

# Avian Influenza in cage-free systems

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# It has not been an easy year with bird flu ...

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Europe

2 minute read · October 3, 2022 2:20 PM GMT+2 · Last Updated 3 months ago

## Worst ever bird flu crisis in Europe raises risks for next season - EFSA

By Sybille De La Hamaide

Source: Reuters



Source: Euronews

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## US Avian Flu Outbreak Worst on Record With 50 Million Dead Birds

- Avian influenza contributed to 50.54 million birds killed
- Highly pathogenic virus prompted turkey, egg prices to soar

Source: Bloomberg

## Colombia confirms 22 avian influenza outbreaks since Oct. 19

Outbreaks were identified in backyard flocks near wild birds

5 December 2022 1 minute read By Global Ag Media South America

Source: Poultry World

NEWS EXPLAINER | 21 October 2022

## Why is bird flu so bad right now?

The virus is running amok around the world. Possible explanations include an enhanced ability to replicate or infect more bird species.

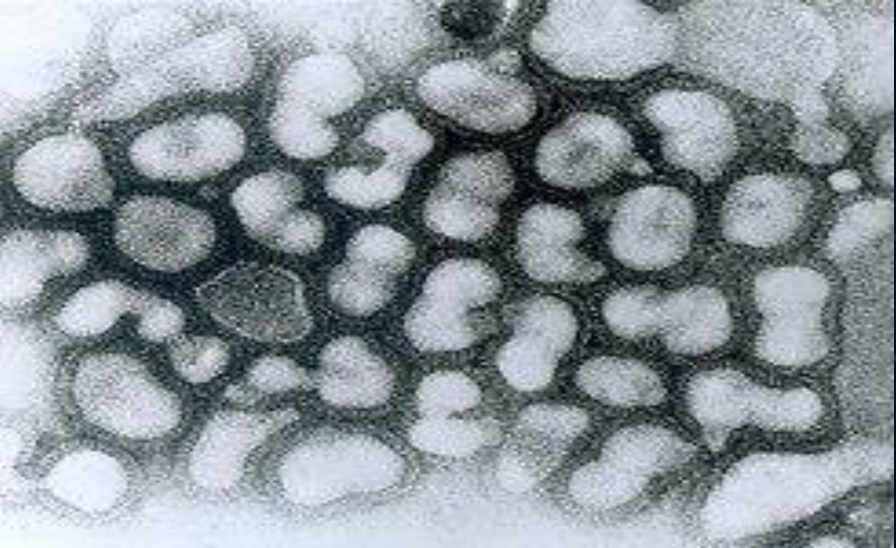
[Saima May Sidik](#)

Source: Nature

# Etiology

## TAXONOMICAL CLASSIFICATION

- Realm: <i>Riboviria</i>
- Kingdom: <i>Orthornavirae</i> Realm: <i>Riboviria</i>
+ Phylum: <i>Duplornaviricota</i> Kingdom: <i>Orthornavirae</i>
+ Phylum: <i>Kitrinoviricota</i> Kingdom: <i>Orthornavirae</i>
+ Phylum: <i>Lenarviricota</i> Kingdom: <i>Orthornavirae</i>
- Phylum: <i>Negarnaviricota</i> Kingdom: <i>Orthornavirae</i>
- Subphylum: <i>Haploviricotina</i> Phylum: <i>Negarnaviricota</i>
+ Class: <i>Chunqiuviricetes</i> Subphylum: <i>Haploviricotina</i>
+ Class: <i>Milneviricetes</i> Subphylum: <i>Haploviricotina</i>
+ Class: <i>Monjiviricetes</i> Subphylum: <i>Haploviricotina</i>
+ Class: <i>Yunchangviricetes</i> Subphylum: <i>Haploviricotina</i>
- Subphylum: <i>Polyploviricotina</i> Phylum: <i>Negarnaviricota</i>
+ Class: <i>Ellioviricetes</i> Subphylum: <i>Polyploviricotina</i>
- Class: <i>Insthoviricetes</i> Subphylum: <i>Polyploviricotina</i>
- Order: <i>Articulavirales</i> Class: <i>Insthoviricetes</i>
+ Family: <i>Amnoonviridae</i> Order: <i>Articulavirales</i>
- Family: <i>Orthomyxoviridae</i> Order: <i>Articulavirales</i>
- Genus: <i>Alphainfluenzavirus</i> Family: <i>Orthomyxoviridae</i>
Species: <i>Alphainfluenzavirus influenzae</i> Genus: <i>Alphainfluenzavirus</i>
+ Genus: <i>Betainfluenzavirus</i> Family: <i>Orthomyxoviridae</i>
+ Genus: <i>Deltainfluenzavirus</i> Family: <i>Orthomyxoviridae</i>
+ Genus: <i>Gammainfluenzavirus</i> Family: <i>Orthomyxoviridae</i>
+ Genus: <i>Isavirus</i> Family: <i>Orthomyxoviridae</i>



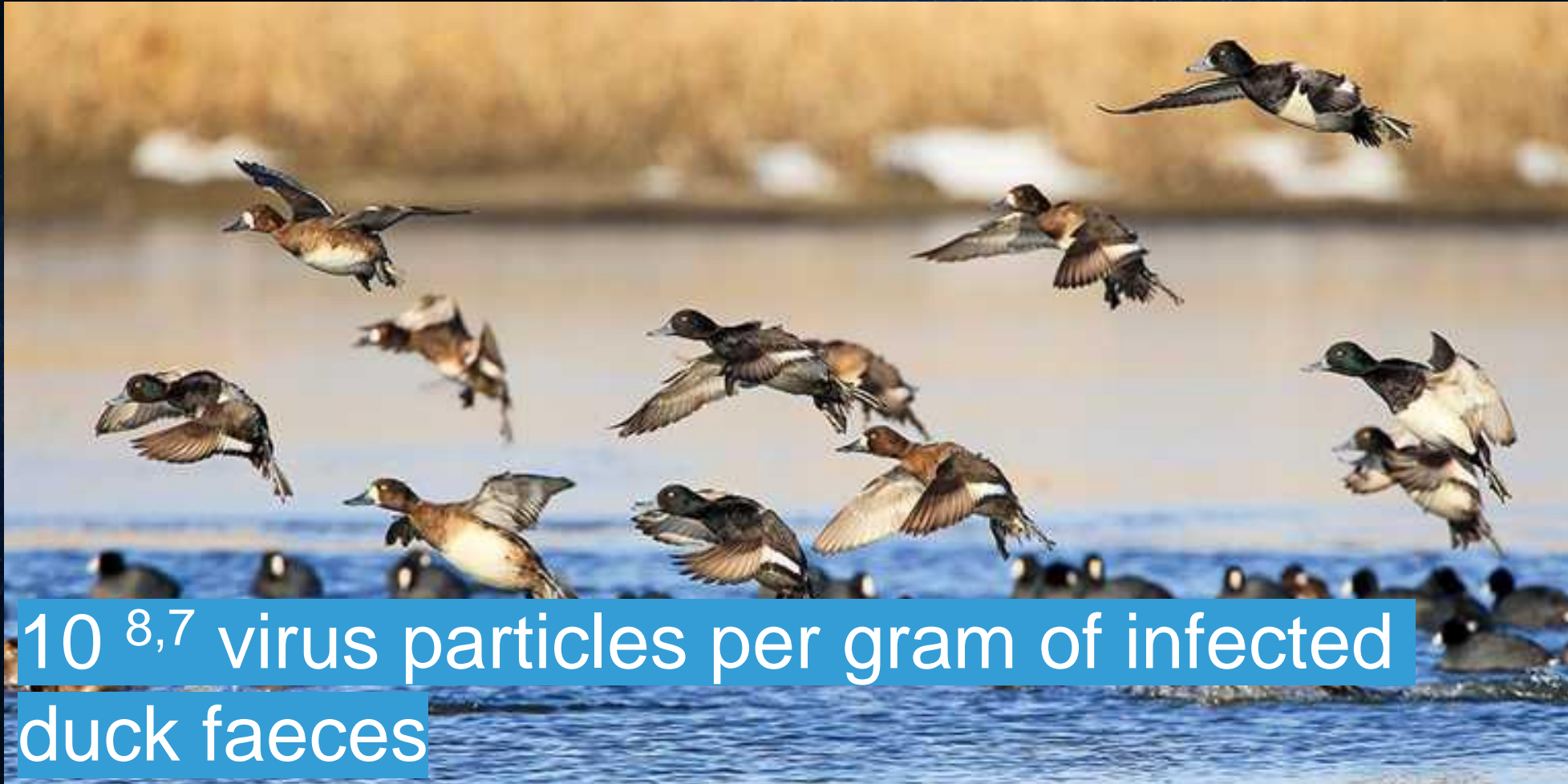
Picture: Wikipedia



**Family:** Orthomyxoviridae  
**Genus:** Alphainfluenzavirus  
**Specie:** Alphainfluenzavirus influenzae

# Ecology

## AIV RESERVOIR



Picture: Dean Pearson

# Ecology

## AFFECTED BIRD FAMILIES

Anseriformes

Charadriiformes

Ciconiiformes

Galliformes

Passeriformes

Columbiformes

Falconiformes

Piciformes

Pelecaniformes

Procellariiformes

Podicipediformes

Gaviiformes

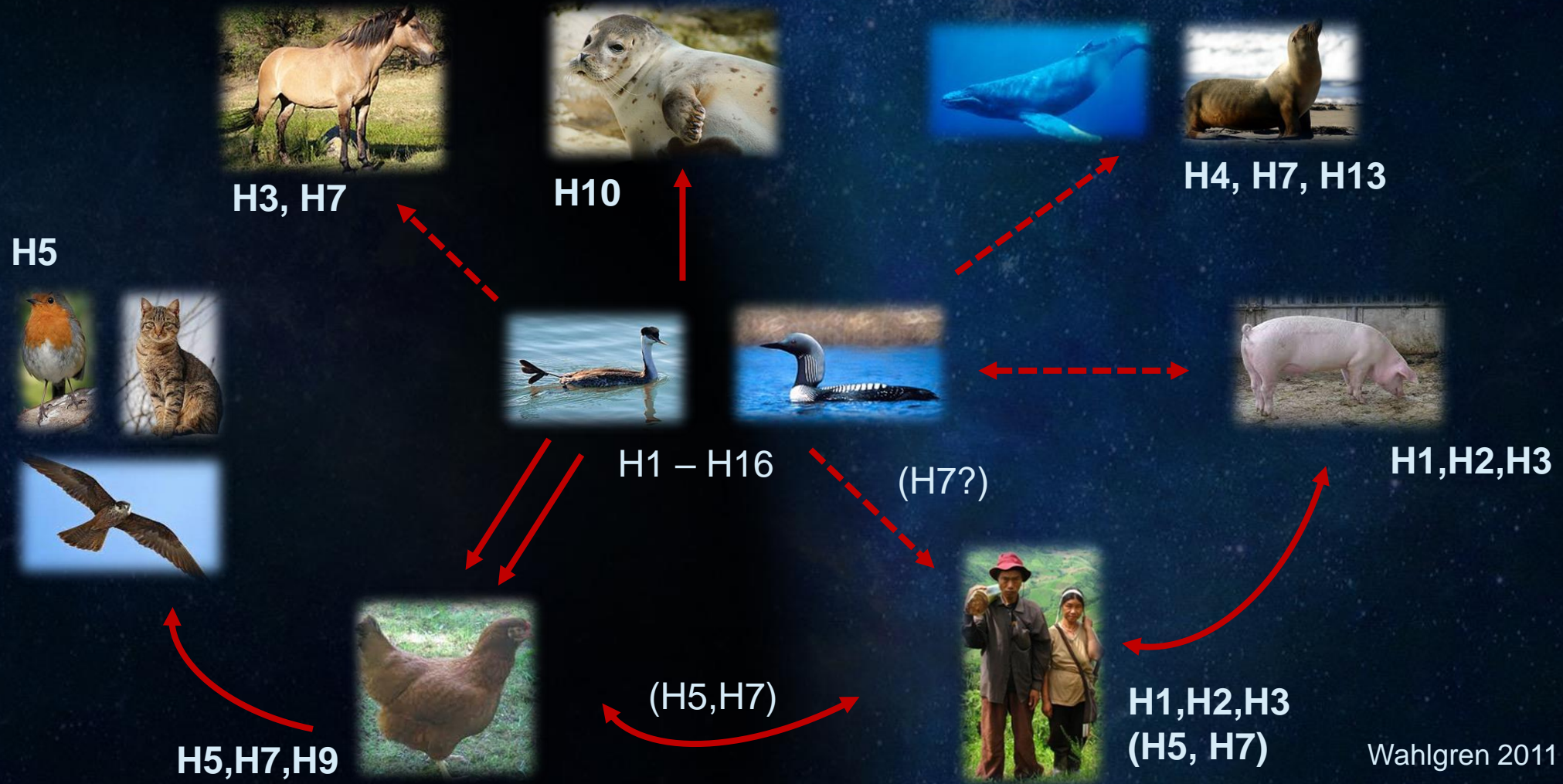
Gruiformes

**60 % of known bird families  
(at least)**



# Ecology

## INFLUENZA IN OTHER SPECIES



Wahlgren 2011

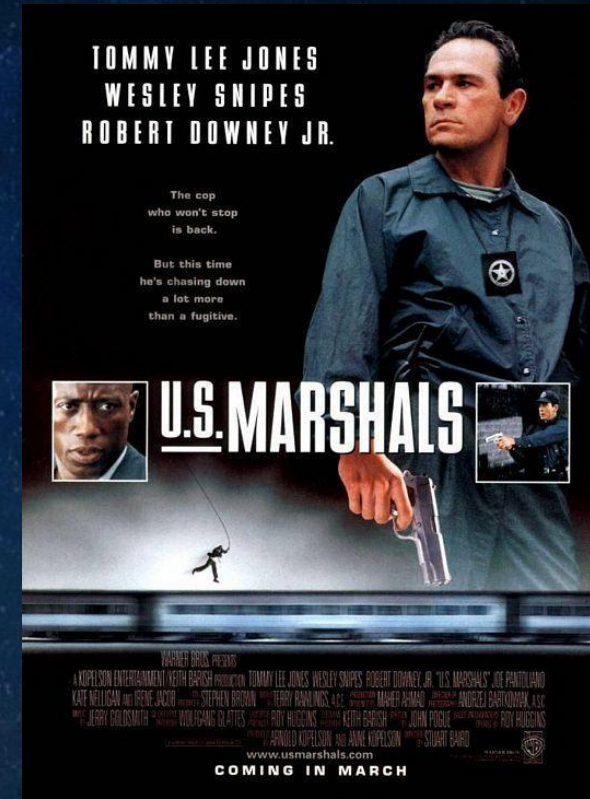
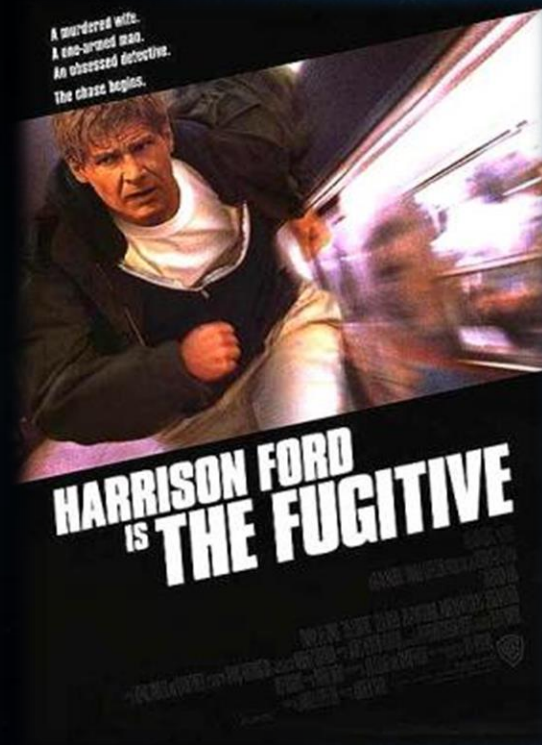
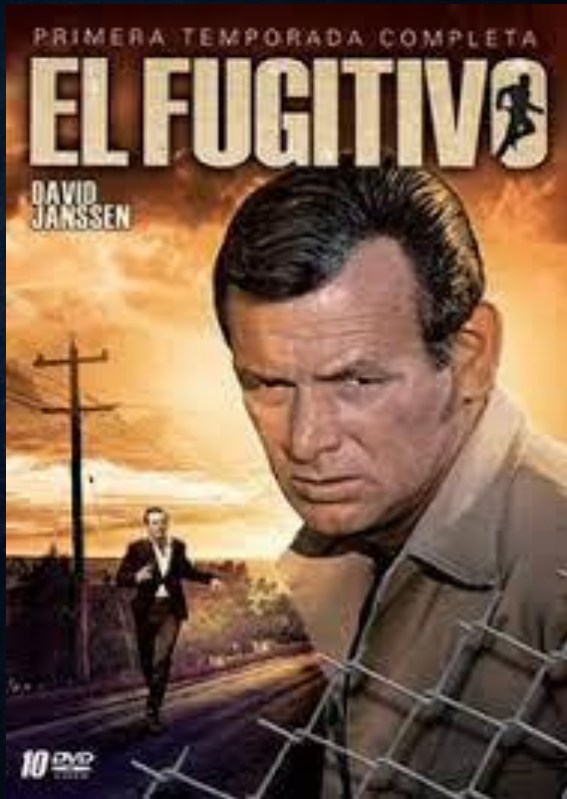
Picture: Wikipedia



# Ecology

## AIV'S SURVIVAL STRATEGY

Always on the run!!!!

