano Emilia (BO), Italy

^c Department of Public Health and Animal Pathology, Faculty of Veterinary Medicine, University of Bologna, Bologna, Italy

^d Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia, Parma, Italy

^e Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia, Brescia, Italy

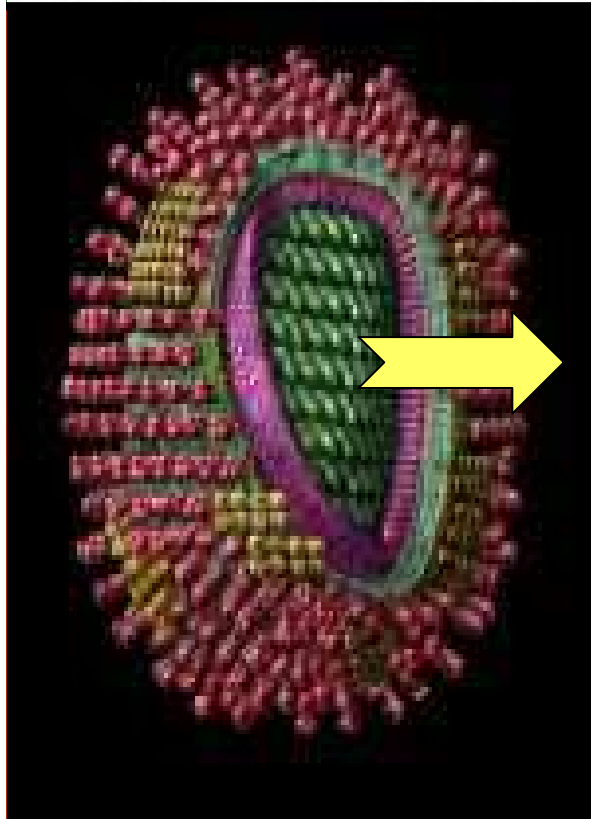
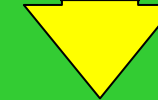
^f Virology Division, Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38105, USA

Received 10 September 2003; returned to author for revision 29 October 2003; accepted 17 February 2004

Available online 21 April 2004



**H7N3
FIRST
ANCESTOR**



**Type A
(8 RNA segments)**

**-PB1, PB2, PB3
-PA)**

4° - HA (Haemoagglutinin)

5° - NP(Nucleoprotein)

6° - NA (Neuroaminidase)

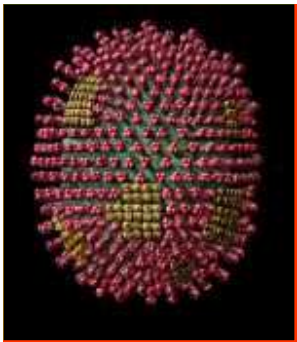
7° - M1, M2 (MatrixProt.)

8° - NS .1, NS 2

Viruses analyzed in this study

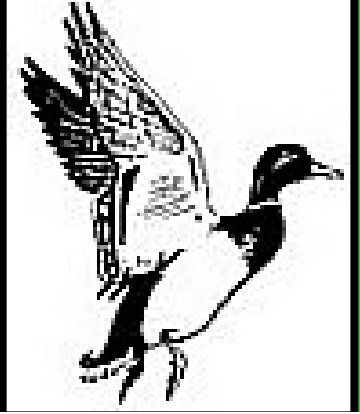
Virus	Subtype	Date of isolation	Location
A/Mallard/Italy/35/99	H2N3	December 1999	Tuscany
A/Mallard/Italy/36/99	H2N3	December 1999	Tuscany
A/Mallard/Italy/208/00	H5N3	August 2000	Tuscany
A/Mallard/Italy/33/01	H7N3	October 2001	Tuscany
A/Mallard/Italy/43/01	H7N3	October 2001	Tuscany
A/Turkey/Italy/214845/02	H7N3	October 2002	Lombardia
A/Turkey/Italy/220158/02	H7N3	October 2002	Lombardia

Note. All isolates were obtained from cloacal swabs. None of the animals from which the isolates were obtained showed any disease signs at the time of sample collection. However, 2 weeks before collection, the two birds that were the sources of A/Turkey/Italy/214845/02 and A/Turkey/Italy/220158/02 had shown mild respiratory symptoms, which were diagnosed and treated as mycoplasmosis.



	30	40	50	60	70	80
Tern/Astr/775/83	H13	AFNAVIHGKVENNKCE	TPPTTPHPVYNCSD	TVITKNHTTINN	ITTVVFQDPETH	FRLPL
Mallard/It/208/00	H5	IFNTVIHEKIGDHQ	TVVYPTITAPVVP	NCSDTIITYNNT	VVNNITTTIIT	KAEKHFKSSL
Mallard/It/35/00	H2	IFNTVIHEKIGDHQ	TVVYPTIAAPVVS	NCSDTIITYNNT	VVNNITTTIIT	KAEKHFKSSL
Mallard/It/36/00	H2	IFNTVIHEKIGDHQ	TVVYPTIAAPVVS	NCSDTIITYNNT	VVNNITTTIIT	KAEKHFKSSL
Mallard/It/33/01	H7	IFNTVIHEKIGDHQ	TVVYPTVTAPVVP	NCSDTIITYNNT	VVNNITTTIVT	KAETHFKSSL
Mallard/It/43/01	H7	IFNTVIHEKIGDHQ	TVVYPTVTAPVVP	NCSDTIITYNNT	VVNNITTTIVT	KAETHFKSSL
Turkey/It/214845/02	H7	IFNTVIHGKIGDHQ	TVVYPTVTAPVV	TKAEPHFKSSL	
Turkey/It/220158/02	H7	IFNTVIHGKIGDHQ	TVVYPTVTAPVV	TKAEPHFKSSL	
Pigeon/Nan/9-366/00	H3	IFNAVIHEKIGDHQ	TVIYPTITPPVVP	NCSDTIITYNNT	VVNNITTTIIT	KAEKHFKSSL
Bantam/Nan/9-058/00	H3	IFNAVIHEKIGDHQ	TVIYPTITPPVVP	NCSDTIITYNNT	VVNNITTTIIT	KAEKHFKSSL
Duck/Ger/1215/73	H2	IFNTVIHEKIGDH	STVVYPTITTPVVP	NCSDTIITYNNT	VINNITTTIITE	AERHFKPSL
Turkey /Min/916/80	H7	VFNTVIHEKIGDHQ	TVIHPTIMTPAVP	NCSDTIITYNNT	VINNITTTIITE	AERLFKPPL
Ruddy Turnstone/NJ/65/85	H7	IFNTVIHEKIGDHQ	TVIHPTITTPAVP	NCSDTIITYNNT	VINNITTTIITE	AERPFPKPL
		** *** *	** *			* * *

Fig. 2. Alignment of the NA stalk region of N3 viruses. The full names of virus strains are as indicated in Fig. 4. Potential glycosylation sites are underlined. Asterisks indicate conserved amino acid residues.



