The Natural and Unnatural History of Avian Influenza Viruses in Wild Birds

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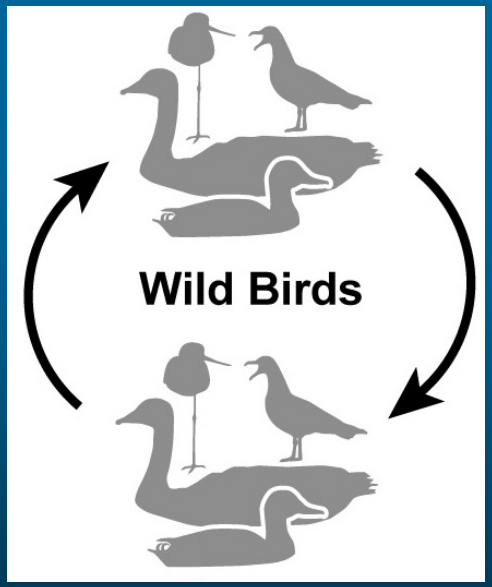
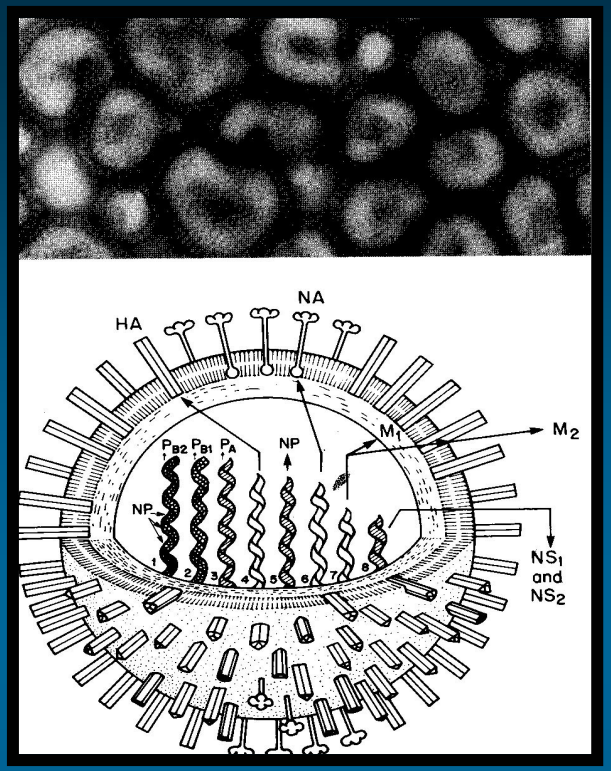
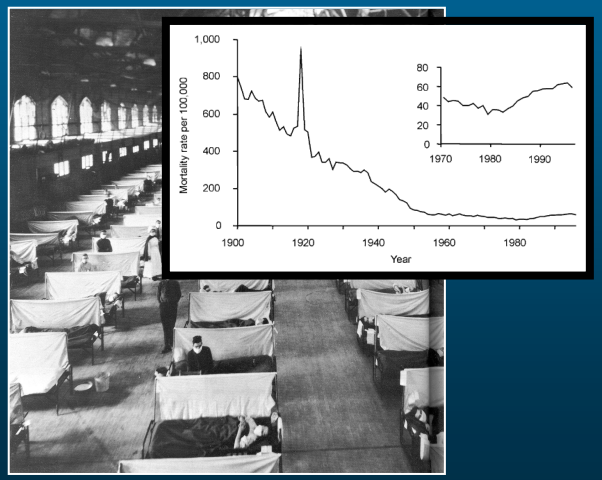


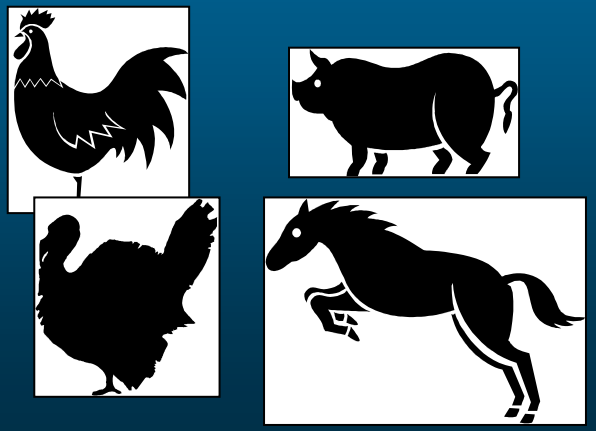
There is a little more to it than this!



Family: *Orthomyxoviridae*
***Orthomyxovirus*:**
Type A

Classification
HA 16 serotypes
NA 9 serotypes





Avian Reservoir(s)

A very brief history of AIV surveillance directed at wild birds.

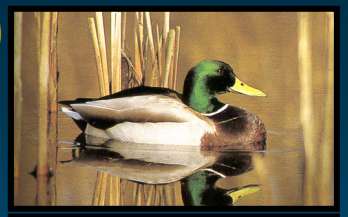
Initial isolation of AIV from a free-living bird: Common tern mortality in South Africa caused by highly pathogenic A/tern/South Africa/1966 (H5N3)

Discovery of the duck reservoir: AIVs isolated from ducks samples as part of Exotic Newcastle disease surveillance in CA during early 1970s.