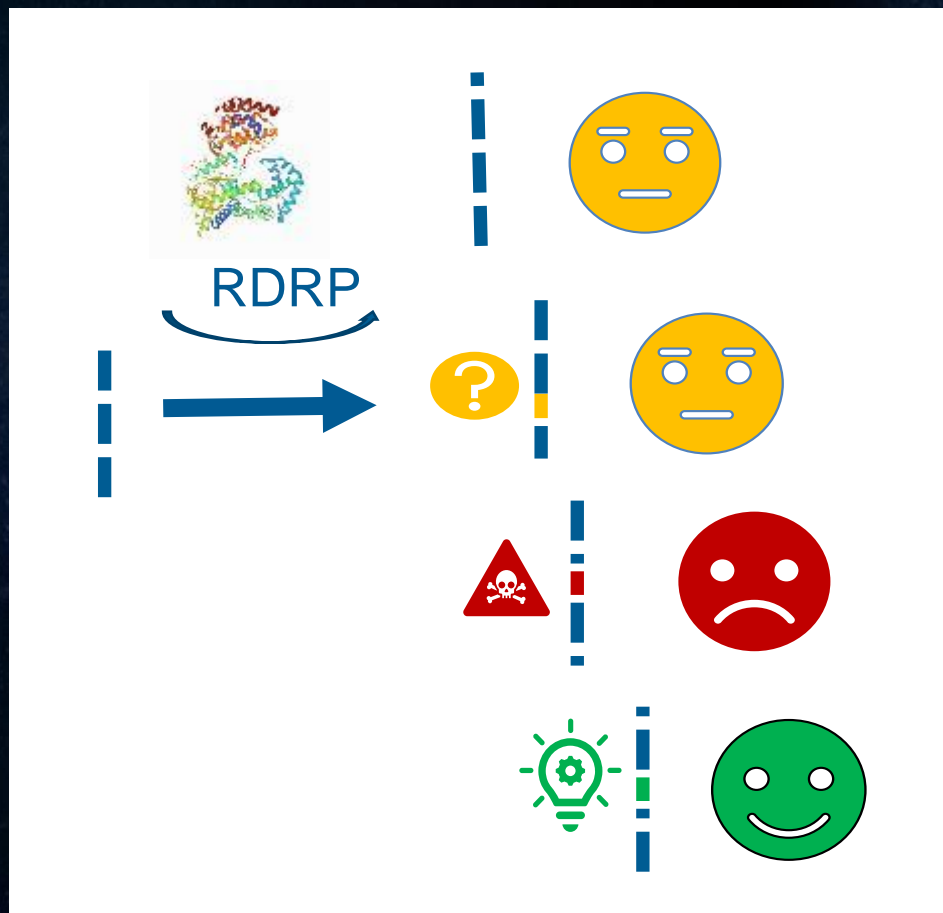




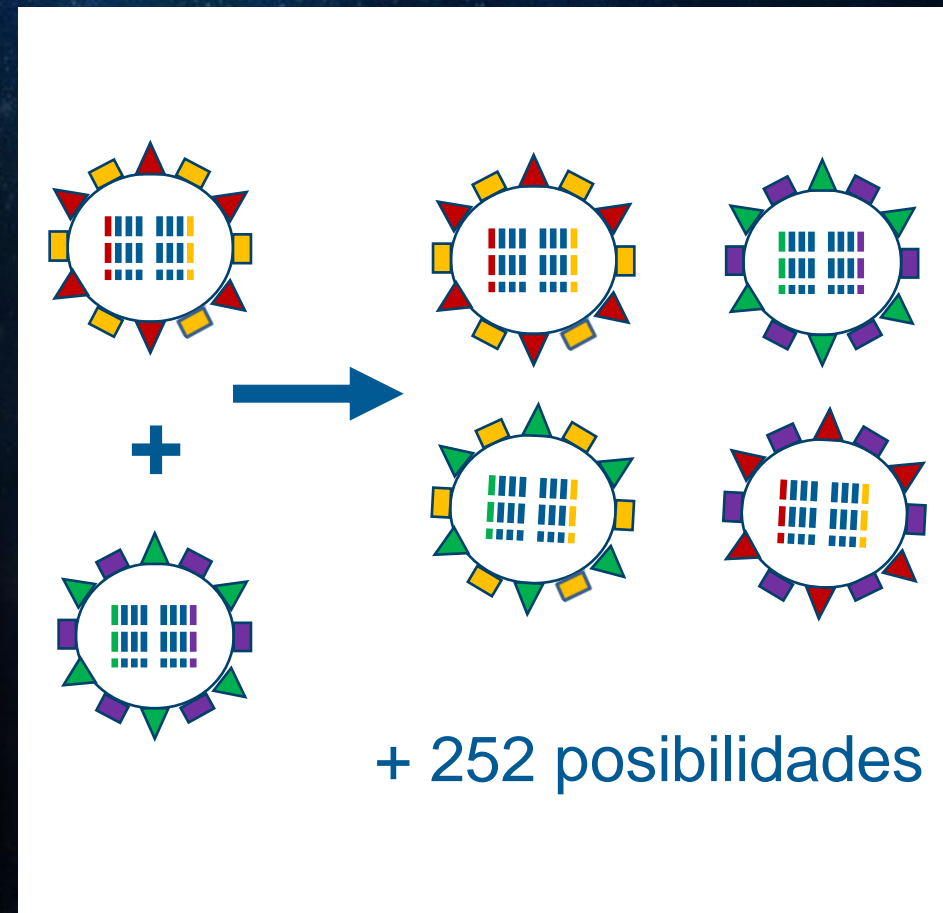
# Ecology

A BREAKAWAY ARTIST ...

## Antigenic drift



## Antigenic shift

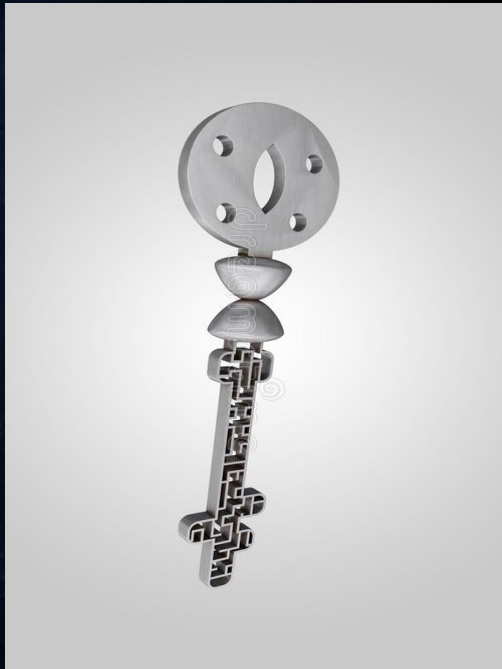




# Pathogenesis

## MAIN PROTEIN SURFACES

### Hemagglutinin (H)



Picture: Wikipedia

### Neuraminidase (N)

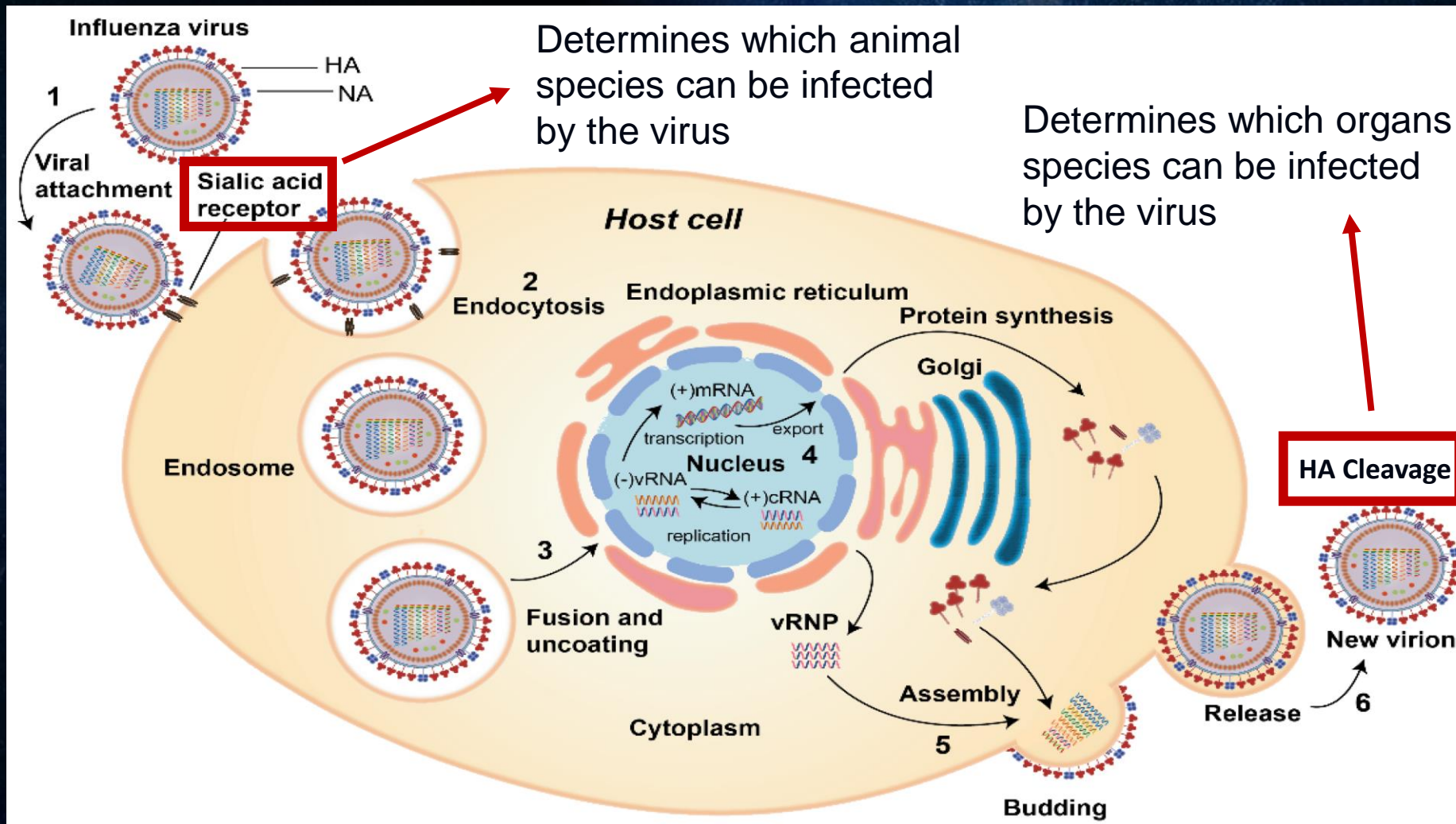


Picture: Wikipedia



# Pathogenesis

## CELL INFECTION CYCLE



Determines

# Pathogenesis

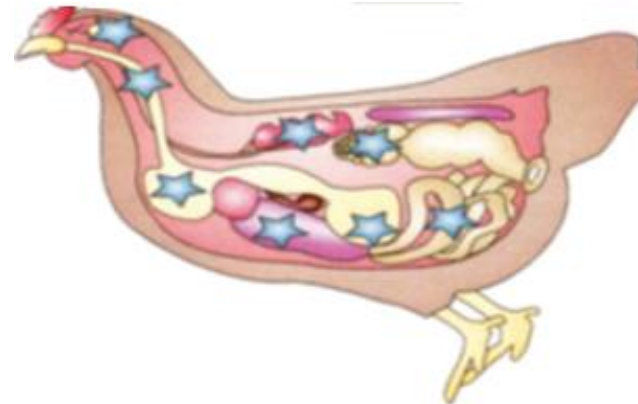
## LPAI vs HPAI

### Low Pathogenic Avian Influenza

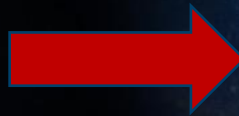


Non OIE list  
Mild respiratory disease  
H1-H16

### High Pathogenic Avian Influenza



OIE list  
High mortality  
H5 or H7



**LPAI H9N2 ??**

# Clinical signs & lesions

## LPAI H9N2 LESIONS



(a) Fibrinous tracheitis with fibrinous plug in the trachea,  
(b) tracheal bifurcation and  
(c) fibrine in bronchi

# Clinical signs & lesions

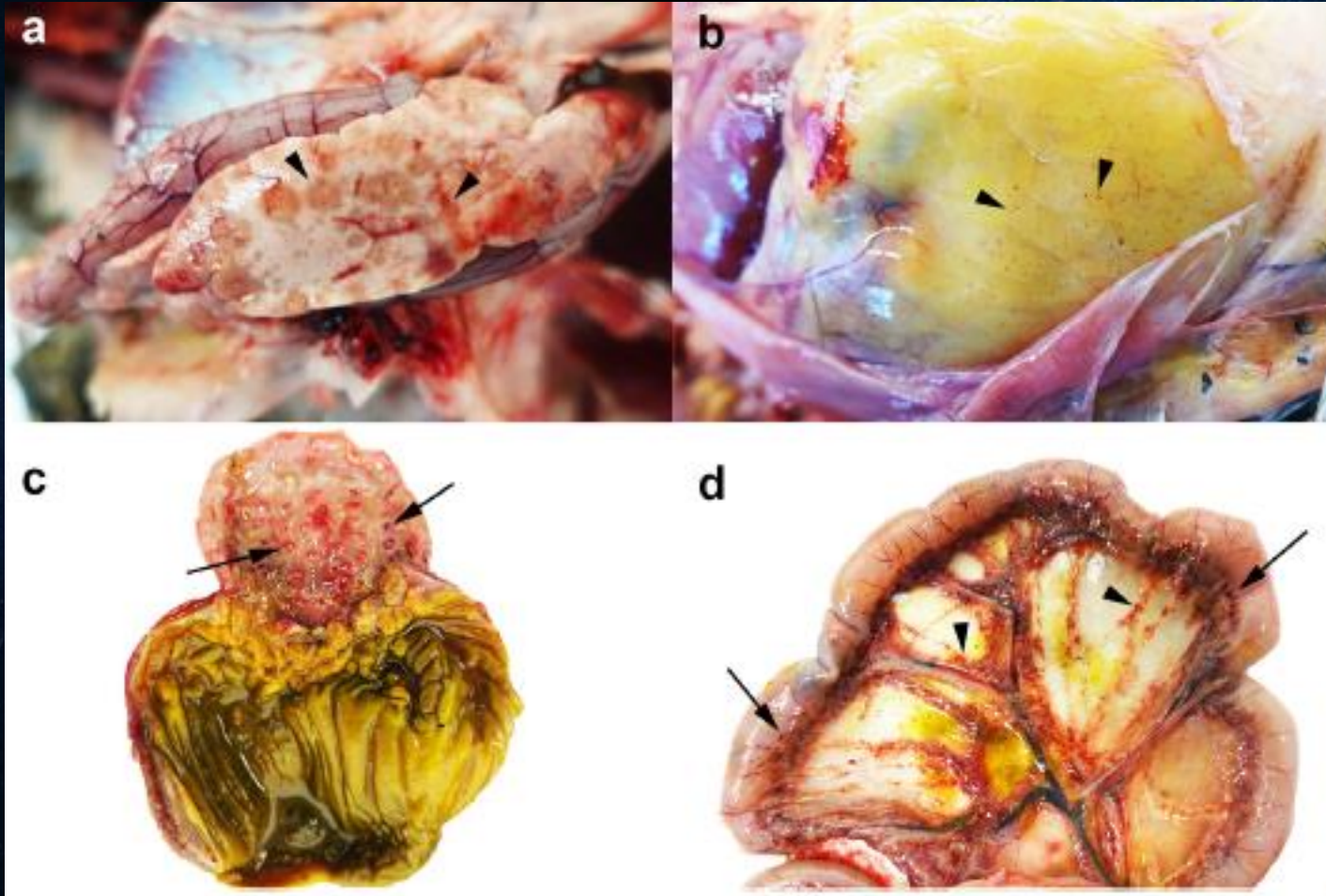
## WORLDWIDE DISTRIBUTION OF LPAI H9N2





# Clinical signs & lesions

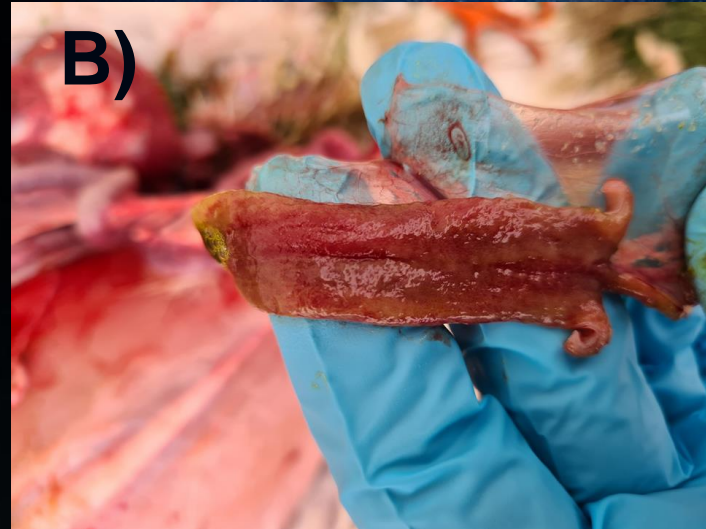
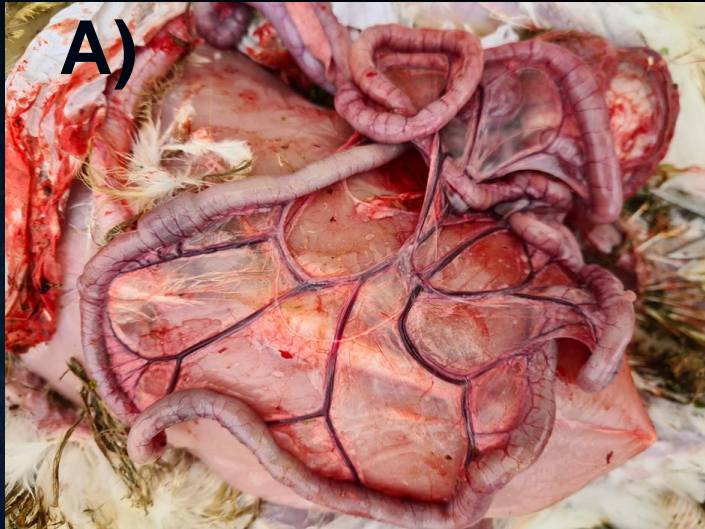
## HPAI H5N1 LESIONS (CHICKENS)



(a) petechiae  
in the coelomic fat  
(c), haemorrhages in  
the proventricular  
mucosa  
(b) haemorrhages in  
the intestinal serosa  
and  
(d) in the mesentery

# Clinical signs & lesions

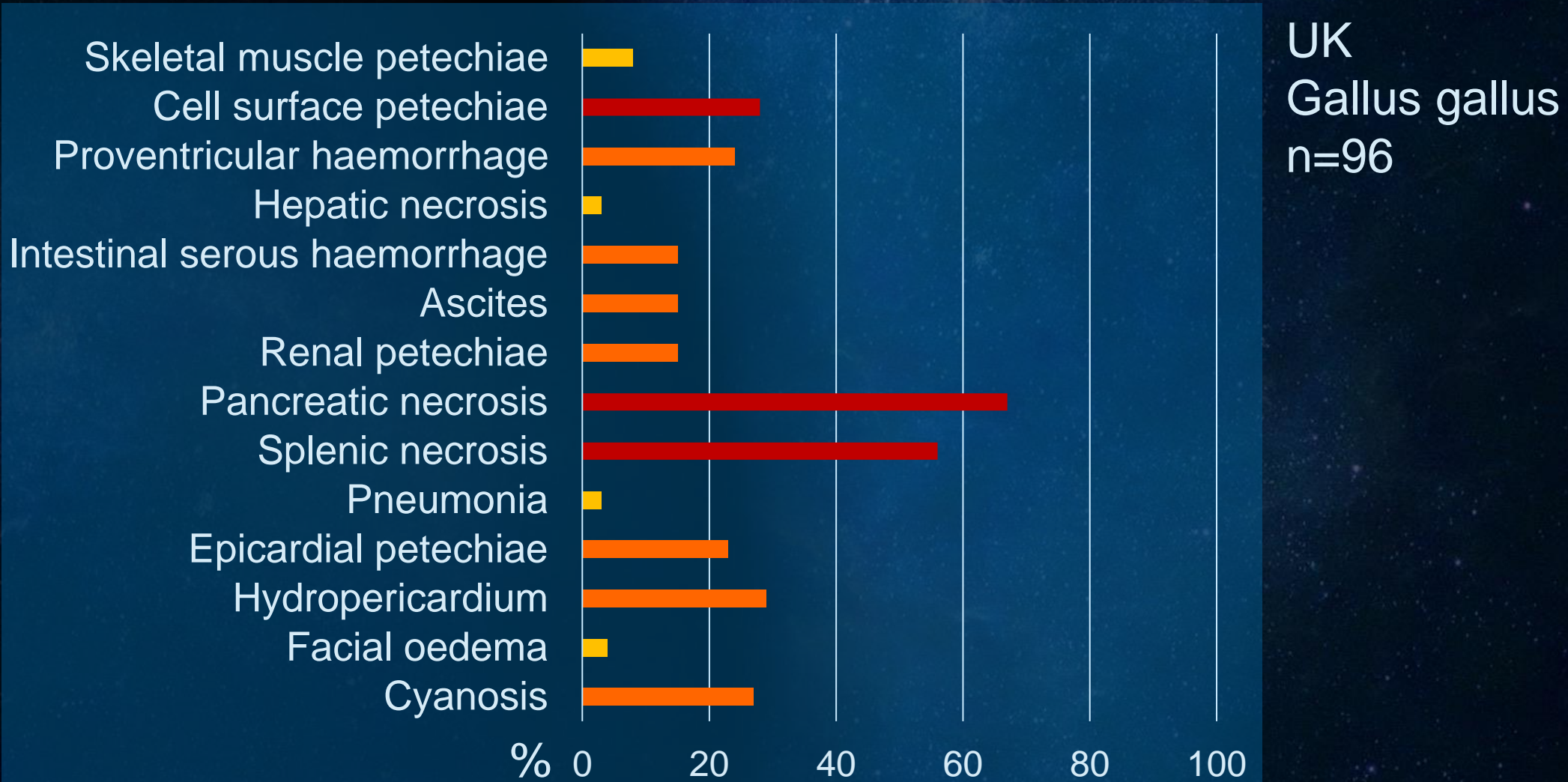
## HPAI H5N1 LESIONS ( TURKEYS )