LPAI AH7N3



October 2001



October 2002



2002/2003

Serological Analysis of Serum Samples from Humans Exposed to Avian H7 Influenza Viruses in Italy between 1999 and 2003

Simona Puzelli,¹ Livia Di Trani,² Concetta Fabiani,¹ Laura Campitelli,¹ Maria Alessandra De Marco,³ Ilaria Capua,⁴ Jean Francois Aguilera,⁵ Maria Zambon,⁵ and Isabella Donatelli ¹

1 Department of Infectious, Parasitic and Immuno-Mediated Diseases

2 Department of Food and Animal Health, **Istituto Superiore di Sanita`**, Rome,

3 Istituto Nazionale per la Fauna Selvatica, Ozzano Emilia, Bologna,

4 Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy;

5 Centre for Infection, Health Protection Agency, London, United Kingdom

References

1. Puzelli S. et al. Serological analysis of serum samples from humans exposed to avian H7 influenza viruses in Italy between 1999 and 2003. *J Infect Dis* 2005; 192:XXX–XX.



Ducks / Sea Gulls

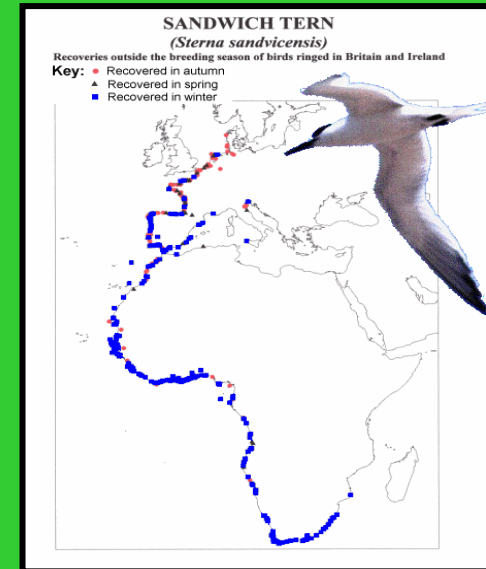
These species have frequently interactions



Sternidae

Emilia Romagna Region 2001, Italy Type A seroprevalence

Species	N° examined	N° Positives	Prevalence (%)
<i>Common Tern</i>	41	1	2,4
<i>Little Tern</i>	13	1	7,7
<i>Sandwich Tern</i>	71	1	1,4
<i>Black Tern</i>	15	2	13,3
Total	140	5	3,6



*(All HI negatives for H5 and H7)

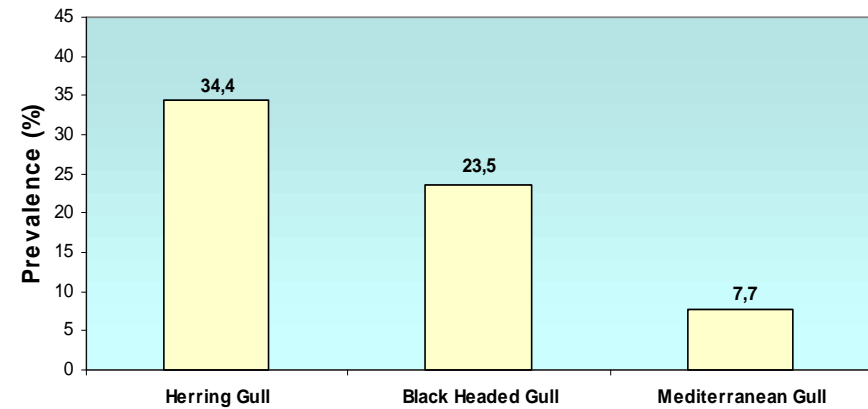




Laridae

Emilia Romagna Region, 2001
Italy, Type A seroprevalence

- Seroprevalences for type A influenza viruses in *Laridae* trapped in Emilia Romagna Region



*One adult of Herring Gull was H7 seropositive

Species	N° examined	N° positives	Prevalence (%)
<i>Mediterranean Herring Gull *</i>	32	11	34,4
<i>Black headed Gull</i>	17	4	23,5
<i>Mediterranean Gull</i>	52	4	7,7
Total	101	19	18,8



**Virological exams
(Italy)**

**Herring Gull
(Juveniles)**

Emilia Romagna Region

in 1999: 64 samples

in 2000: 70 samples

**All negatives to Avian influenza
virus**

Nestling of Herring Gull



Passeriformes

Sampled in Italian wetlands

Acrocephalus scirpaceus

All cloacal swabs
result negative to Avian
influenza virus

Passer montanus

Vet. Res. Comm. (De Marco et al, 2003)



Modern Birds

Neognathia

After Olson, 1985, Feduccia, 1980, 1996, Ericson, 1996, Caspers, Uit de Weerd, Wattle, & de Jong,, 1997, van Tuinen, Sibley & Hedges, 2000, Carcía-Moreno & Mindell, 2000 and van Tuinen, Butvill, Kirsch & Hedges, 2001)

Neognathia Huxley, 1867 [Neognathe] (neognathous modern birds)

--o Galloanserae

Galloanserae

| |-- Galliformes (fowls, chickens)

| |-- "Anserimorphae" (duck-like birds; sorsamaiset linnut)

| |?- †Dromornithiformes [*sensu?* Murray & Megirian [from Grellet-Tinner, 2001]

| `-- Anseriformes (ducks, swans & geese)

--o Neoaves

| |-- Mesitornithidae Wetmore, 1960 [Mesiornithes Wetmore, 1960])

| |-- *Monias benschi* Oustalet & Grandidier, 1903

| `--o *Mesitornis* Bonaparte, 1855

| |-- *M. variegata*

| `-- *M. unicolor*

`--o Gruimorphae

|?- †*Anisolornis excavatus*

|?- †Diatrymiformes [Neornithes *incertae sedis*]

|?- Gruiformes Bonaparte, 1854 [Grues Bonaparte, 1854] (cranes and relatives)

|?+-- Phoenicopteriformes (flamingoes;)

| `-- Podicipediformes (grabs)

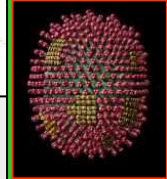
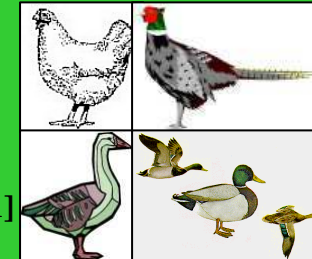
|?- Charadriomorphae (shorebird-like modern birds;)

|?- "The Land-bird Assemblage"

|?- Eurypygoidea Selby, 1840 [Eurypygae Fürbringer, 1888] (sunbitterns)

|?- Rhynocheti [Rhynochetoidea Sharpe, 1891] (kagut; kagus)

`?- Otides Wagler, 1830 (bustards;)



Neoaves

Most evidence of the monophyly of Galloanserae come from molecular studies, and the group is not well supported by morphological grounds. However, the most recent, although preliminary, phylogenetical analysis of modern birds based on morphology (Livezey & Zusi, 2001, Meyr & Clarke, 2003) seem to confirm the monophyly of Galloanserae.

Galliformes

(*Sedentary land based birds*)

February/March 1992/93/94

Samples size:

394 Pheasants

(*Phasianus colchicus torquatus*)

trapped in the wild

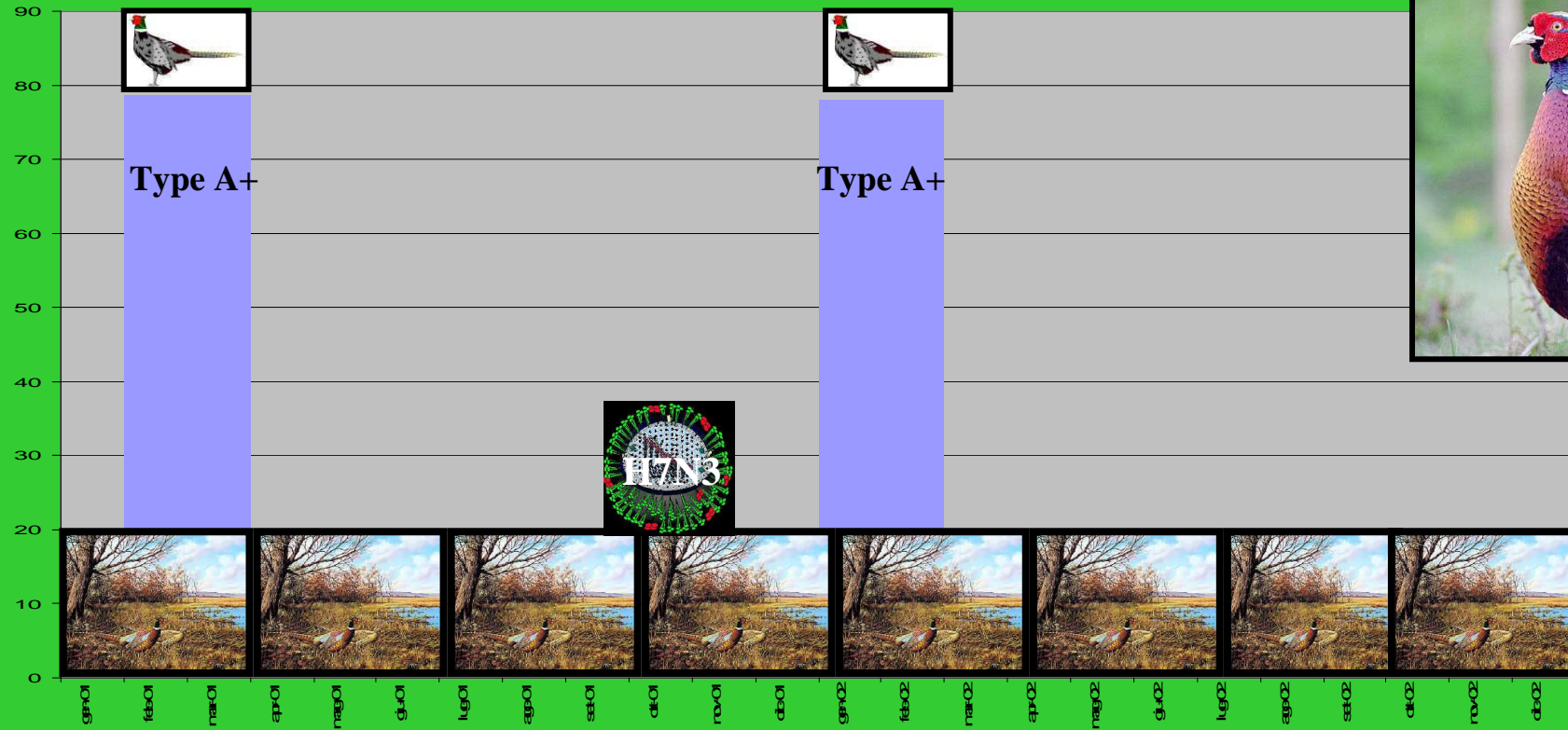
**30 Coming from
reared groups**

**All sera were found
negatives to type A Influenza viruses**

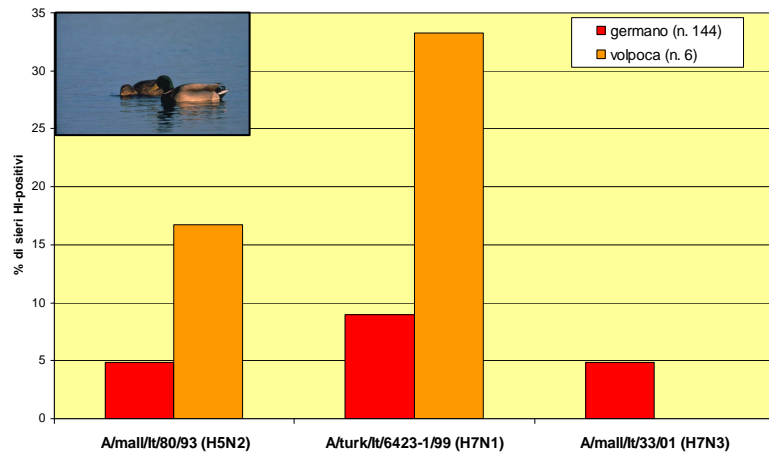


(De Marco *et al*, 2003 Vet. Res. Comm.)