- (a) Intestinal serosal haemorrhage in the mesentery
- (b) intestinal serosal haemorrhage
- (c) haemorrhages in the proventricular mucosa
- (d) haemorrhagic tracheitis

# Clinical signs & lesions

## GROSS LESION OBSERVED IN BIRDS WITH HPAI H5N1





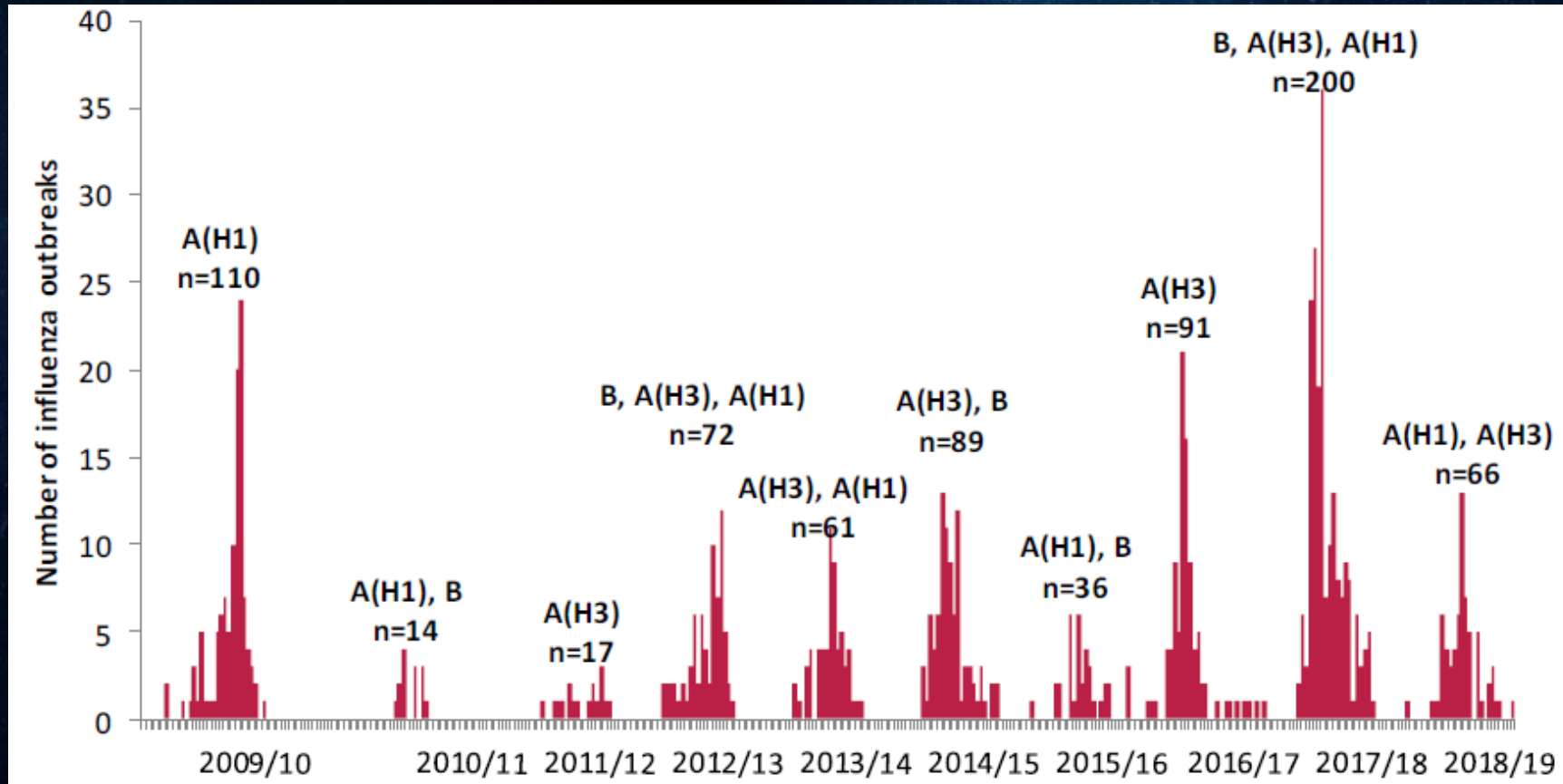
Is Avian influenza  
a zoonotic disease?

Picture:  
Wikipedia

# Flu in humans

## SEASONAL FLU

Every winter, H1 - (H2) - H3



Source: HSE

# Flu in humans

## PANDEMIC FLU



**H1N1 Spanish flu**

1918

**H1N1 Russian flu**

1976

**H2N2 Asian flu**

1957

**H3N2 Hong Kong flu**

1968

1910

1920

1930

1940

1950

1960

1970

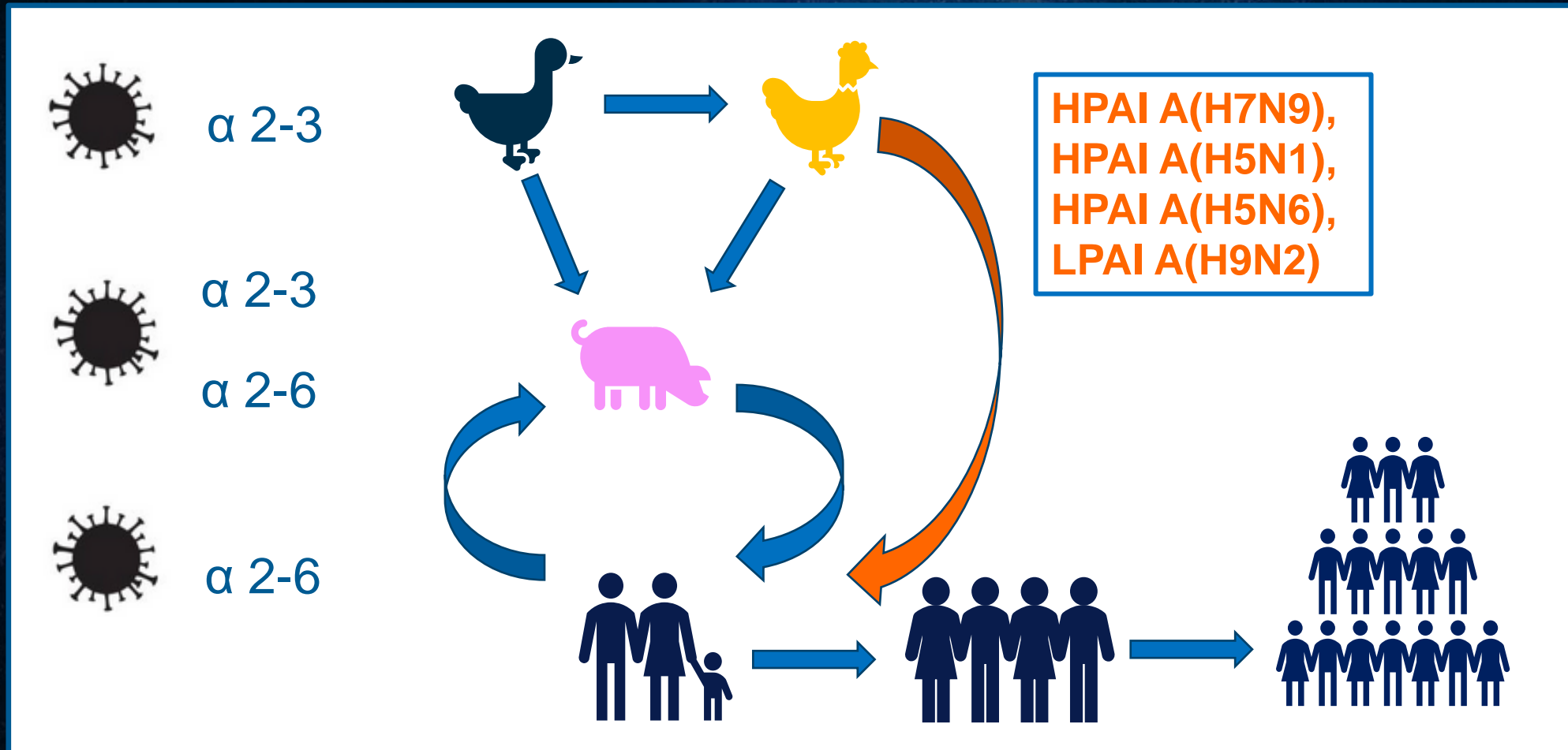
1980

1990

2000

# Flu in humans

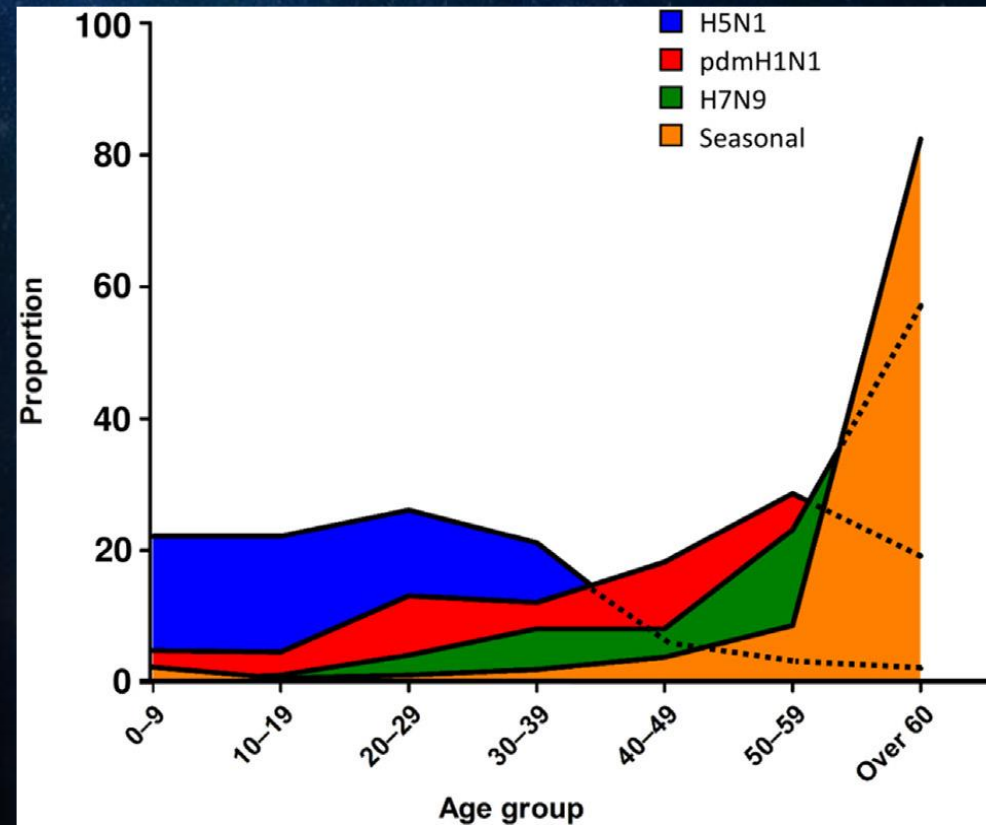
## BIRD-TO-HUMAN TRANSMISSION OF INFLUENZA



# Flu in humans

## AVIAN FLU IN HUMANS

Virus	H5N1	H7N9	H9N2
Host & sialic acid			
$\alpha$ -2,3-Gal	Mild Moderate	Mild	Mild
$\alpha$ -2,3-Gal	Severe	Mild	Mild Moderate
$\alpha$ -2,3-Gal $\alpha$ -2,6-Gal	Moderate	Mild	Mild
$\alpha$ -2,6-Gal	Severe	Moderate Severe	Mild Moderate
$\alpha$ -2,6-Gal	Severe	Severe	Mild Moderate



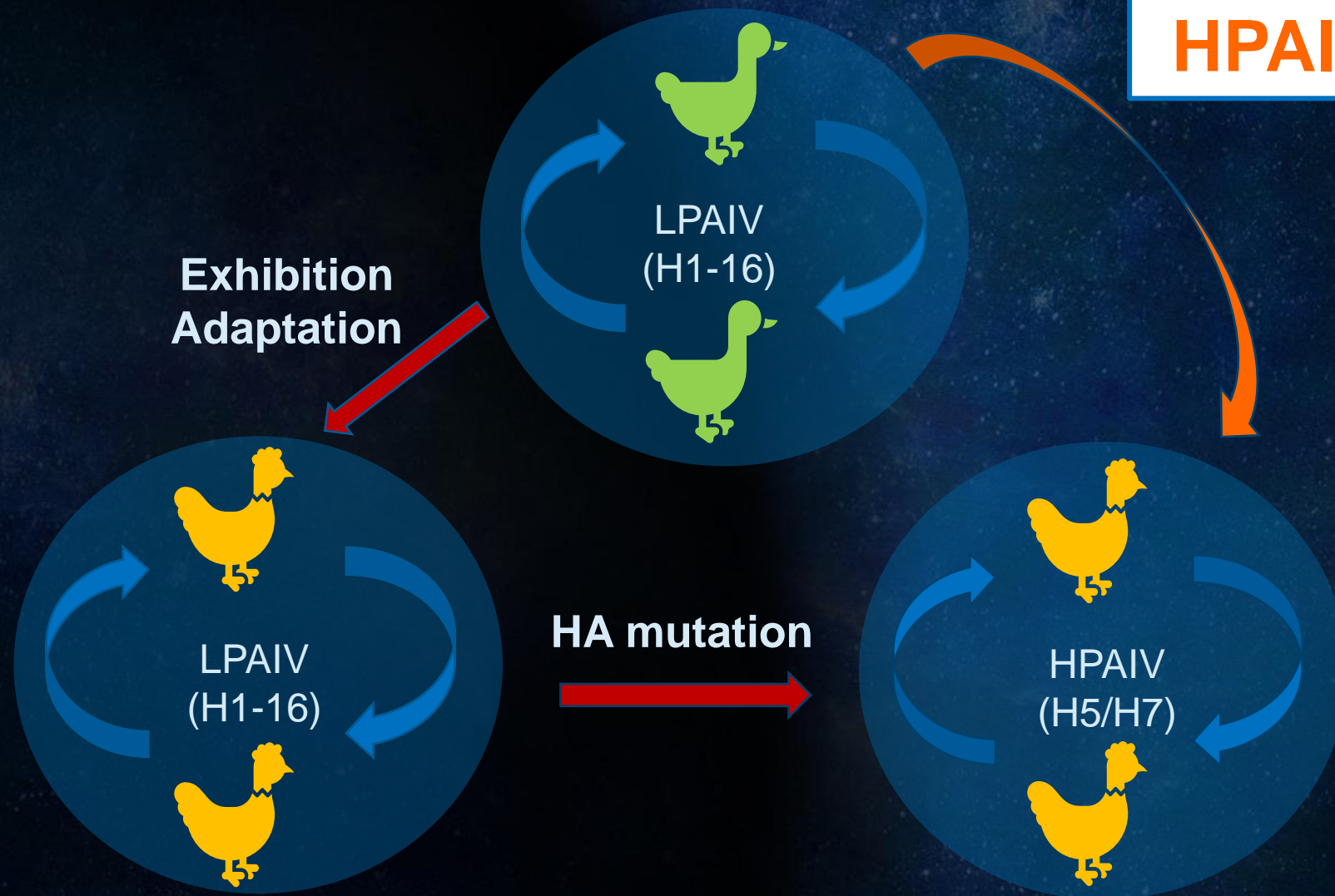




# Epidemiology

## INFECTION CYCLE

HPAI H5Nx ??