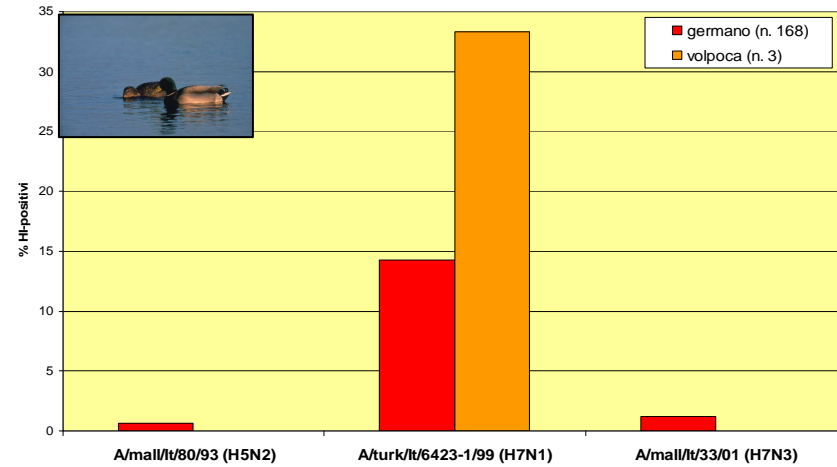
SIEROPREVALENZE PER VIRUS INFLUENZALI DI TIPO A 2001-2002



ANNO 2001: PERCENTUALI DI SIERI DI ANATIDI NP-POSITIVI TESTATI IN HI NEI CONFRONTI DI ALCUNI CEPPI H5 E H7



ANNO 2002: PERCENTUALI DI SIERI DI ANATIDI NP-POSITIVI TESTATI IN HI NEI CONFRONTI DI ALCUNI CEPPI H5 E H7



Owls

- **114 Owls**

All sera were negative to
type A Influenza
virus



Birds of prey

- **33 Birds of Prey**

- **(Falcons and others)**



1 Buzzard and 1 Peregrine Falcon
were positive to type A
Influenza virus

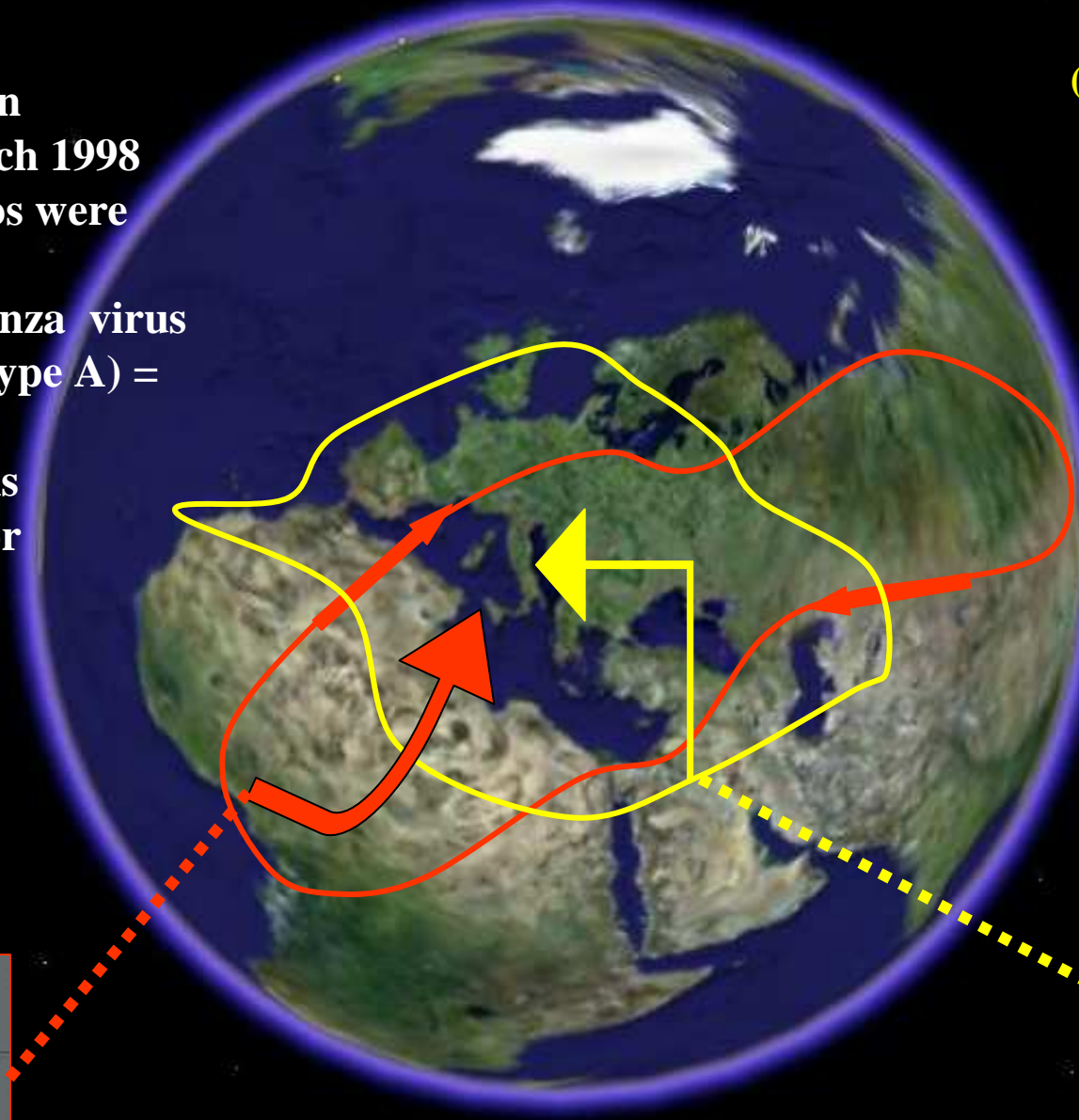
Trans-saharians migratory duck (*Anas querquedula*) 126 Garganeys

Trapped in Italian Wetlands in March 1998
126 Cloacal Swabs were negatives for type A Influenza virus seroprevalence (type A) = 9.6%
All sampled ducks were negative for the H7 subtype

Transaharian migratory birds: The Ethiopian Region Garganeys and Quails

Galliformes (Migratory land based birds) Samples size: 258 Quails (*Coturnix coturnix c.*)

Trapped in Italy during May 1998
All sera were found negatives to type A Influenza viruses



Focolai e Censimenti aerei....



Ministero della salute: Ordinanza del 11/02/2006

**MISURE URGENTI DI PROTEZIONE PER CASI DI INFLUENZA AVIARIA AD ALTA
PATOGENICITÀ**

NEGLI UCCELLI SELVATICI

ZONA DI PROTEZIONE:

**ABBATTIMENTO E DISTRUZIONE DEGLI ANIMALI SENSIBILI INFETTI,
SOSPETTI DI INFEZIONE E DI CONTAMINAZIONE**



ne trovate qualcuno in questa immagine?

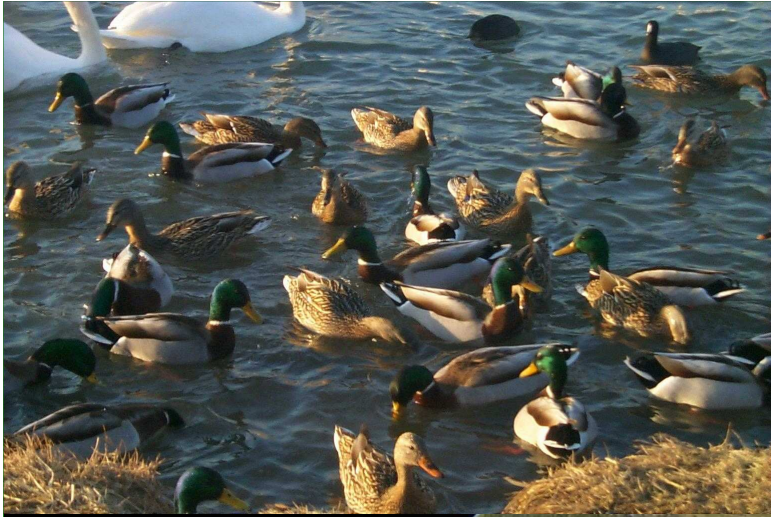
*Circolare del Dipartimento della Sanità pubblica vet. 15 Febbraio 2006
Agonizzanti...ovvero Stato preagonico
autorizzato dai Serv. Vet. Locali senza il rispetto della precedente
procedura...ma con*



*Ordinanza del Sindaco su parere favorevole dei Serv. Vet. Locali sentite
le unità di crisi regionali e locali*

Why to kill?





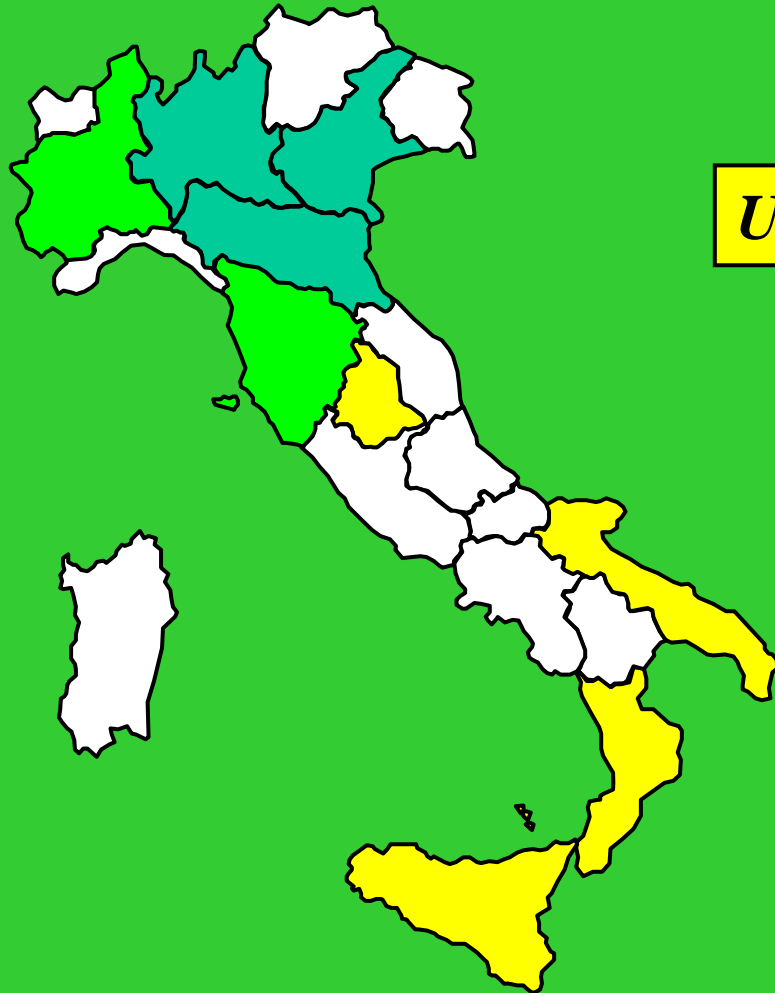
The Anas castanea example



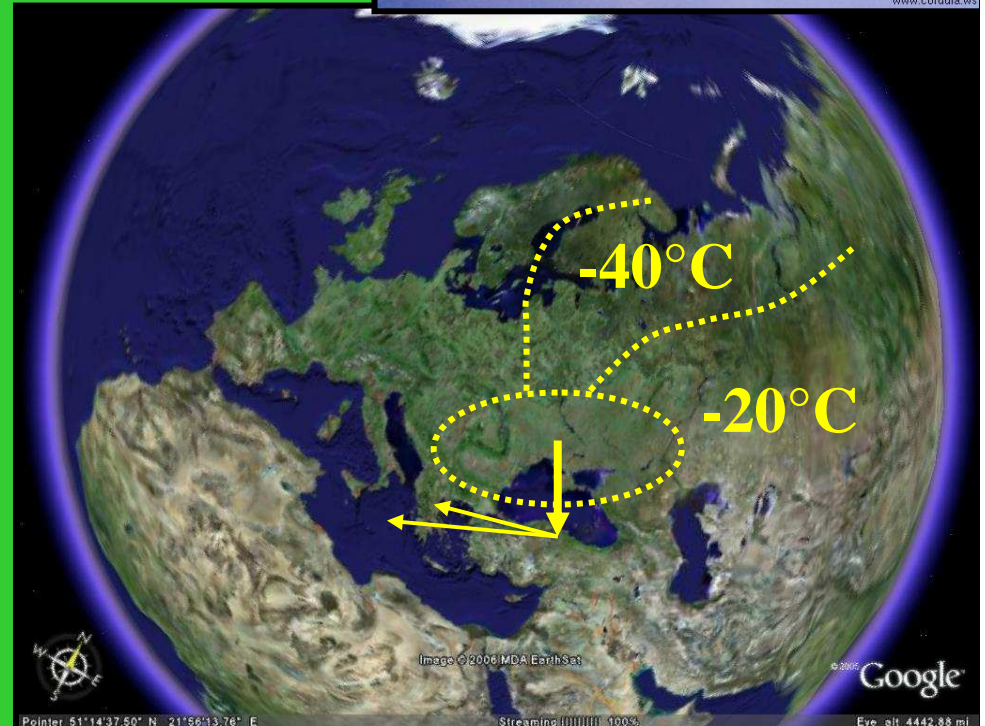
*Risk Areas?
Theoretical.....*



Out breeding and wandering birds



Unpredictable



Avian Influenza Surveillance Program (Wild)