# Epidemiology

## FIRST H5N1 DETECTION IN EUROPE



Laying hens

$10^{6,5}$  ELD50  
H7N7 (O/SP)

26% mortality  
10 dpi

$0^{6,5}$  ELD50  
H5N1 (O/SP)

100% mortality  
2 dpi



Barbarie duck

$10^{6,5}$  ELD50  
H5N1 (O/SP)  
6 weeks

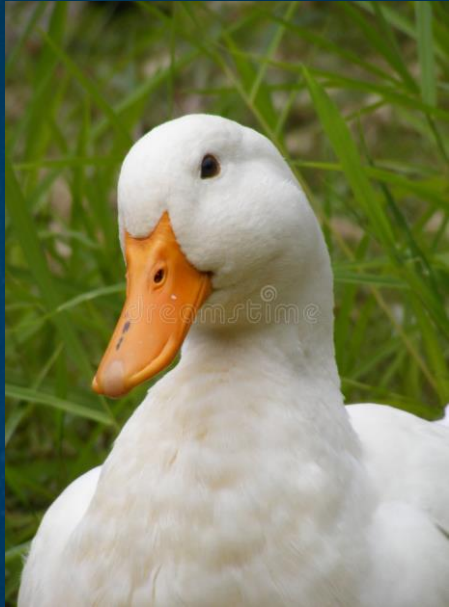
100% mortality  
18 dpi

$10^{6,5}$  ELD50  
H5N1 (O/SP)  
18 weeks

Nervous signs  
10dpi  
0% mortality  
18 dpi

# Epidemiology

## INITIAL INTRODUCTION INTO AI-FREE COUNTRY



Migratory  
waterfowl and  
other wild birds



Poultry



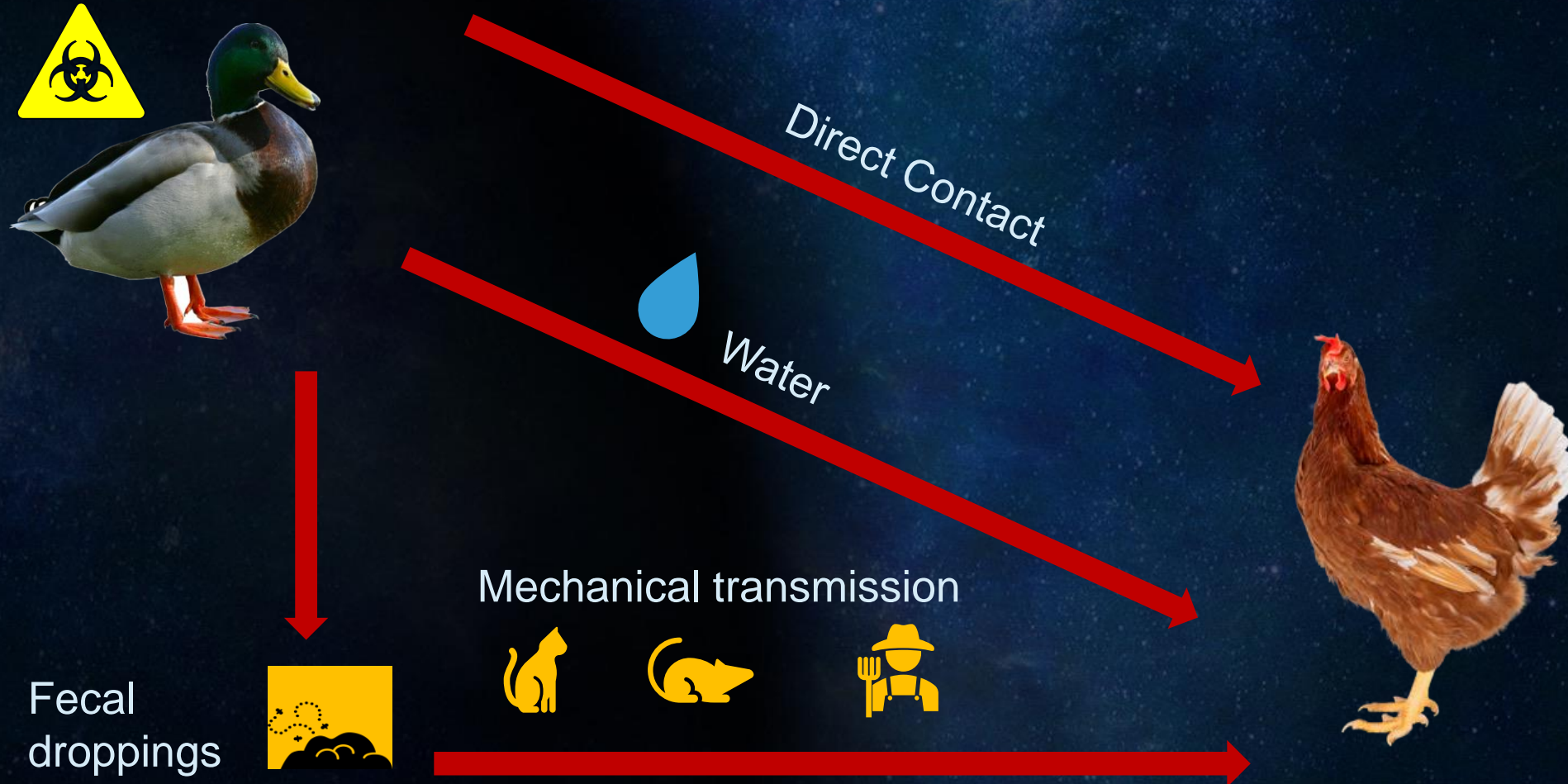
Companion  
or pet birds



Domestic pigs

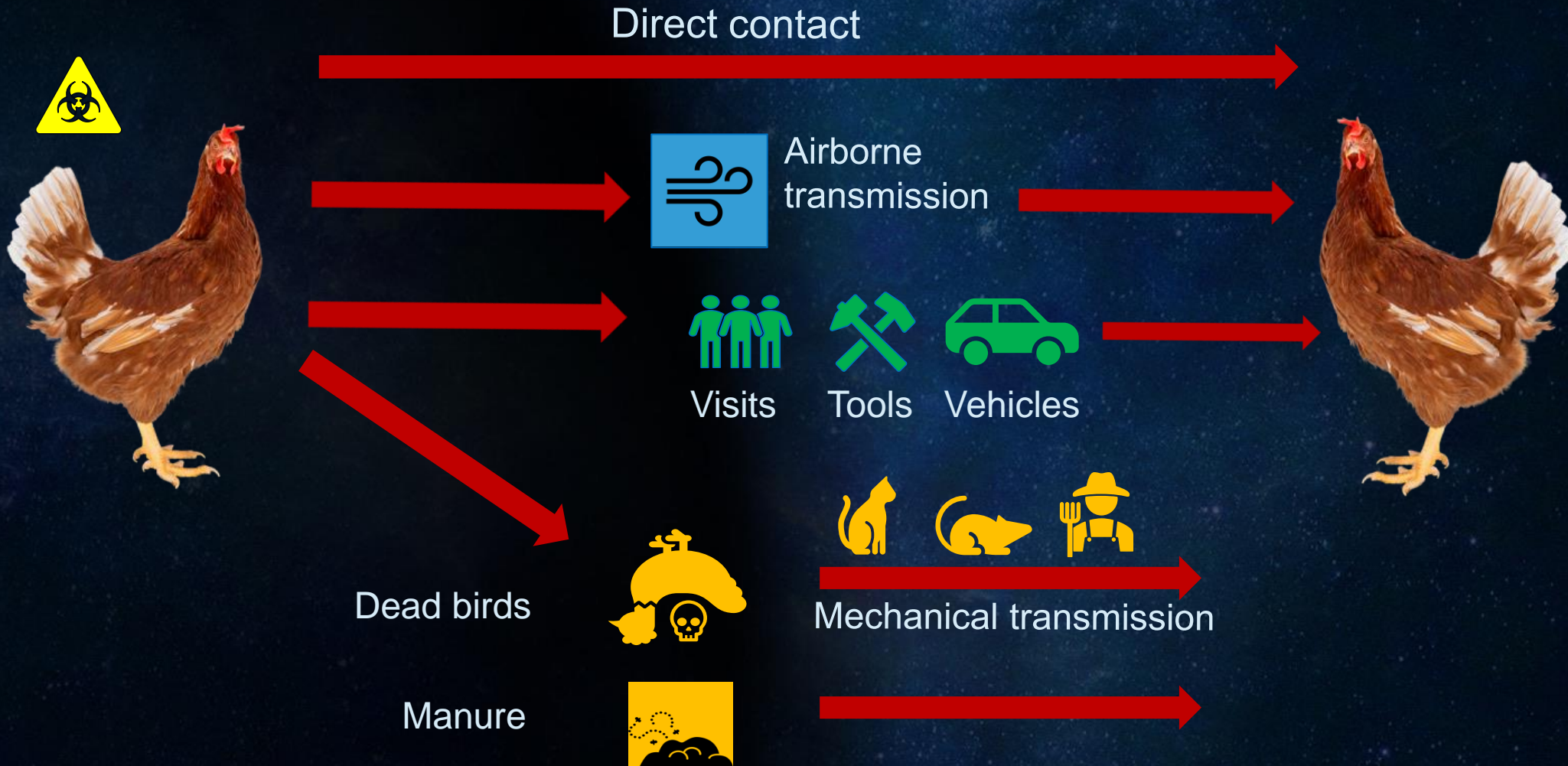
# Epidemiology

## TRANSMISSION FROM MIGRATORY BIRDS



# Epidemiology

## TRANSMISSION FROM INFECTED POULTRY



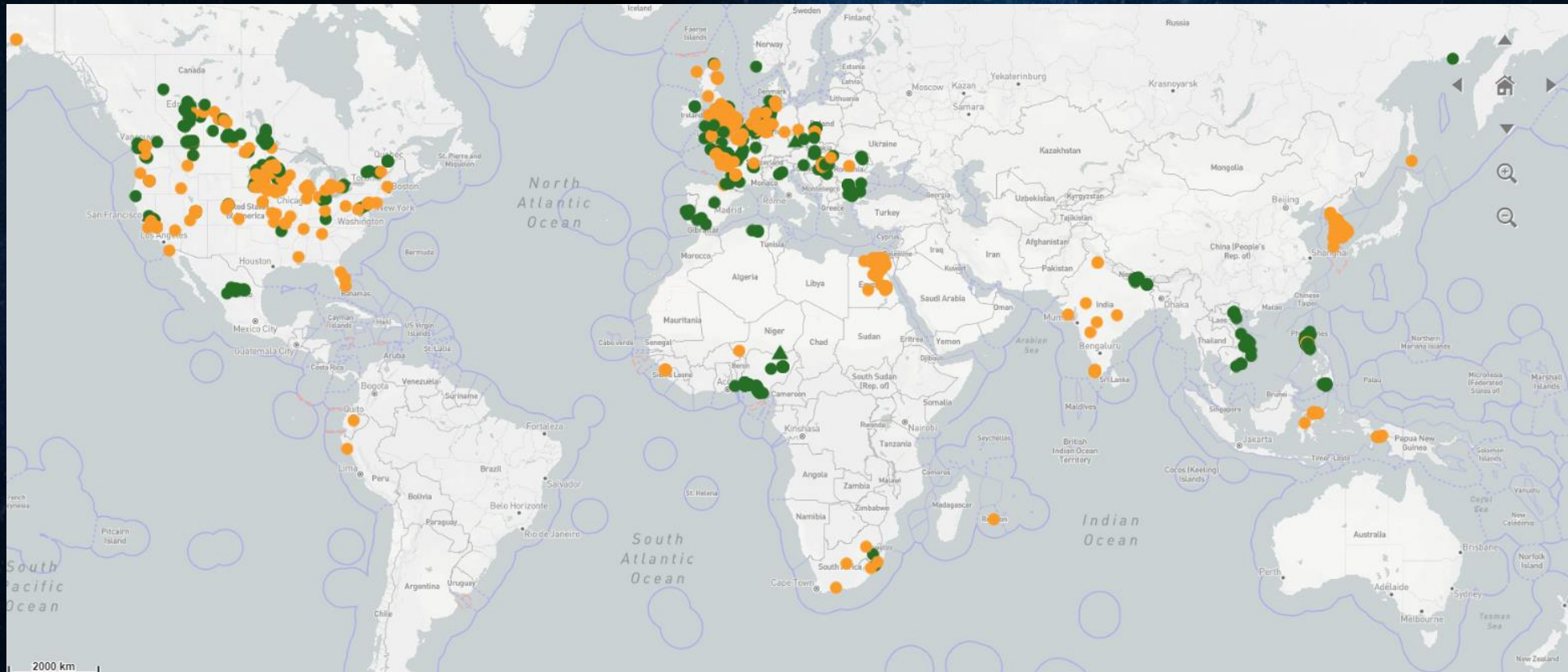
# Epidemiology

## HPAI POSITIVE COUNTRIES



# Epidemiology

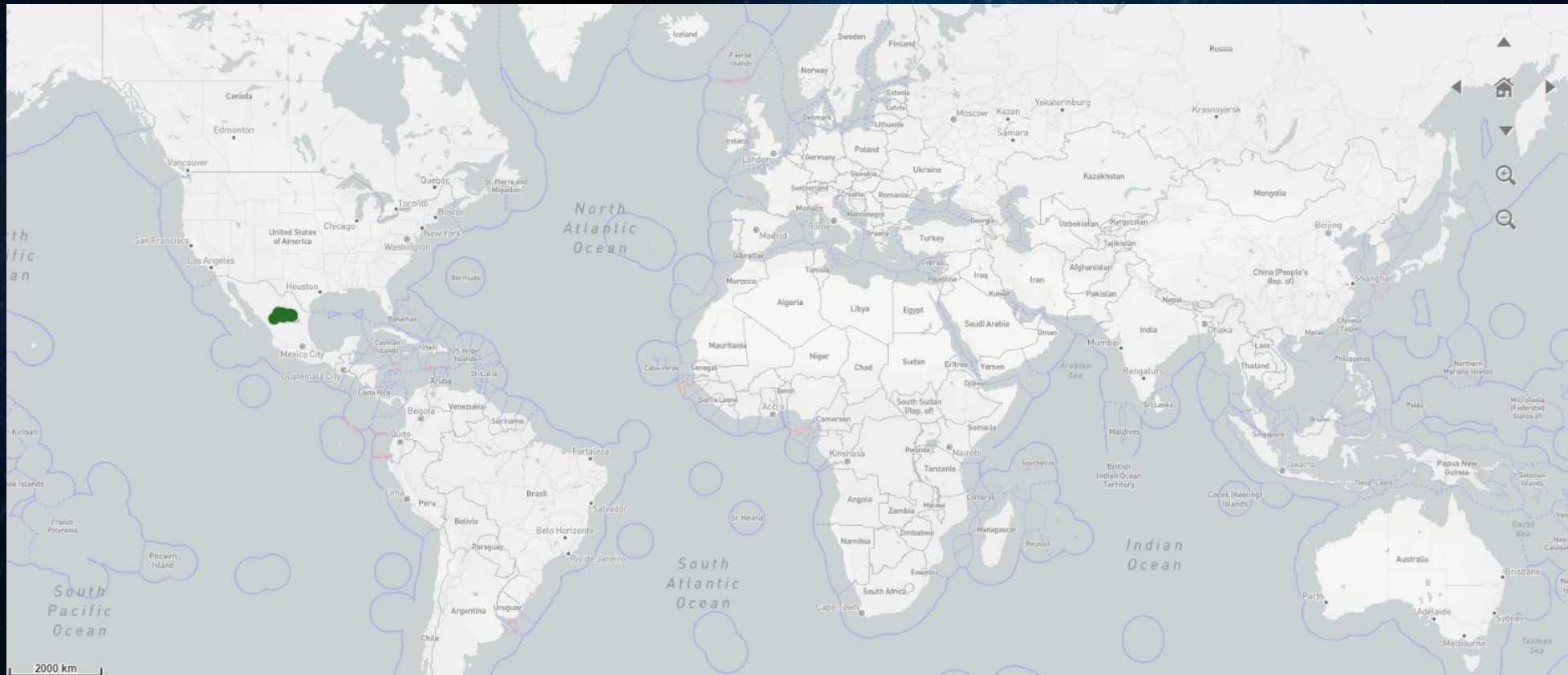
## HPAI CASES IN POULTRY BY 2022





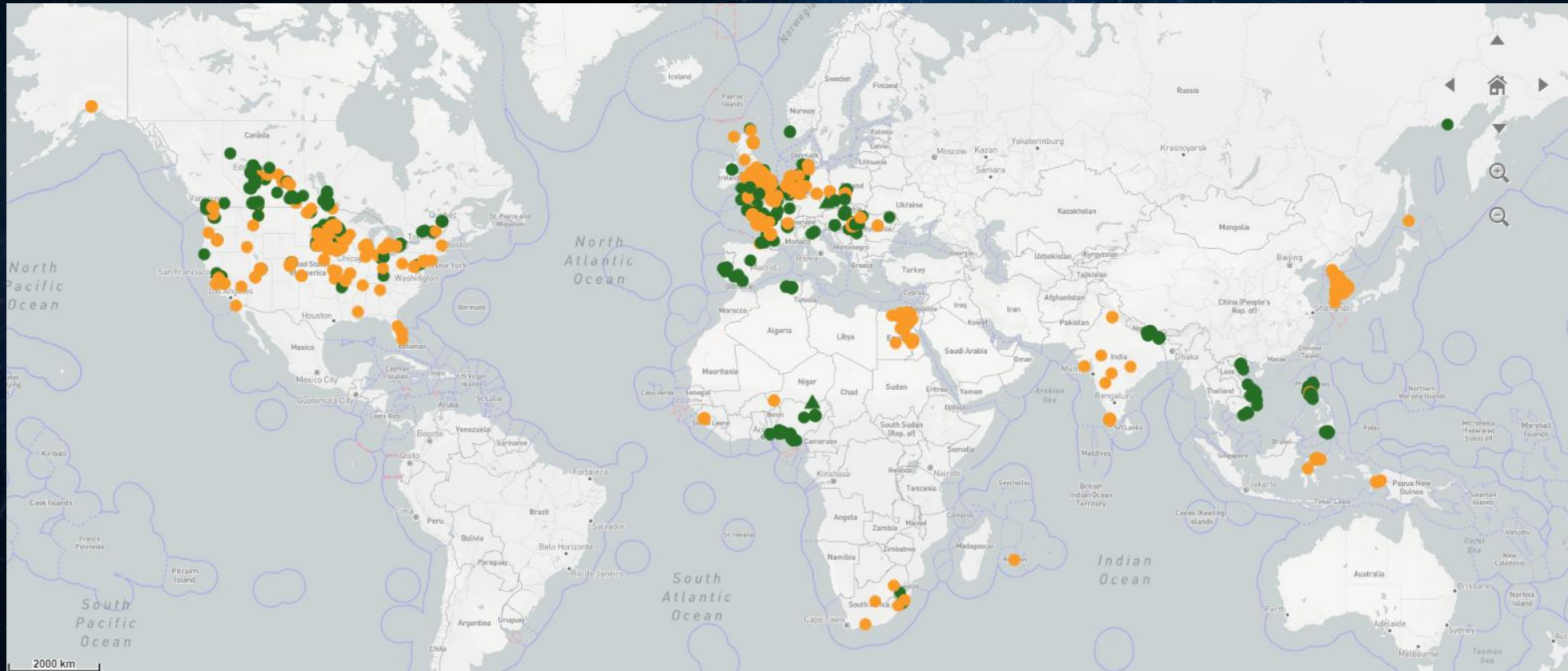
# Epidemiology

## HPAI H7 OUTBREAKS IN POULTRY IN 2022



# Epidemiology

## HPAI H5 OUTBREAKS IN POULTRY IN 2022



# Epidemiology

## 2.3.4.4b H5N1 HPAIV



Article

### Epidemiological Features of the Highly Pathogenic Avian Influenza Virus H5N1 in a Densely Populated Area of Lombardy (Italy) during the Epidemic Season 2021–2022

Silvia Bellini <sup>1,\*</sup>, Alessandra Scaburri <sup>1</sup>, Erika Molica Colella <sup>1</sup>, Monica Pierangela Cerioli <sup>1</sup>, Veronica Cappa <sup>1</sup>, Stefania Calò <sup>1</sup>, Marco Tironi <sup>1</sup>, Mario Chiari <sup>2</sup>, Claudia Nassuato <sup>2</sup>, Ana Moreno <sup>1</sup>, Marco Farioli <sup>2</sup> and Giuseppe Merialdi <sup>1</sup>



Communication

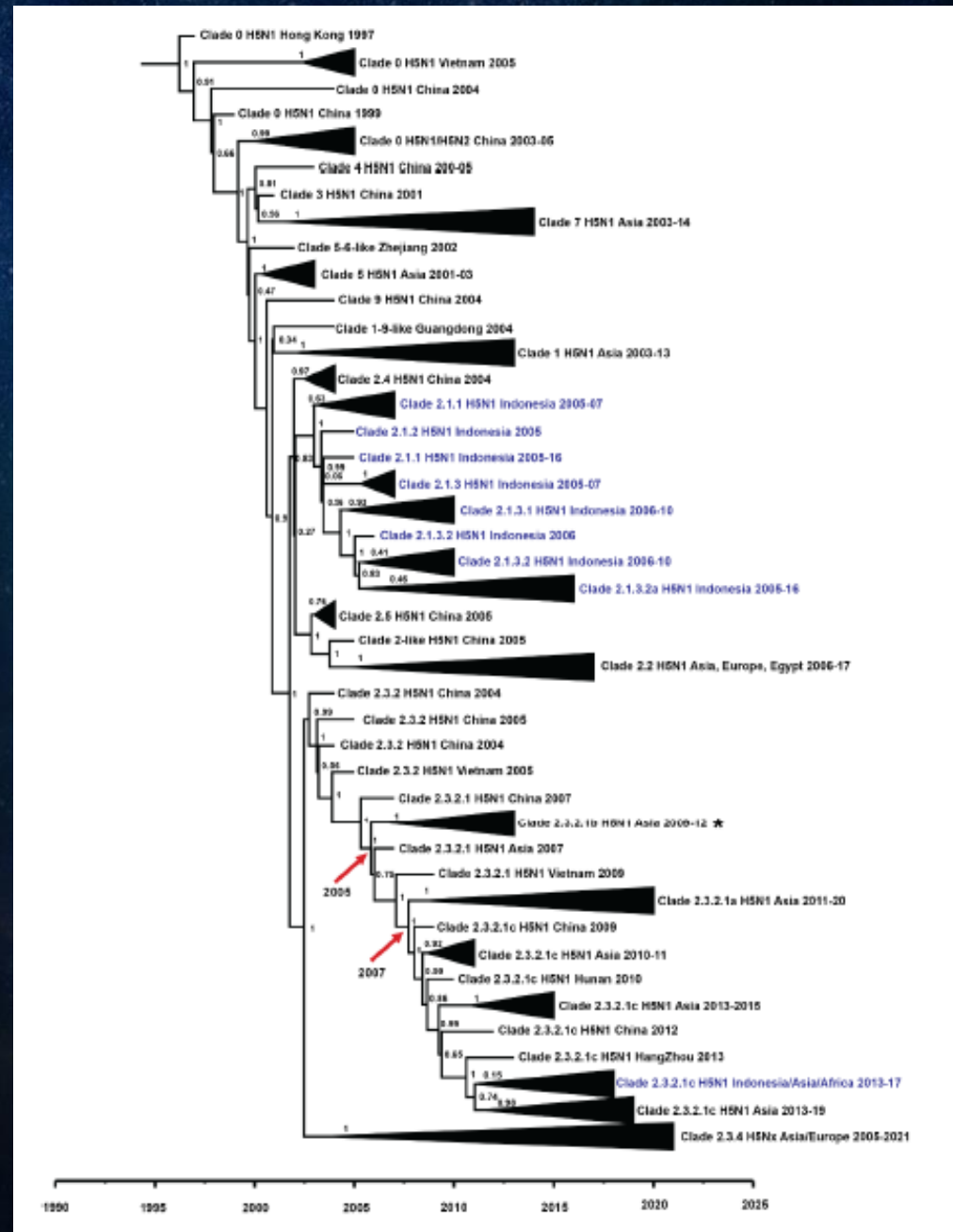
### Detection of New H5N1 High Pathogenicity Avian Influenza Viruses in Winter 2021–2022 in the Far East, Which Are Genetically Close to Those in Europe

Norikazu Isoda <sup>1,2</sup>, Manabu Onuma <sup>3</sup>, Takahiro Hiono <sup>1,2</sup>, Ivan Sobolev <sup>4</sup>, Hew Yik Lim <sup>1</sup>, Kei Nabeshima <sup>3</sup>, Hisako Honjyo <sup>3</sup>, Misako Yokoyama <sup>3</sup>, Alexander Shestopalov <sup>4,\*</sup> and Yoshihiro Sakoda <sup>1,2,4</sup>

DISPATCHES

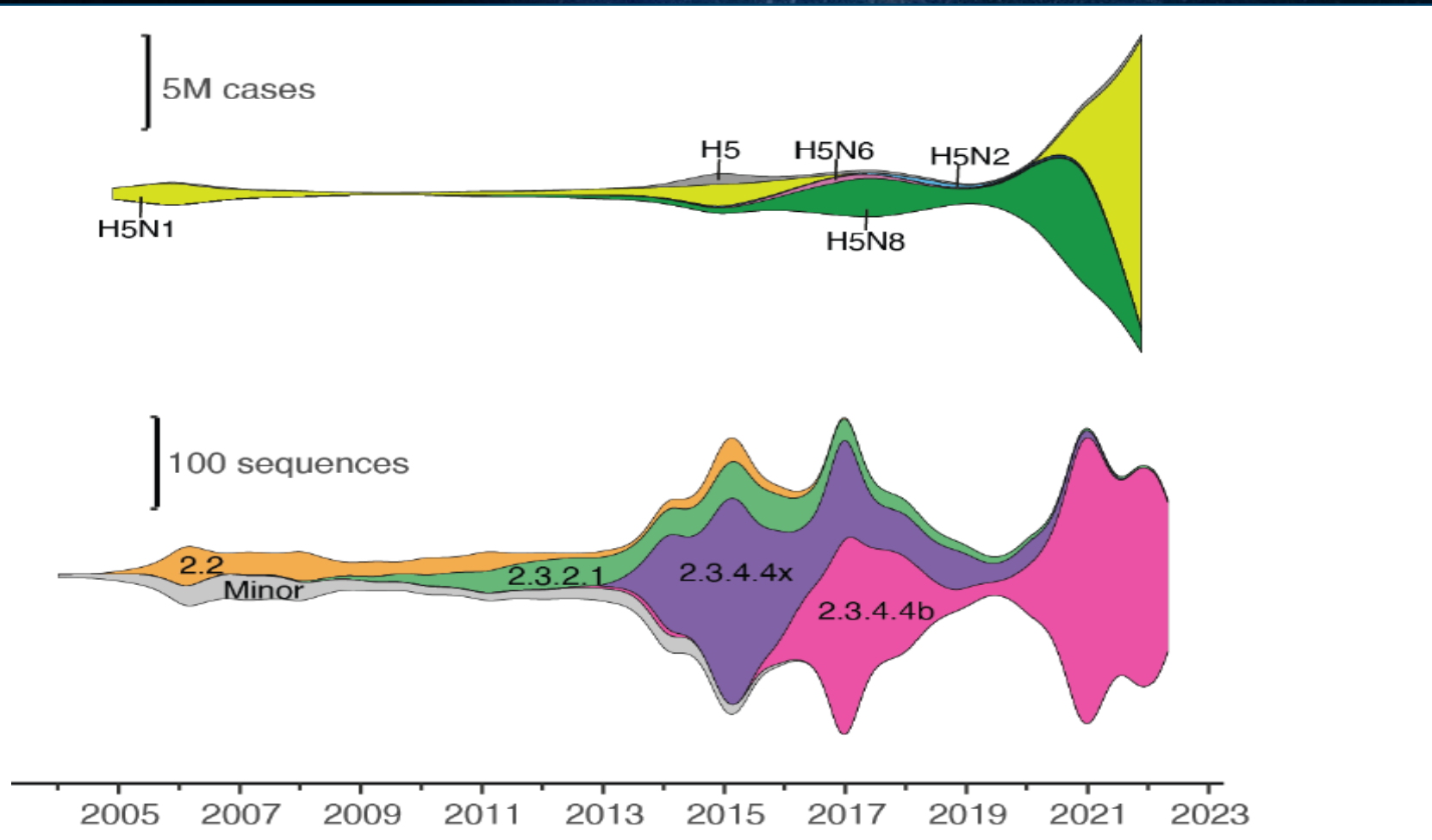
### Highly Pathogenic Avian Influenza A(H5N1) Clade 2.3.4.4b Virus in Poultry, Benin, 2021

Idrissa Nonmon Sanogo, Fidelia Djegui, Yao Akpo, Corneille Gnanvi, Gabriel Dupré, Adam Rubrum, Trushar Jeevan, Pamela McKenzie, Richard J. Webby, Mariette F. Ducatez



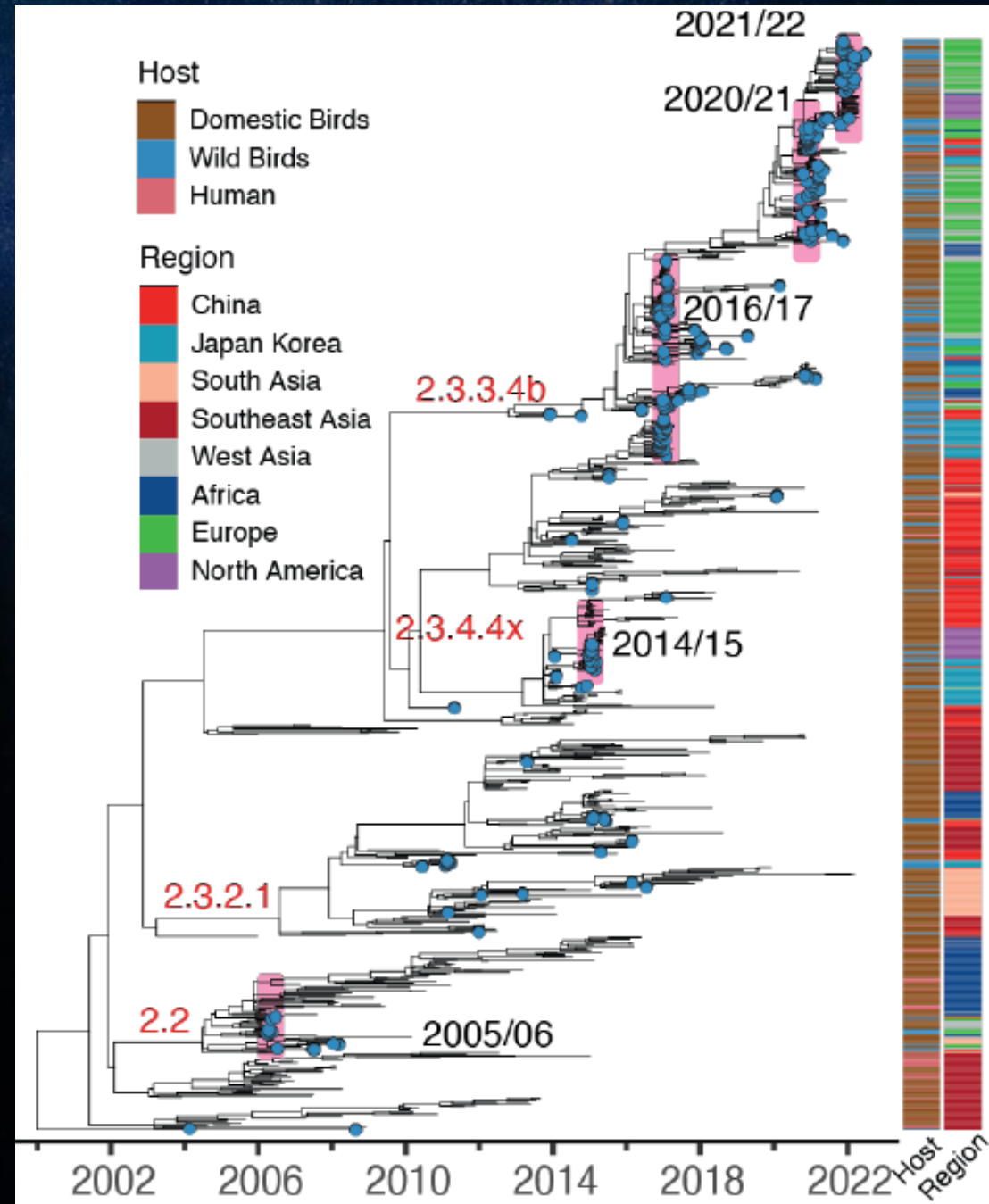
# Epidemiology

## 2.3.4.4b H5N1 HPAIV



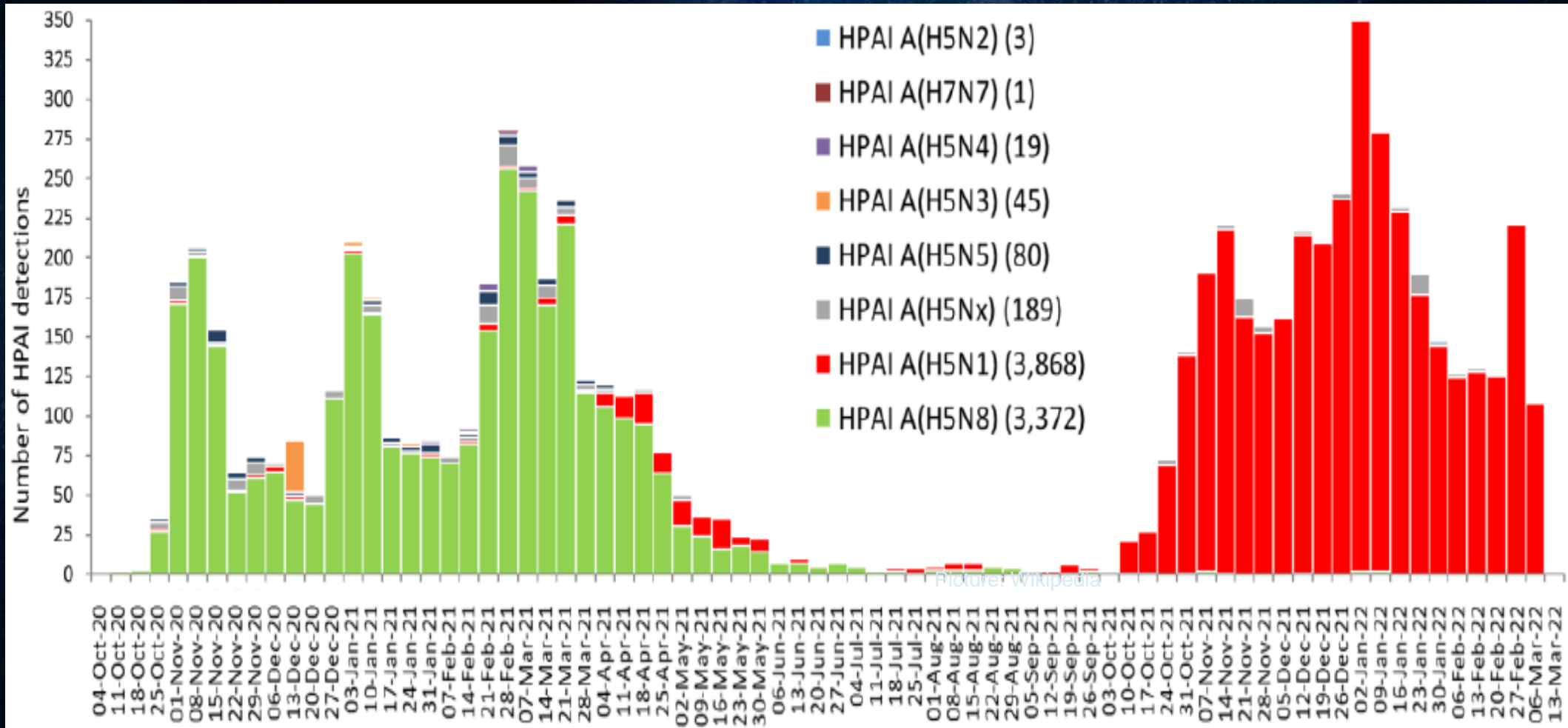
# Epidemiology

## CLADE H5Nx EVOLUTION



# Epidemiology

## EVOLUTION OF ISOLATED SUBTYPES IN EUROPE



# Epidemiology

## 2.3.4.4b H5N1 HPAIV: SILENT INFECTIONS

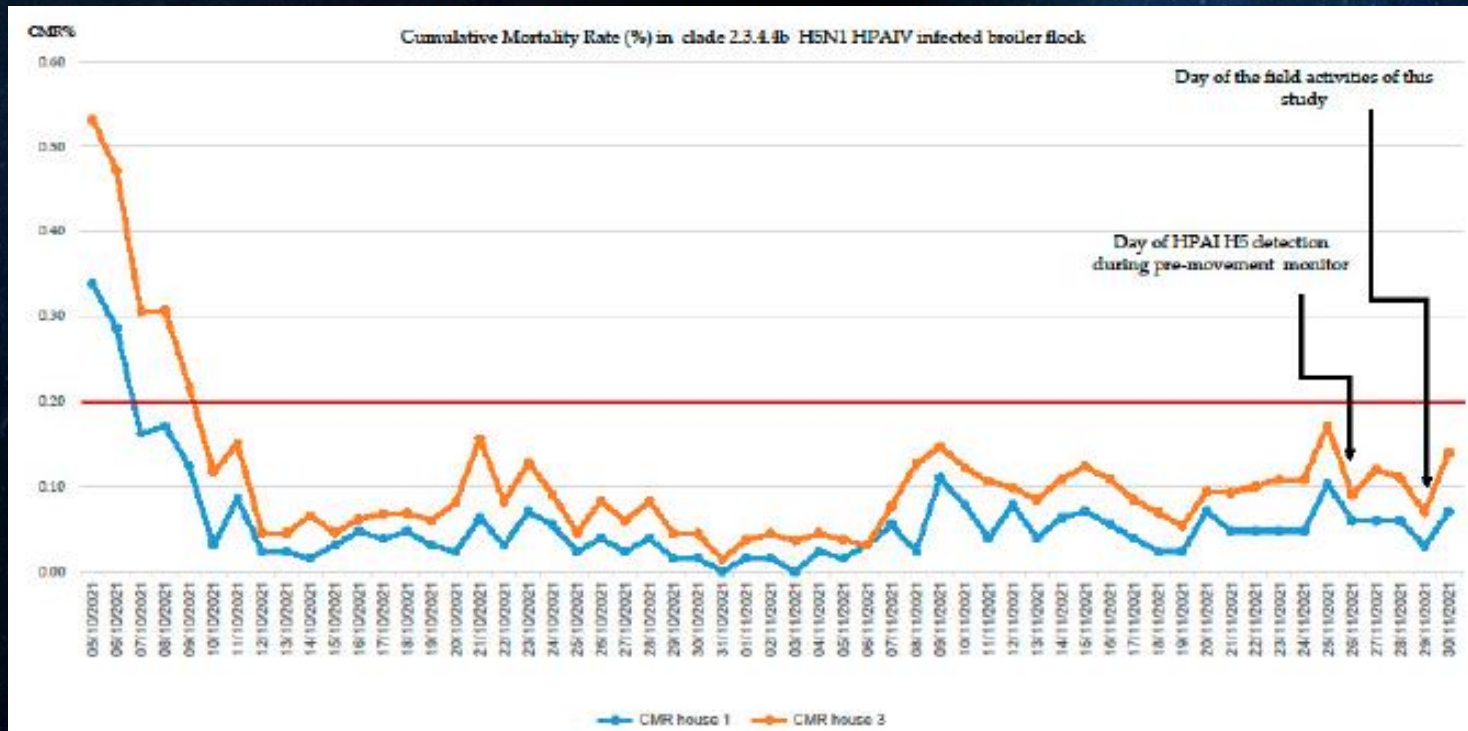
**House 1** Pool PCR 3/6  
**House 2** Pool PCR 0/6