Why (monitoring wild birds)?
and Why not? (*Sentinel ducks, Fecal dropping*)
when, why



Environmental viruses

Viral resistance : (from Stallknecht, 1999/ 2006)

Drinking water : 4 days at 22°C

30 days at 0°C

Distilled Water : 102 days at 28°C

207 days at 17°C

Experimental conditions : 9 days at 28°C

100 days at 17°C



Viral resistance in Faeces:

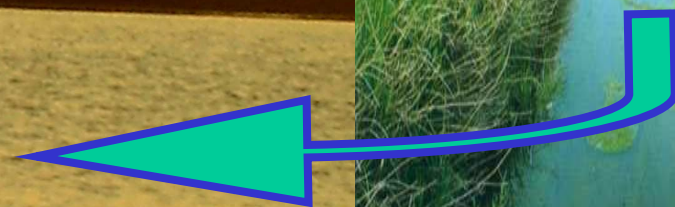
35 days at 4 °C, 7 days at 20 °C

(data from Webster)

Max infectivity

salt water with PH 6.2

water without salt PH 8.2





dogs



Hunters



Hunted birds

Hunter's and health risk



Decoys



What can the virus see

Reservoir



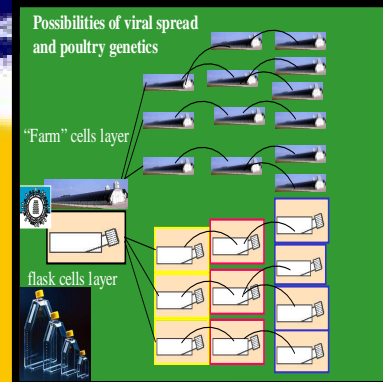
watching the Europe?

Epiphenomena



Wild ducks 7.5 mil. susceptible/year
In all the Palearctic (50.000 in Italy)

Aquatic birds 23.5 mil.
(230 species In all the Palearctic)



Potential Domestic Reservoir:
9.2 mil. (7 million of raised Ducks) /year.
90% of these ducks are reared in the
Lombardia and Veneto Italian Regions.

Domestic Epiphenomena : 4 Billion
Susceptible host/year in Europe,
over 560 milion/year in Italy

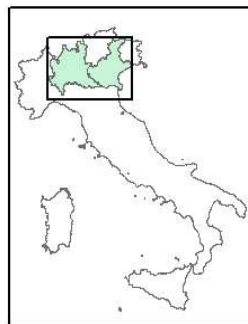
Dispersione della popolazione avicola

- Legend
- Species
- Turkey
 - ▲ Chicken
 - △ Other birds
- Municipality
- Province
- Region

3.300 allevamenti avicoli

95 milioni di volatili accasabili/ciclo

**65% della produzione
avicola nazionale**

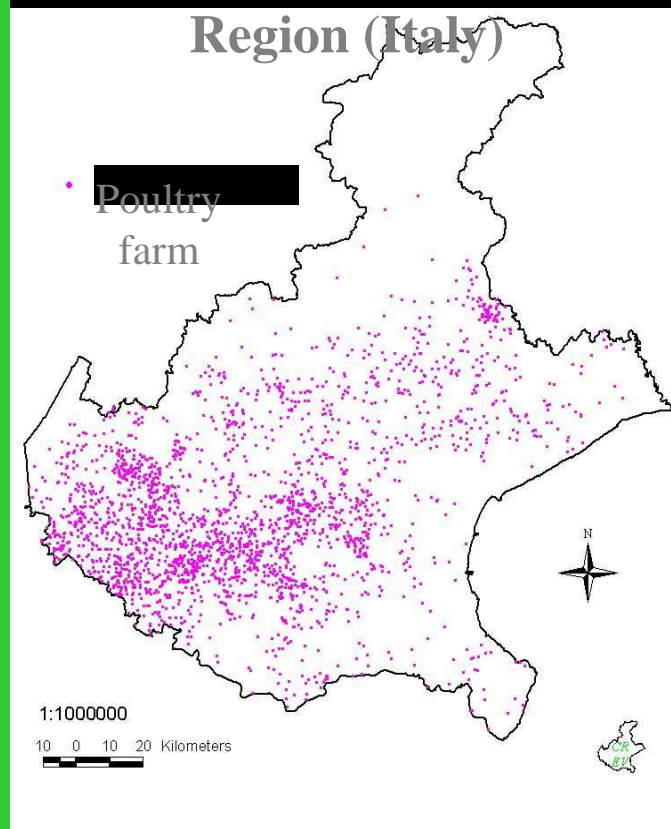


Reference system: ED50
Coordinates projection:
Gauss-Boaga fuso Ovest



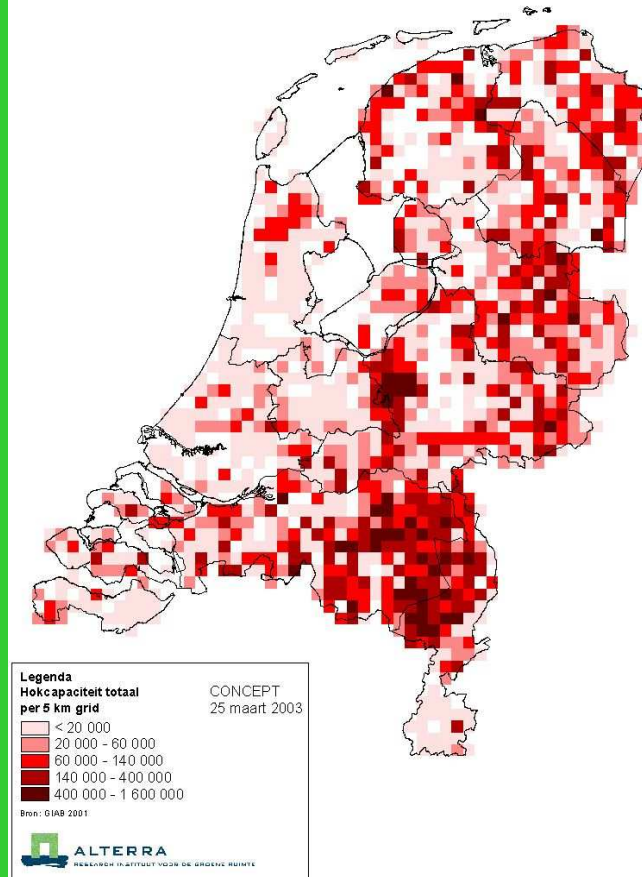
Aree ad alta densità avicola in Europa

Spatial distribution of poultry farms in Veneto Region (Italy)