**House 1**

ELISA 0/35  
Cloacal PCR 7/60  
Tracheal PCR 10/60  
Organs PCR 5/5

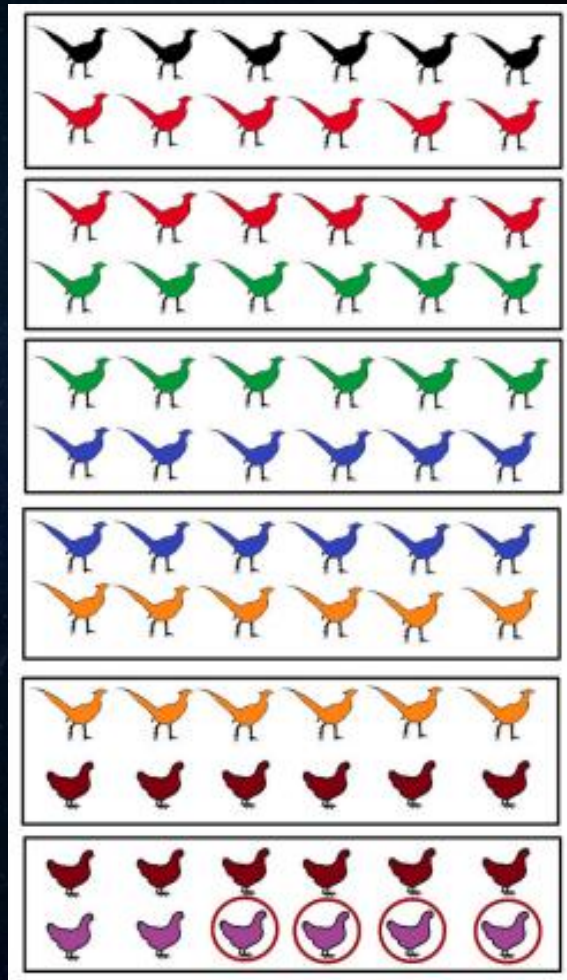
**House 2**

ELISA 0/35  
Cloacal PCR 0/60  
Tracheal PCR 0/60  
Organs PCR 1/5

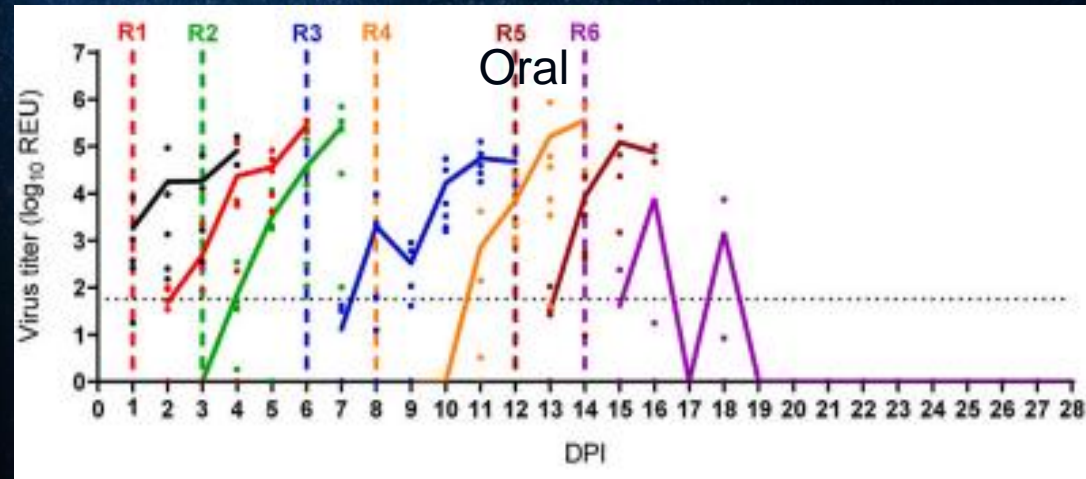


# Epidemiology

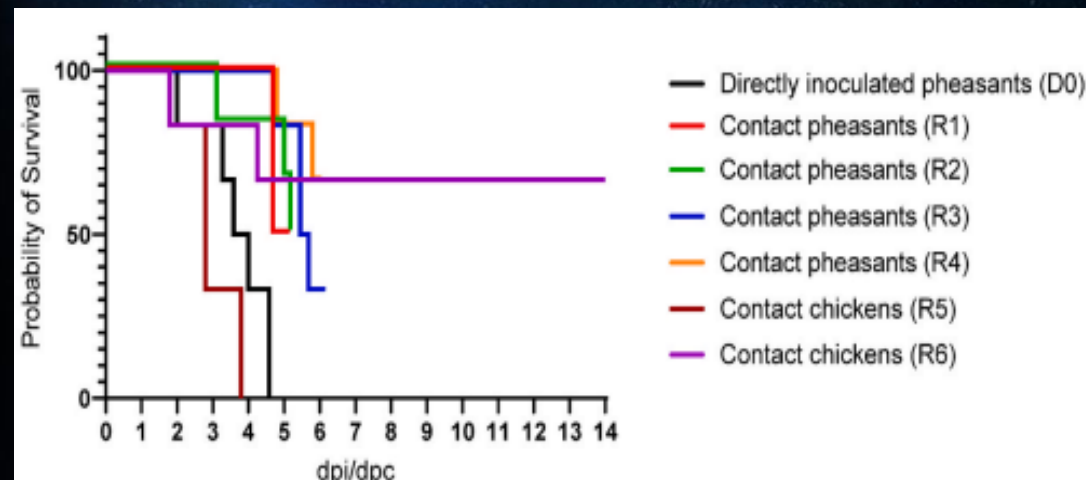
## 2.3.4.4b H5N1 HPAIV: INFECTIONS IN PHEASANTS AND CHICKENS



Viral excretion



Mortality





# Epidemiology

## NEWLY INFECTED SPECIES?

> *Vet Microbiol.* 2022 Jul;270:109461. doi: 10.1016/j.vetmic.2022.109461. Epub 2022 May 13.

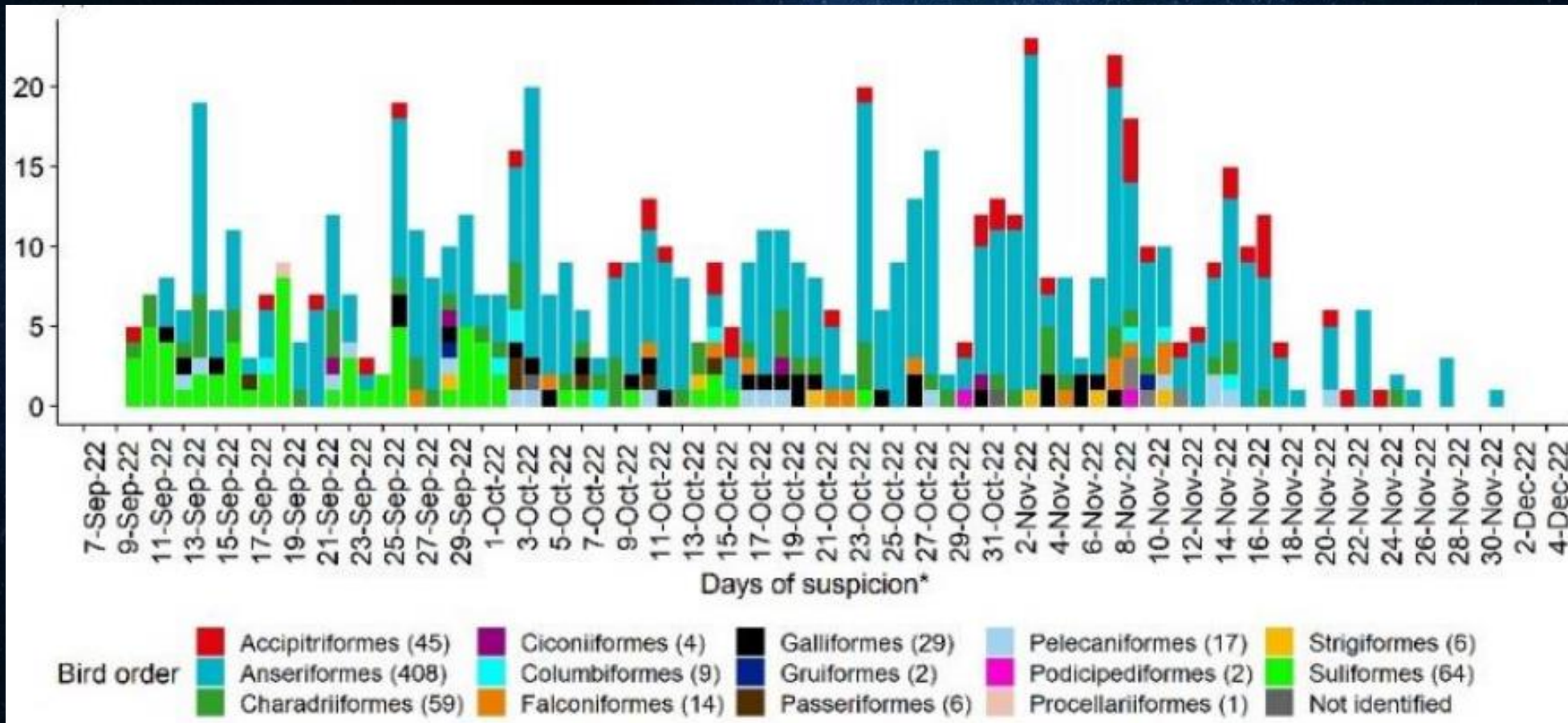
### **Genuine lethal infection of a wood pigeon (*Columba palumbus*) with high pathogenicity avian influenza H5N1, clade 2.3.4.4b, in Germany, 2022**

Martin Peters <sup>1</sup>, Jacqueline King <sup>2</sup>, Peter Wohlsein <sup>3</sup>, Christian Grund <sup>2</sup>, Timm Harder <sup>4</sup>



# Epidemiology

## HPAI-ISOLATED BIRD FAMILIES IN EUROPE 2022

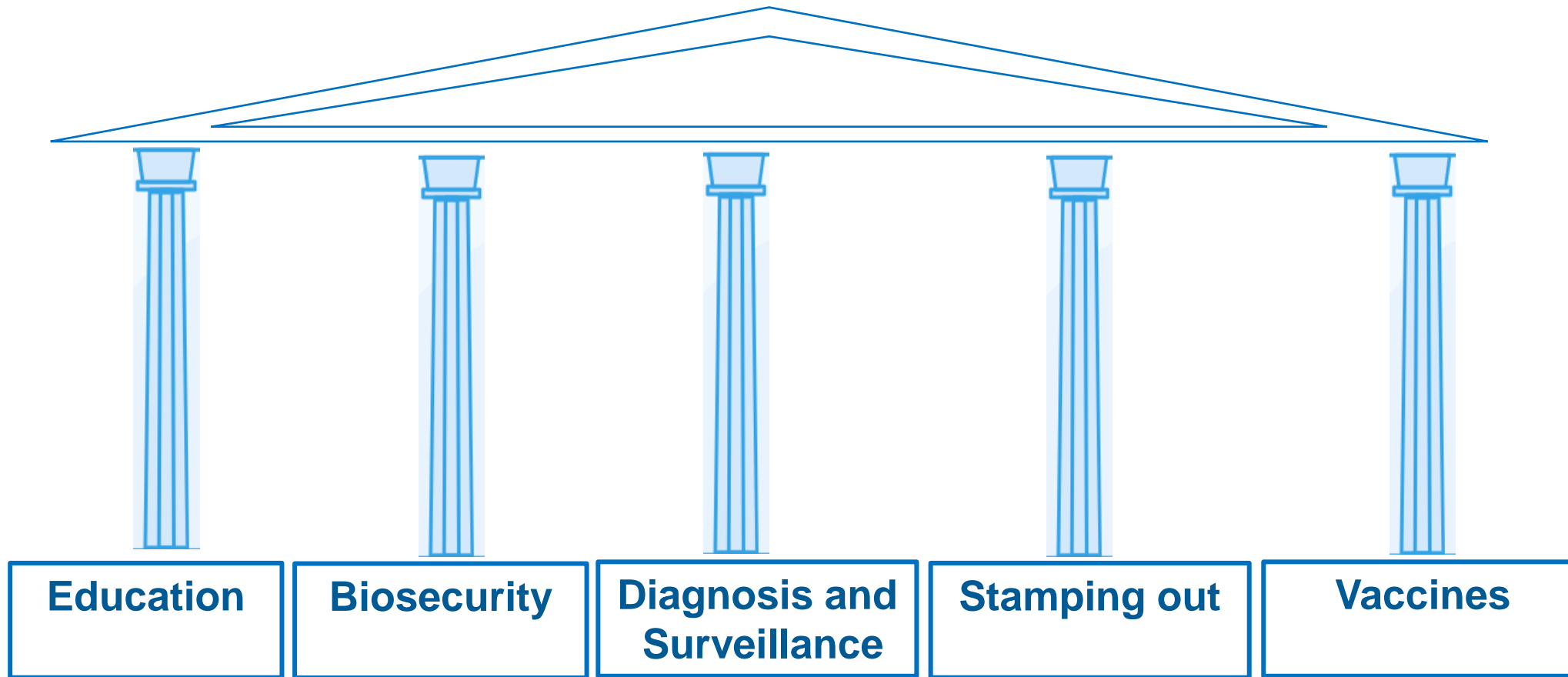


# AND ... WHAT DO WE DO NOW?

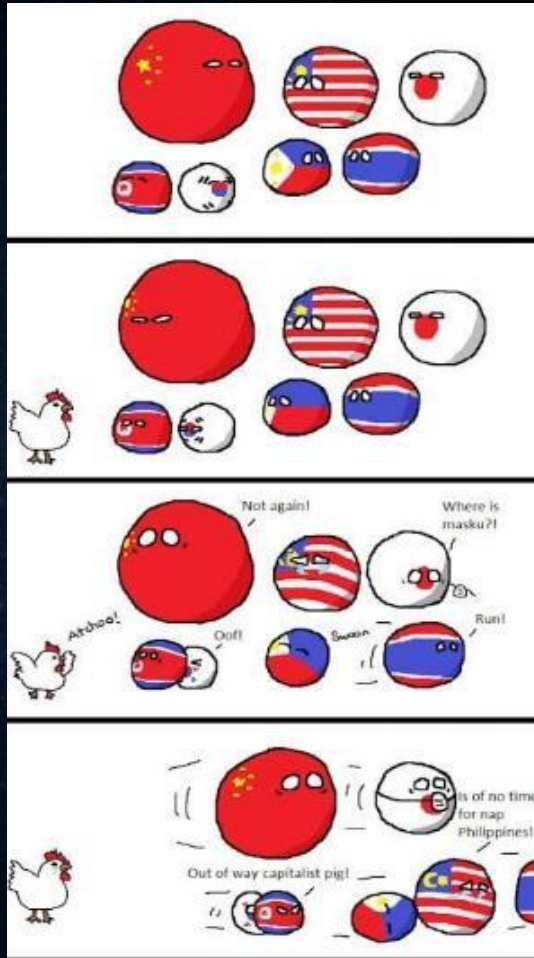


RESISTANCE  
IS FUTILE,  
MY YOUNG  
DUCKLING

# Avian influenza control programmes



# Education



Polandball -Gesundheit

Or



Hongkong Government

# Education

*The key to your profit*

**ENG**

Technical Tip

## Biosecurity in times of bird flu

**B**ird flu or avian influenza is undoubtedly one of the most problematic bird diseases for the egg production sector today. Given this context, biosecurity programmes are proving to play a fundamental role in preventing the disease from entering different countries as well as the appearance of secondary outbreaks.

This article briefly explains the general characteristics of biosecurity programmes and how bird flu spreads, then gives a list of biosecurity steps of special importance in periods when there is a risk of bird flu cases appearing.

### Biosecurity programmes in poultry farms

Biosecurity plays a crucial role in controlling bird flu (and practically any avian disease). In disease-free areas, it is the main tool to prevent the disease from entering farms in the territory. However, the other pillars of the programme are also essential, since they complement the biosecurity and work in synergy with it. For biosecurity programmes to have a real impact on the birds' health, they must have a series of characteristics:

**1**

They must be part of the companies' organisational culture. Biosecurity is not simply about taking isolated measures on some farms, but rather about the company itself working in a way that minimises the risk of diseases entering and spreading. This implies a lot of changes in terms of facilities, procedures, logistics, staff training, etc.

**2**

They have to be introduced and work in the long term. It is very difficult to raise real biosecurity levels in the short term if there is no background work behind it, and the farmer, facilities, the staff and the company as a whole will not be prepared for a moment of great pressure such as a bird flu outbreak.

**3**

Biosecurity programmes must be all-encompassing. In other words, they must cover all the risks of diseases entering or spreading on farms. Biosecurity can be divided into three categories: location, facilities and operations. We can then identify different programmes to control specific risks of diseases entering (visits, pest control, water and feed, replacement of birds, removal of by-products, staff training, L&D protocol, etc.). They all work together, but the programme is only as strong as its weakest link.

Of course, for certain diseases, there will be specific parts of the biosecurity programme that may be more important than others. This will depend mainly on each particular disease's mode of transmission.

### Understanding how bird flu is transmitted

Adapted from Wahlgren 2011

The avian influenza virus (AIV) or bird flu is highly diffusible and infectious. It can infect most known families of birds, which includes Anseriformes (ducks, geese and swans), Charadriiformes (gulls), Ciconiiformes (herons), Columbiformes (pigeons), Falconiformes (birds of prey) and Galliformes (partridges and pheasants) and more.

However, different strains show varying degrees of adaptation to different host species. Transmission between different bird species also can occur, especially between closely related ones. Furthermore, direct transmission to mammals, though less common, has also been documented.

### Adapting biosecurity in periods of high bird flu risk:

Biosecurity programmes must be set up to prevent the risk of the disease entering before periods of high bird flu risk begin. In any case, during such periods there are some especially critical measures that must be reinforced:

#### Risks of AIV being introduced due to migratory birds

- Avoid contact with wild birds; any direct contact with such birds and poultry must be avoided. To do so, buildings must be made "wild bird proof". This means:
  - The farm's walls and roofs must be completely closed and not allow any type of wild bird to enter.
  - Windows and air inlets must be protected with bird mesh or an equivalent system. Such protection must be fitted well and not leave gaps allowing wild birds to enter.
  - The access door must remain closed when not in use, and in any case it must not give direct access to the birds' area. Doors that are not for daily use must be bolted.
  - In the case of birds in systems with access to outdoor zones, authorisation must be requested from the official veterinary services to be exempted from the obligation to use such zones where contact with wild birds is impossible to control.

**Avoid attracting wild birds to the farm:** farms must be "unfriendly" territories for wild birds. To do so:

- Prevent areas of stagnant water from appearing. The land in the surrounding areas must be levelled to prevent stagnant water building up. The possibility of using drains and guttering to drain off water from the buildings' perimeter should be considered.
- Prevent birds from nesting in farm buildings or nearby structures. It is not advisable to have trees or brushy areas of vegetation in the surrounding area.
- Ensure the silos are sealed tight so that birds cannot access the feed. Likewise, spillages of feed that may attract birds must not be allowed.
- Prevent chicken manure from building up in the areas surrounding the buildings, since it always contains undigested cereal grains, which attracts various types of birds.
- In a free range bird system, avoid having drinkers or feeders in uncovered zones in outdoor corrals.
- Remove unnecessary ledges or horizontal surfaces that may be used by wild birds to perch on. Systems such as laser cannons or other devices can also be used to scare away birds.
- Farms should not be built in the immediate vicinity of marshes, lakes or other areas where wild birds often make their migratory stops.

# Biosecurity

## BIOSECURITY LEVELS



Country level



County level

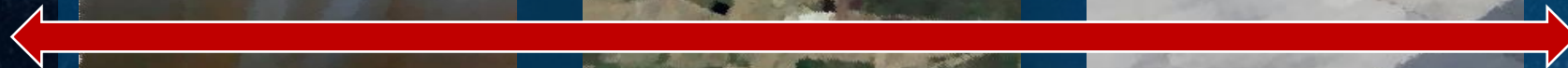


Farm level

Location

Equipment

Operation



# Biosecurity

## FARM LOCATION REALLY MATTERS



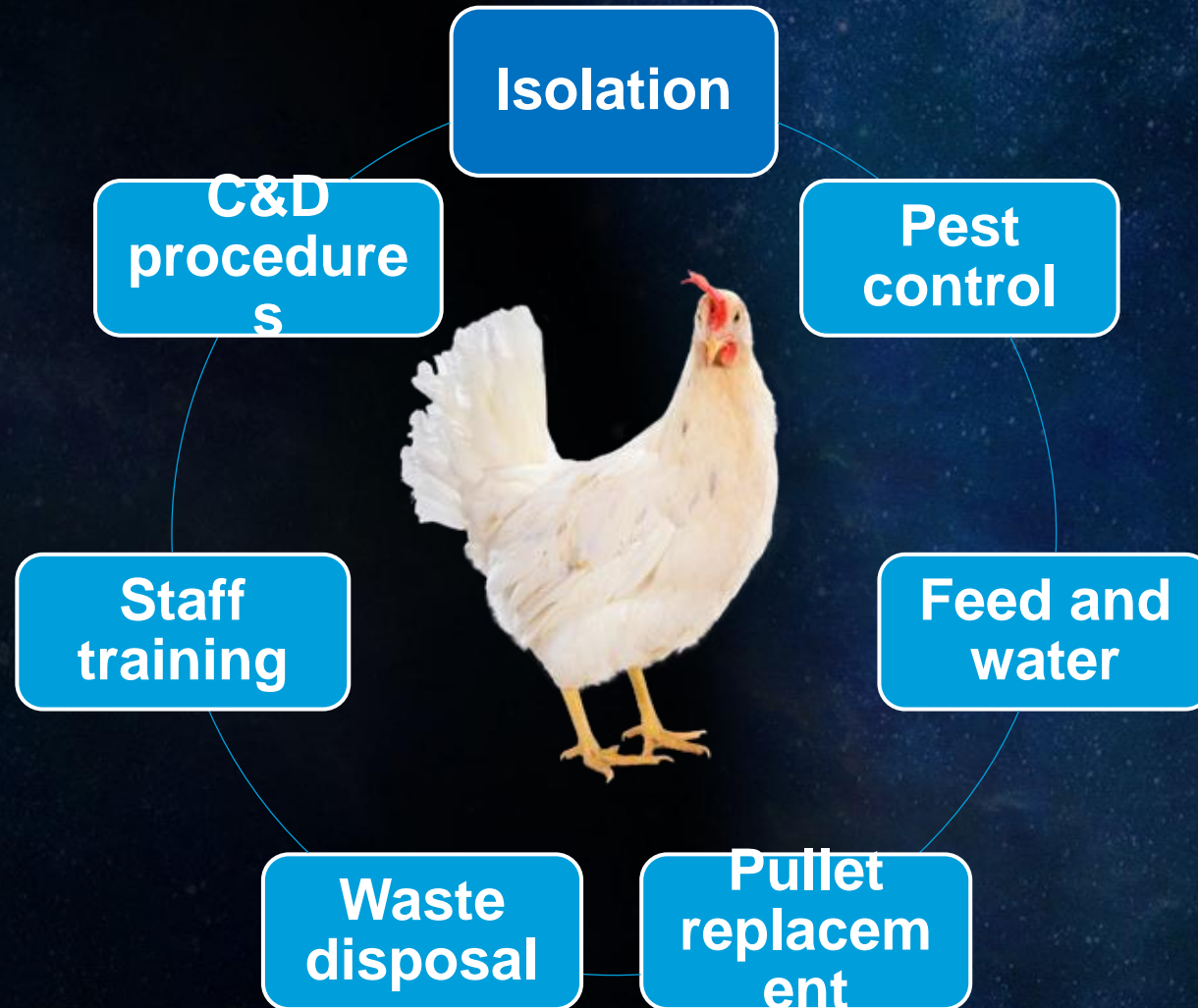
200 m

Picture: Google maps



# Biosecurity

## BIOSECURITY PROGRAMS



# Biosecurity

## VISITOR POLICY ( The easiest and best to apply )



# Biosecurity

## A DECISION TREE FOR ACCEPTING VISITORS



# Biosecurity

## THE VEHICLES ARE NOT FOR DRIVING ON THE FARM



External parking



No entrance to all avoidable vehicles



Complete disinfection for all entering vehicles

# Biosecurity

## ENTRANCE TO A FARM (IDEALLY)

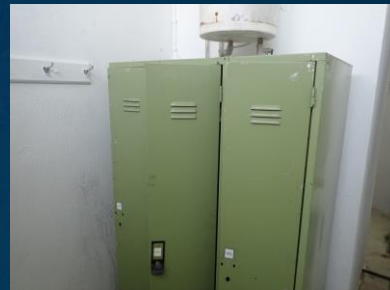


Logbook

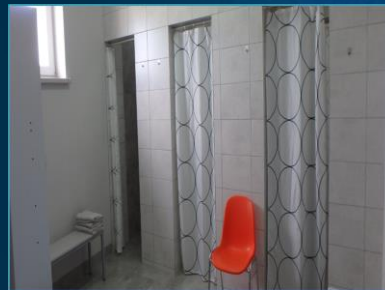
Fecha	Nombre	Empresa	Matrícula vehículo	Motivo de la visita	Última visita en granja	Autorización



Locker



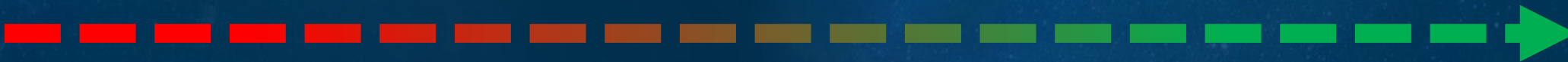
Shower



Farm Cloths



Hygiene block



# Biosecurity

## TOOLS AND PERSONAL BELONGINGS STAY OUT



Leave your personal belongings outside...



Disinfect any items before bringing them into the farm.



Stuff coming from other farms **MUST** be rejected

# Biosecurity

## NOT TO DO FOR POULTRY WORKERS



No visit to other farms.



No keeping backyard poultry at home.



No bird related hobbies.

# Biosecurity

RODENTS: WE CANNOT COEXIST!



Erysipelas

(Meerburg 2012)

Salmonellosis

(Meerburg 2012)

Fowl Cholera

(Meerburg 2012)

...

Mechanical  
transmission





# Biosecurity

## GETTING RID OF RODENTS

### Passive control



### Active control



# Biosecurity

## WILD BIRDS: THE FLYING RODENTS ( EVEN WORSE)



Poultry houses  
MUST be bird-proof



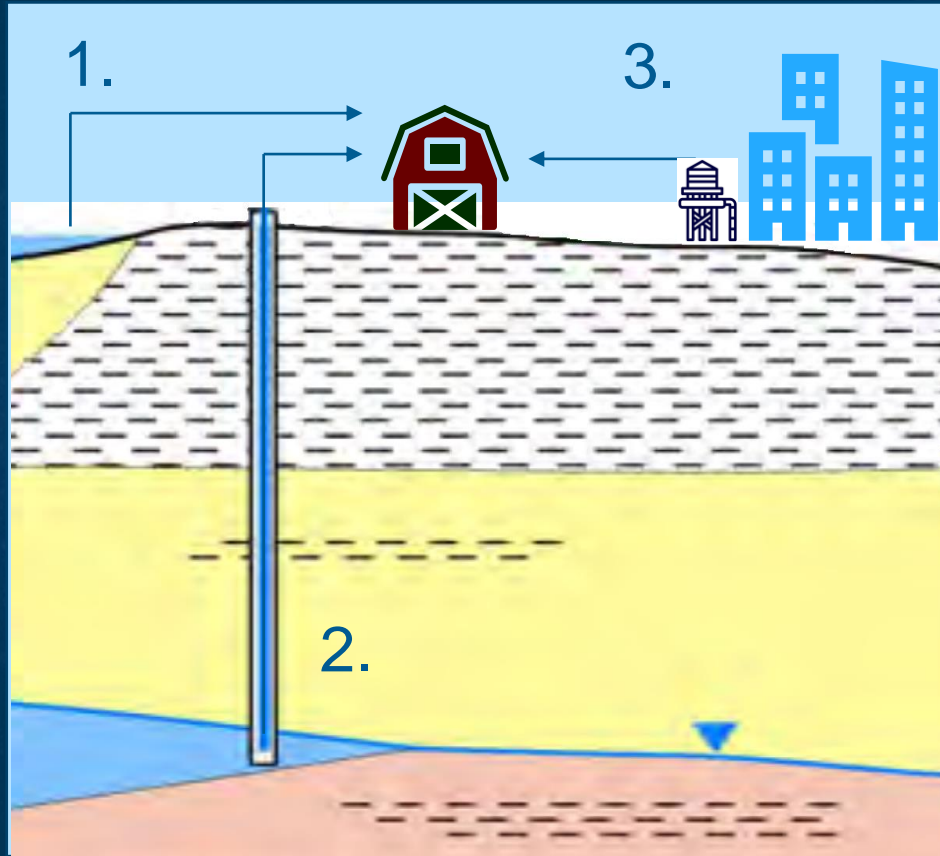
Do not attract birds  
by feed spillage or  
others



Do not allow birds to  
nest in your premises

# Biosecurity

## WATER SOURCE REALLY MATERS



1. **Surface waters**

2. **Well**

3. **Public water network**

Microbiological quality

Chemical Quality

Pre-treatment

# Biosecurity

DEAD BIRDS ARE NOT A SUB PRODUCT  
THEY ARE A BIOLOGICAL RISK

Remove all dead birds  
from the house daily



Store them in a  
correct container



Destroy them totally  
as soon as possible



# Biosecurity

MANURE IS A SUB PRODUCT  
BUT IT IS STILL A BIOLOGICAL RISK



Remove it from the house as soon as possible



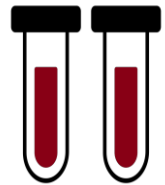
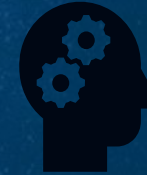
Treat the manure before spreading it on the fields.



Do not spread poultry manure around other poultry houses

# Diagnosis and surveillance

## SAMPLING FLOCKS



### Blood

@ Flock surveillance

### Serology

- ELISA
- HI ( H1 - H16)
- NI ( N1 - N9)

LPAI infections  
monitoring programs

Vaccination monitoring



### Tracheal swabs

### Cecal tonsils

### Cloacal swabs

@ diagnosis  
@ surveillance

### Virology

- SPF chicken embryos
- Tissue cultures

Case confirmation

Clade determination

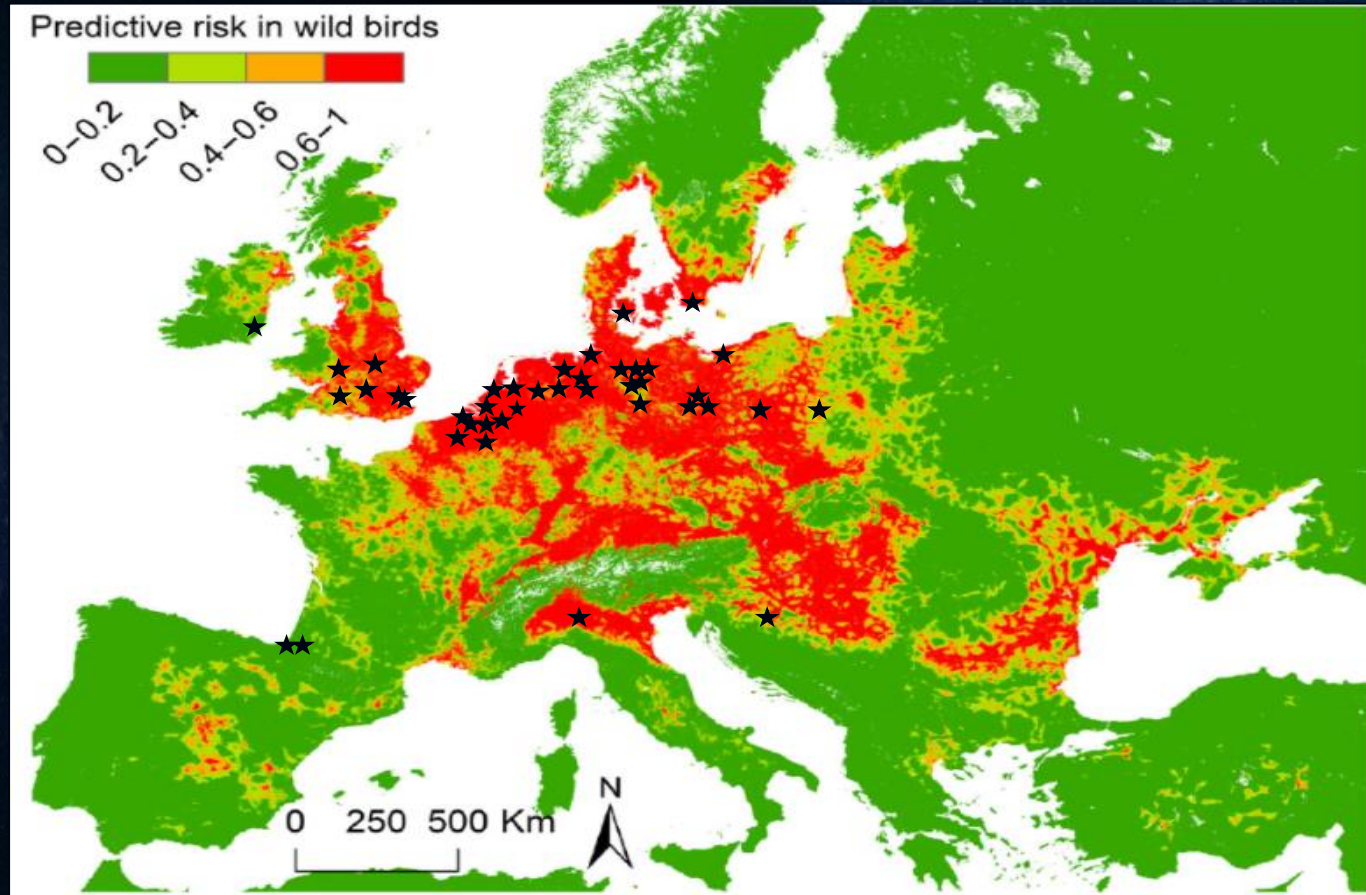
### Molecular biology

- RT- PCR
- Sequencing

Epidemiology studies

# Diagnosis and surveillance

## PREDICTIVE RISK MAPS



# Stamping out WORKING FOR BIOCONTAINMENT

## RESTRICTIONS



## SACRIFICES



## DESTRUCTION



## C&D PROCEDURE



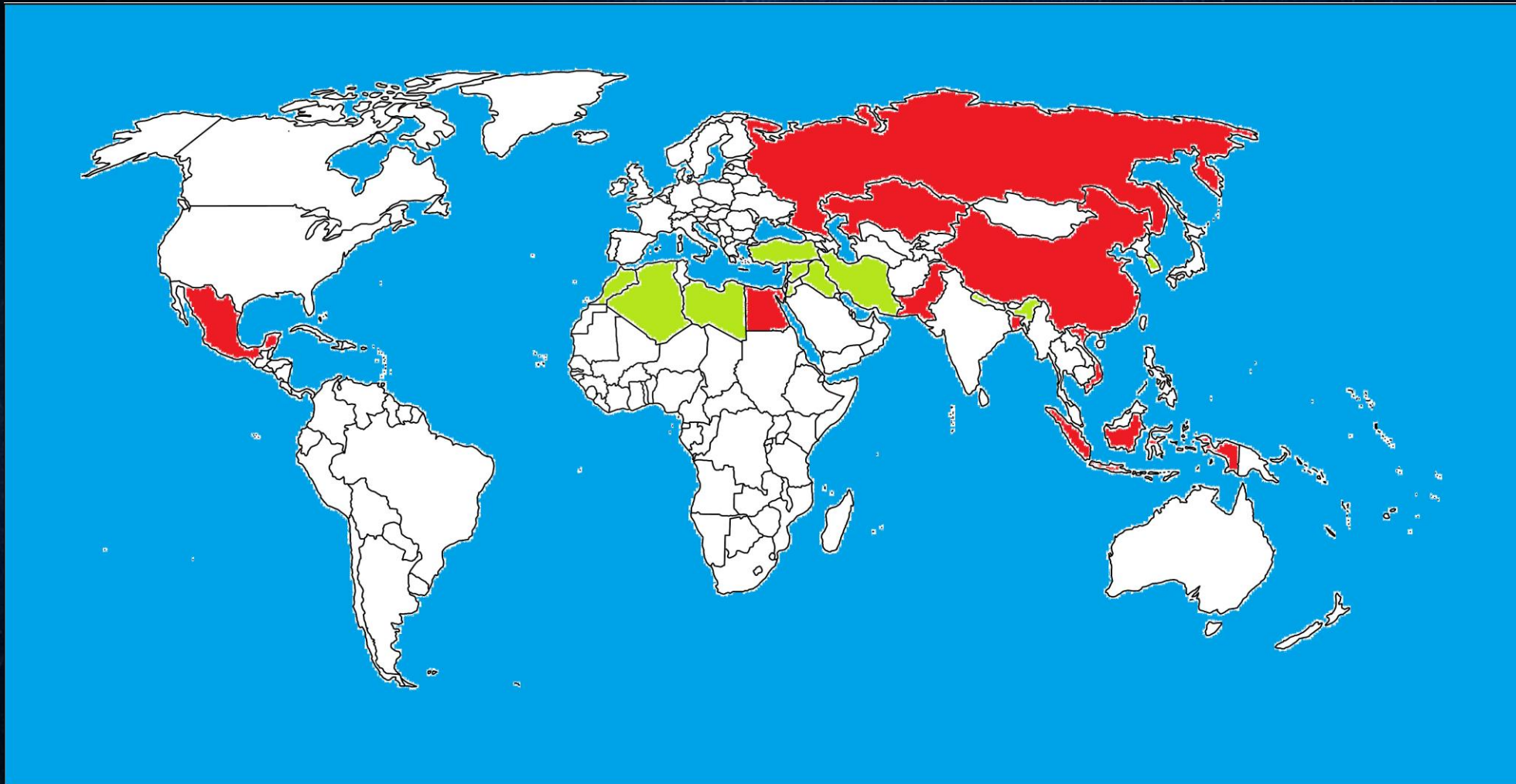
Completely prevent the spread of an exotic virus.




Time is of crucial importance. Logistics is the key point.