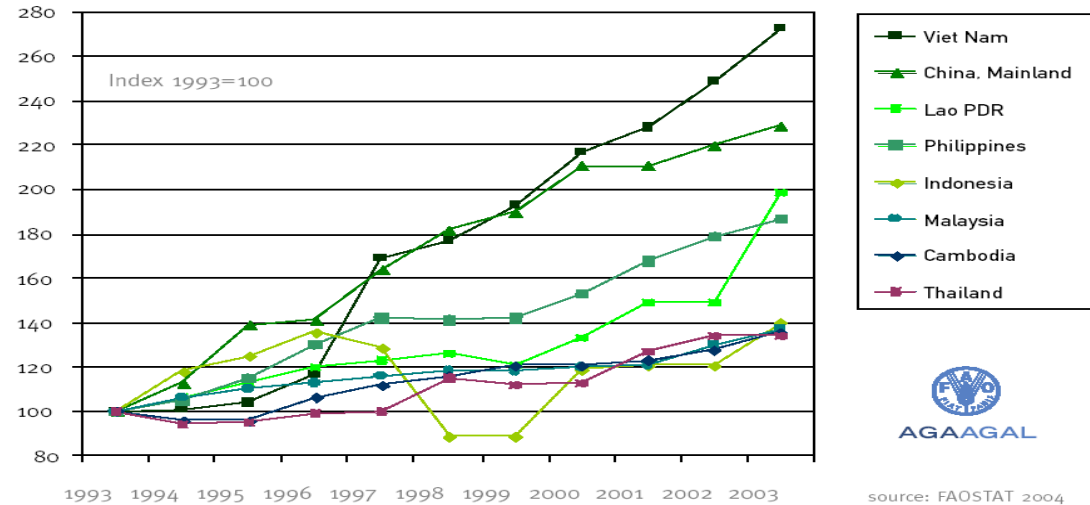
12 allevamenti /km² (provincia di Verona)

Totale hokcapaciteit kippen (vleeskuikens, ouderdieren en leghennen)



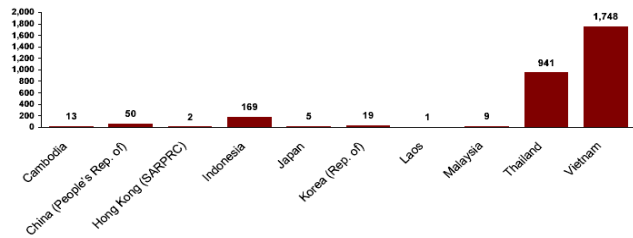
Olanda (Geldersvalley)
20-25 allevamenti

Growth in Poultry Meat Production SE Asia



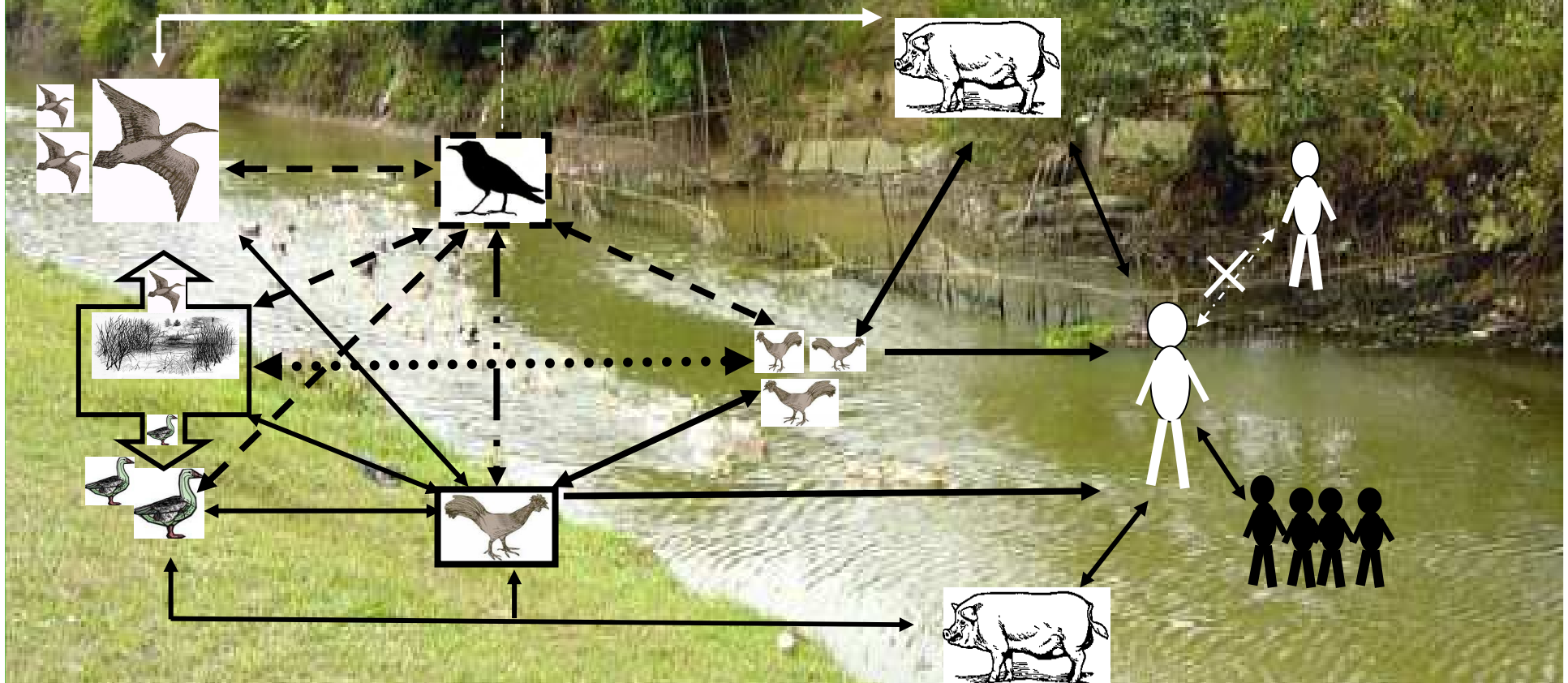
source: FAOSTAT 2004

Outbreaks of Highly pathogenic avian influenza in Asia
(as of 19 November 2004)