# Vaccination programmes



 Vaccination programme  
against HPAI

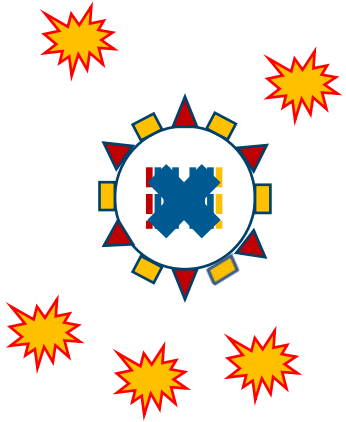
 Vaccination programme  
against LPAI H9N2

# Vaccination programmes

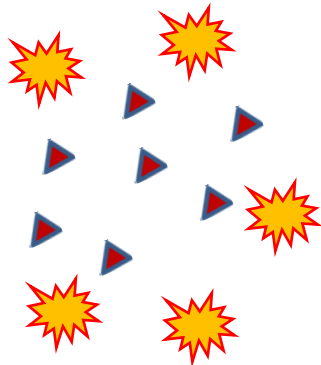
## TYPES OF AVIAN INFLUENZA VACCINES

### Inactivated vaccines

Oil emulsified  
inactivated  
Whole AIV

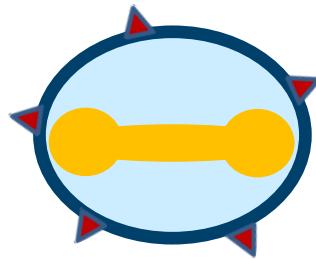


Oil emulsified  
HA protein

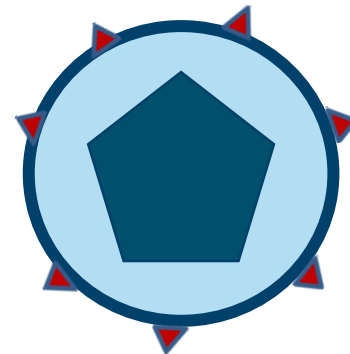


### Recombinants vaccines

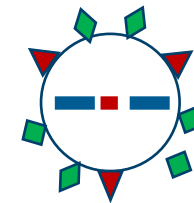
Recombinant  
fowl pox virus



Recombinant  
HVT virus



Recombinant  
ND Virus



# Vaccination programmes

## WHAT TO EXPECT FROM AI VACCINATION ?



### CAN

- Reduce replication of AIV in respiratory & GI tract
- Prevent illness and death in poultry
- Reduce transmission to birds and humans



### CAN'T

- Infection is still occurring to infection
- Interferes with monitoring programmes
- Poor protection against AIV from other serotypes/clades

# Vaccination programmes

## Vaccination programmes

Vectored HVT-AI  
@hatchery

1:32 HI: Prevents mortality  
1:128 HI: Prevents oral shedding

Revaccination if less than 80% population have protective titers



Inactivated Whole AIV @ week 1

Inactivated Whole AIV @ week 6

Inactivated Whole AIV @ week 16



Inactivated Whole AIV @ week 16

0 7 14 21 28 35 42 49 56 63 70 77 84 91 98



# Vaccination programmes

## MEXICO: H7N3 VACCINATION PROGRAMME

2014



2016

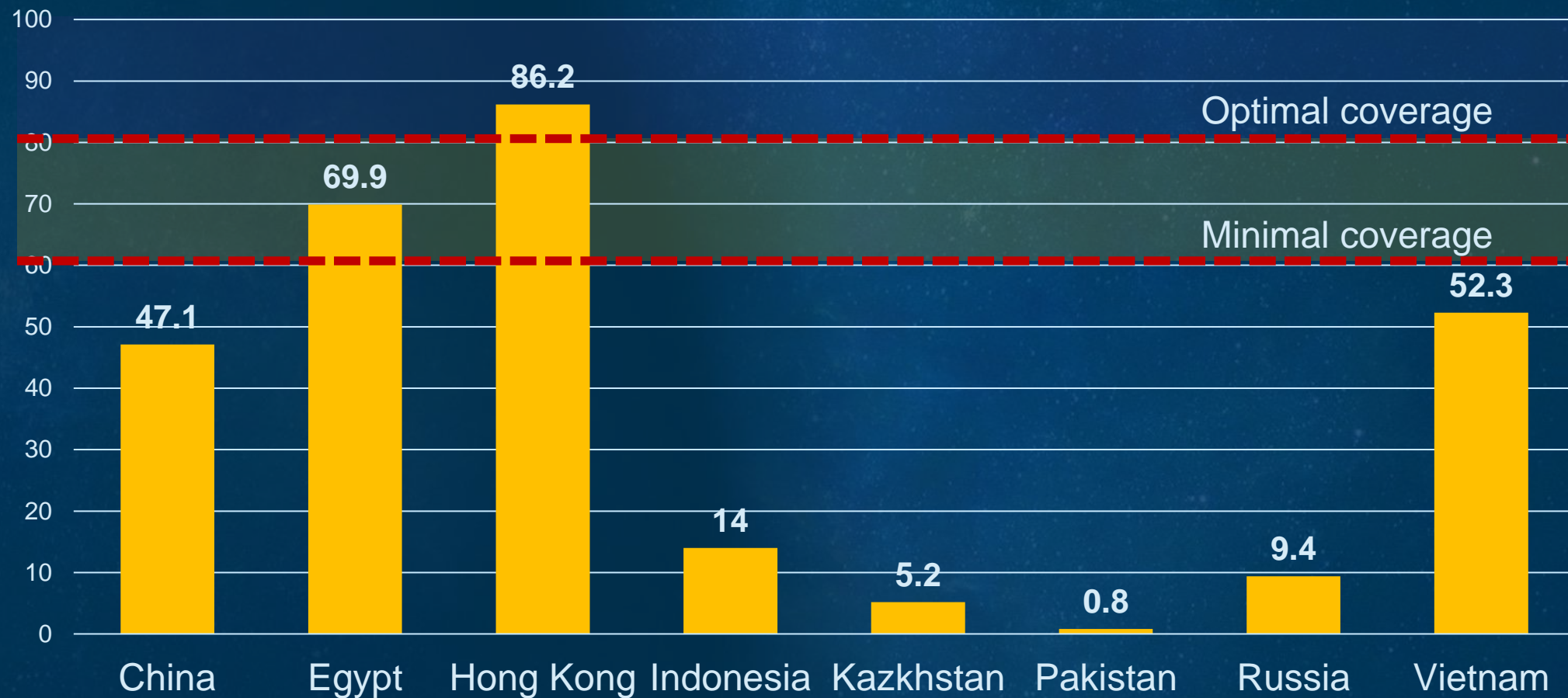


2018



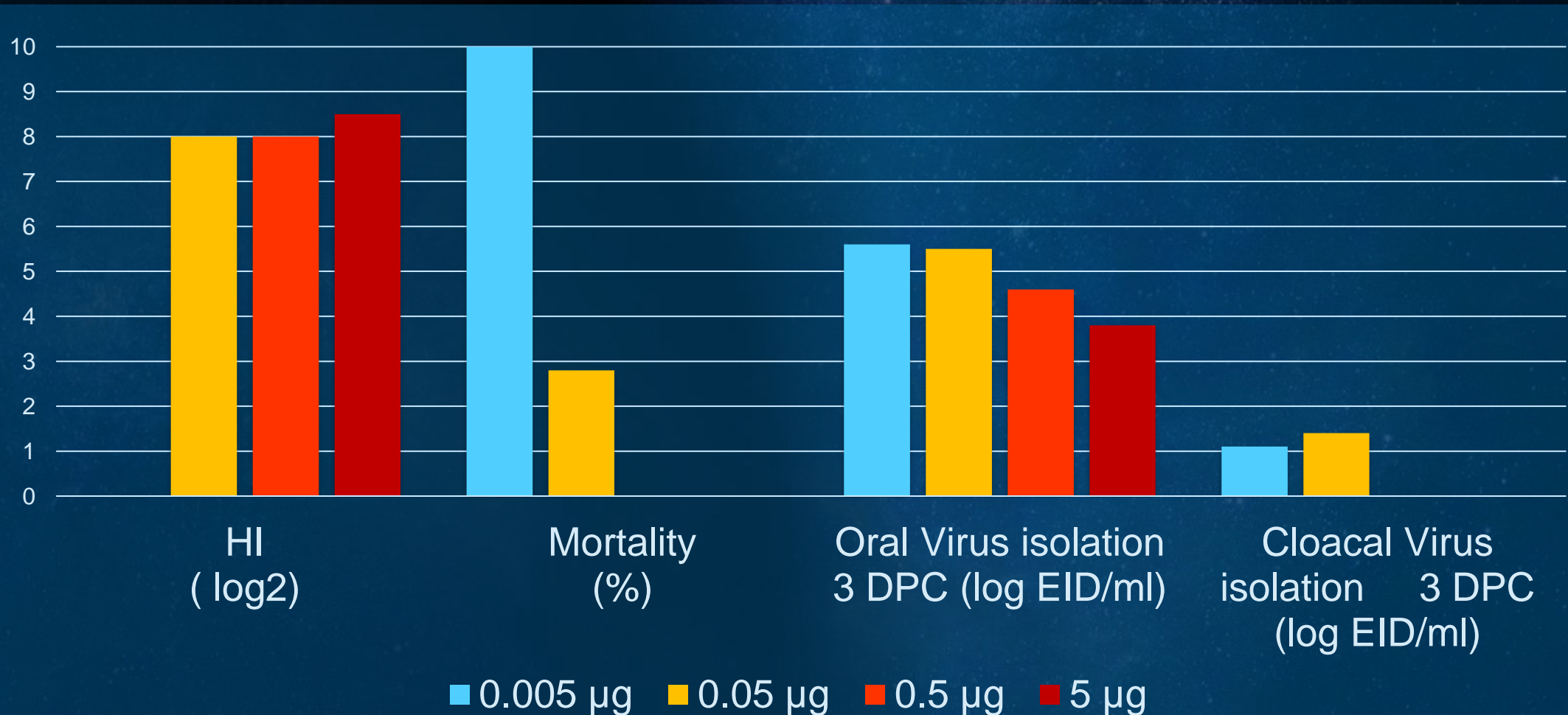
# Vaccination programmes

## AVERAGE AI VACCINE COVERAGE RATE FOR YEARS 2001-2010 OF USAGE



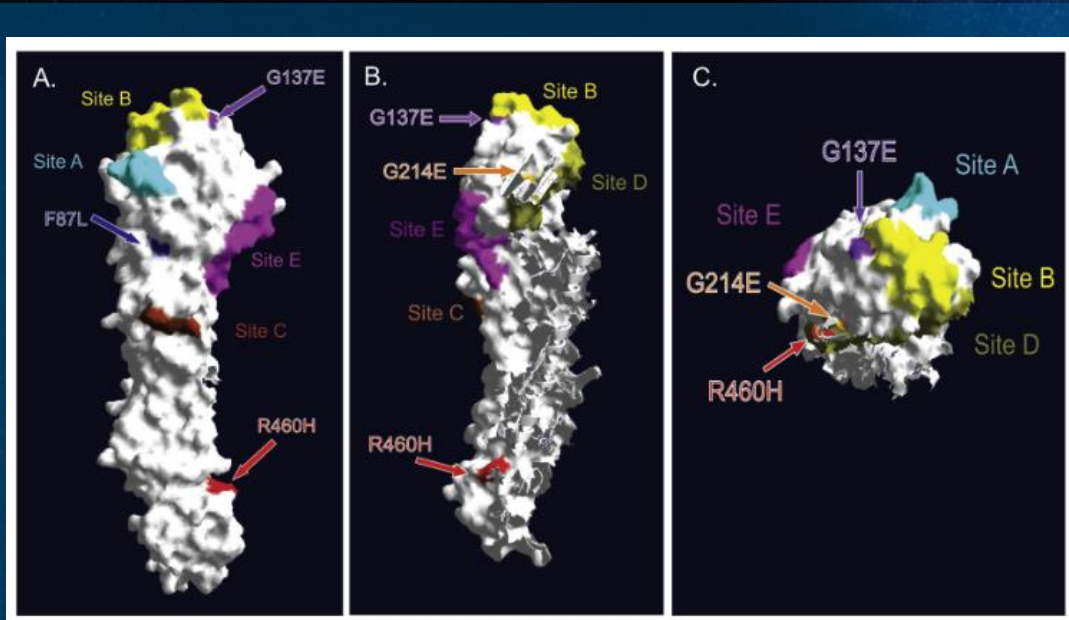
# Vaccination programmes

## VACCINE POTENCY & PROTECTION



# Vaccination programmes

## VACCINE POTENCY & ANTIGENIC SCAPE



AIV can scape from vaccines protection by mutation at critical antigenic site

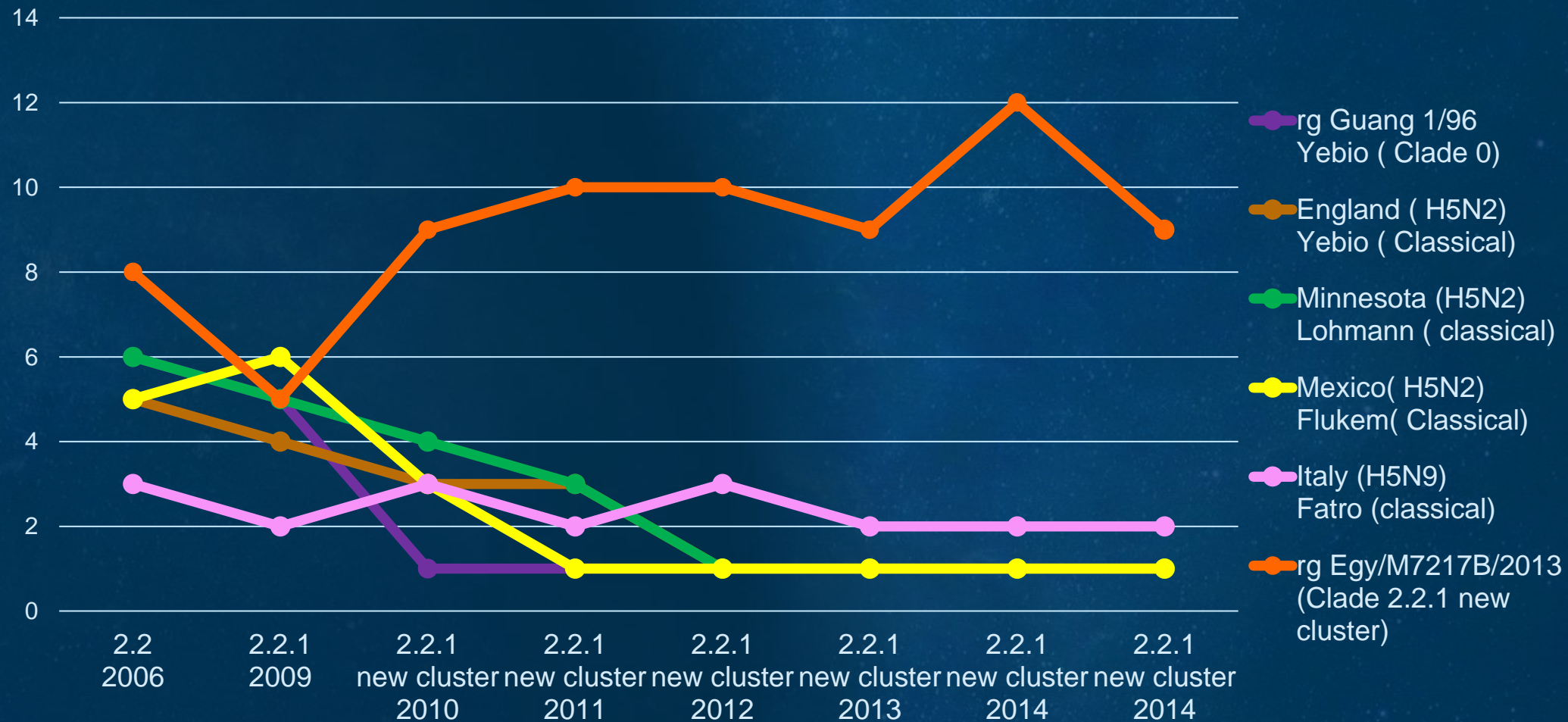
1. Update in vaccine seed strain can be needed time by time.

2. High titers from Antigenically relevant vaccines slow down antigen escape dynamics.



# Biosecurity

## ANTIGENIC SCAPE: VACCINE RESPONSE EVOLUTION IN EGYPT



# Vaccination programmes

## VACCINE TRIAL - 2.3.4.4B H5N1 HPAIV



<u>Vaccine</u>	<u>H5</u>	<u>Inoculated birds</u>	<u>Contact birds</u>	<u>R (Interval method)</u>
No	-	100% Mort	100% Mort	3.64 (1.89-6.99)
HVT- H5	2.2 ( 2006)	0% Mort	0% Mort	0 (0-NA)
HVT- H5	2.3.2	0% Mort	0% Mort	0 (0-NA)
DNA	2.3.4.4a	70% Mort	70% Mort	2.15 (1.03-4.50)
Inac ( 1 dose)	H5N2 ( LP)	50% Mort	50% Mort	0.92 (0.37-2.27)

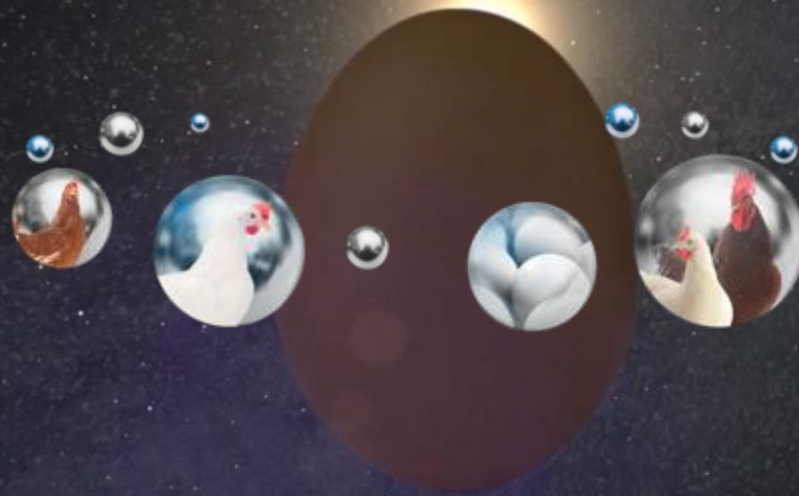
# Take home message

**1. AIV** is a virus with a **great capacity for mutation** and evolution. This must be **taken into account in its control.**

**2. The AI control programmes** are based on several points. **Biosecurity and Surveillance** are **fundamental.** **Vaccination** may be advisable in **some cases.**

**3. A properly implemented vaccination programme** is a **great help** in controlling AI in **endemic areas** but cannot solve the disease on its own.

# Thank you for your attention



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# Patogenia

## PRINCIPALES SUPERFICIES PROTEÍNAS



Disolventes  
orgánicos

Desoxicolato sódico  
Dodecil sulfato sódico)



Desinfectantes  
(Sensible a la  
materia organica)

Aldehídos  
Beta- propiolactona  
Etilenimina binaria



Desinfectantes  
( MUY sensible a la  
materia organica)

Fenólicos  
Amonio cuaternario  
Agentes oxidantes  
Ácidos diluidos  
Hidroxilamina



Disolventes  
orgánicos

Desoxicolato sódico  
Dodecil sulfato sódico)