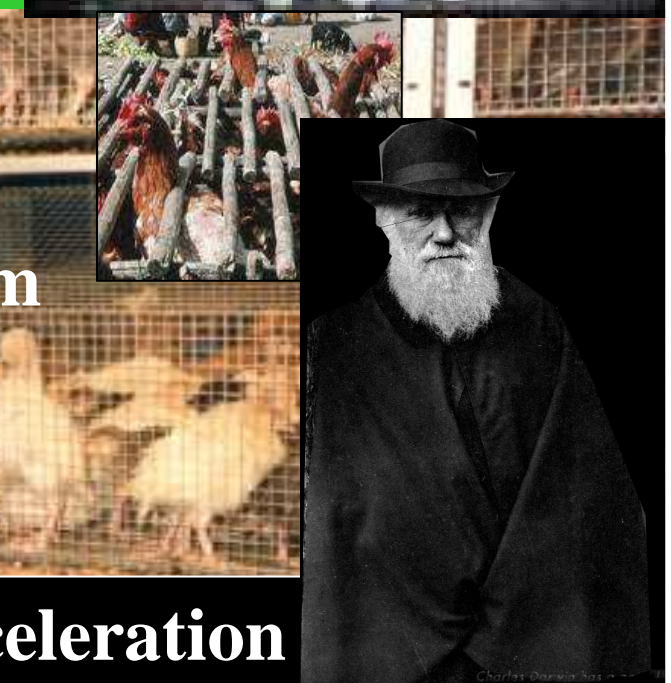
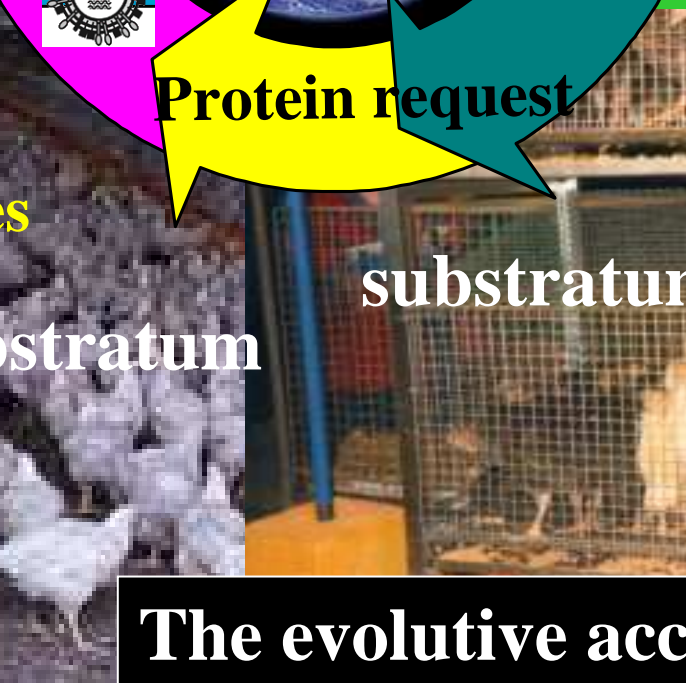
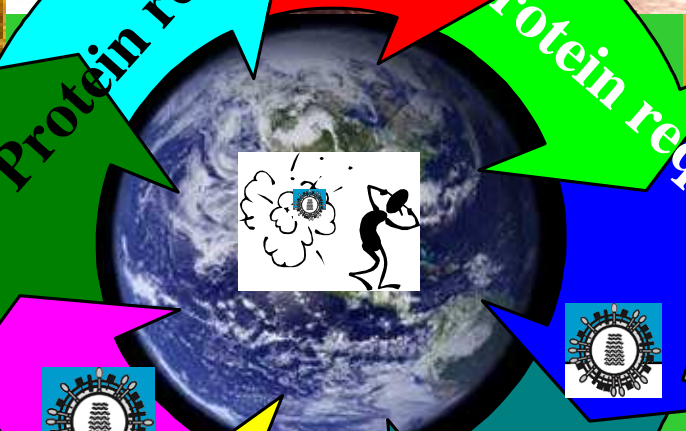
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The South East Asian melting pot



Protein sources

substratum



Protein sources

substratum

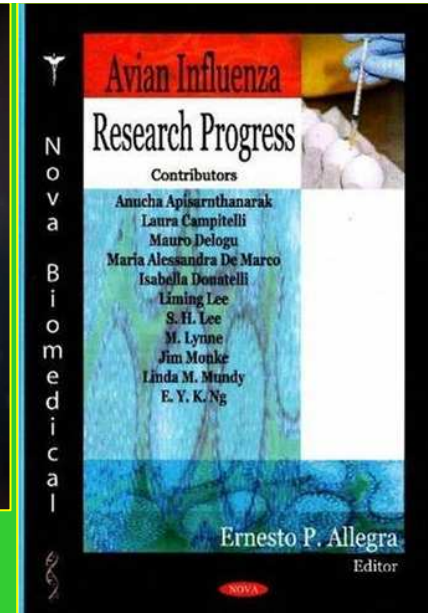
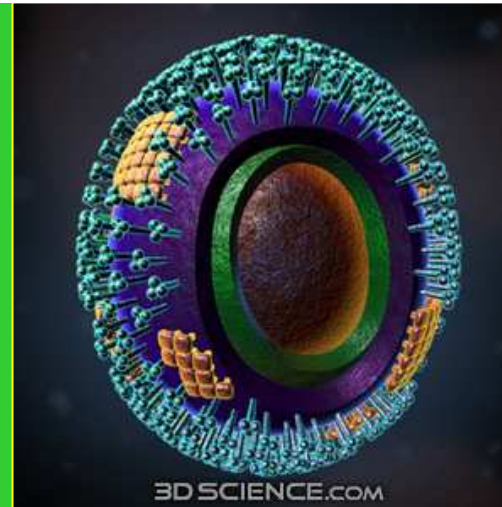
Protein sources

substratum

substratum

The evolutive acceleration

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