"Golden age" of wildlife surveillance for AIV: From late 1960s to mid 1980s numerous publications relating to AIVs in free-living birds.

Avian Hosts and Avian Reservoir(s)

Isolations from wild avian species



Order: Anseriformes, isolations from 30+ species

Order Charadriiformes, isolations from 20+ species

Order: Gaviiformes , 4 species, Red-throated loon, Arctic loon

Order: Procellariiformes, 93 species, Wedge-tailed shearwater

Order: Podicepediformes, 20 species, Pied-billed grebe

Order: Pelecaniformes, 54 species, Great cormorant

Order: Ciconiiformes, 109 species, Squacco heron, Gray heron, Glossy ibis

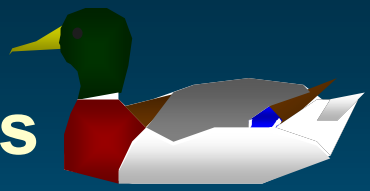
Order: Gruiformes, 185 species, Common moorhen, Eurasian coot, American coot

Order: Columbiformes, Collared dove

Order: Piciformes, Great spotted woodpecker

Order: Passeriformes, 24 species

Epidemiology of avian influenza viruses in the Anseriformes



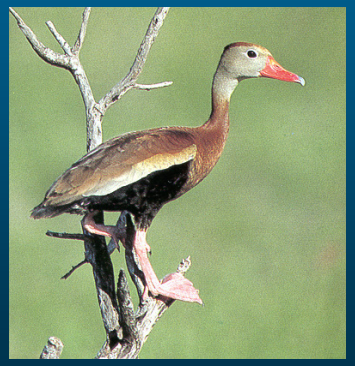
Who: What species are infected?
What: What viruses are they infected with?
When: Is there a temporal pattern?
Where: Is there a spatial pattern?
How: How are these viruses transmitted and maintained in these species?

Anseriformes: A duck just isn't a duck!

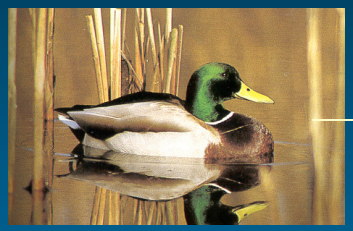
Anatidae: Ducks, geese, and swans (145 species)

Subfamilies within Anatidae:

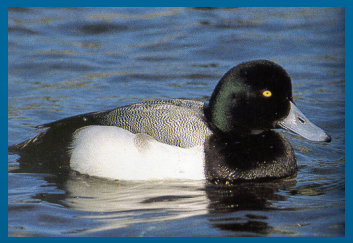
Dendrocygninae: Tree ducks (4 species) AOU North American Checklist
Anserinae: Geese and Swans (4 genera, 15 species)
Anatinae: Ducks (15 genera, 46 species)



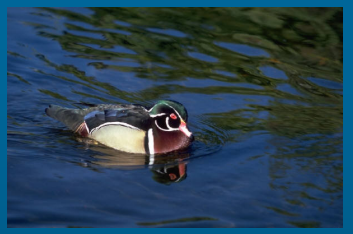
Who: What species are infected?



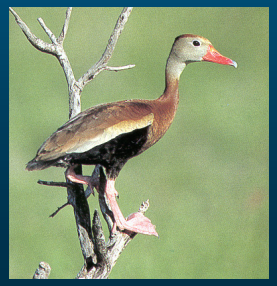
Mallard #1

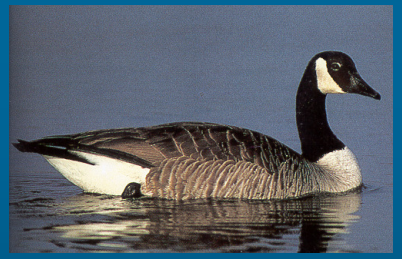


Open water/pelagic habitats



Atypical migration/range patterns





Habitat use variation

Olsen et al. Global patterns of Influenza A virus in wild birds. Science 312:384-388. (limited to studies where specific species given)

36 species 3,275/34,503 (9.5%)

Mallard (12.9%), northern pintail (11.2%), blue-winged teal (11.5%), American black duck (18.1%), common teal (4%)

8 species 47/4,806 (1.0%)

3 species 94/5,009 (1.4%)

Who Again:

What segment of the population is affected?
Juveniles—Why?

Spring: Breeding-Birds paired up on breeding grounds

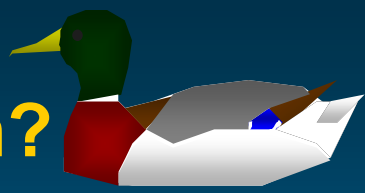
Early Summer: Hens and ducklings, Drakes gather together

Late Summer: Pre-migration staging (gathering of the masses)

Fall: Migration (timing species dependent)

Winter: (Large concentrations on wintering areas)

What: What viruses are they infected with?



All HA and NA subtypes

Dominant HA subtypes: H3, H4, H6

Dominant NA subtypes: N2,N6, N8

Differences between New World and Old World viruses

(This may include subtype diversity)

HA diversity in AIVs isolated from ducks

Alberta (Kawaoka et al, 1988) (n=2599),	H3 (33%), H4 (19%), H6 (42%)
New York (Diebel et al, 1985) (n=160)	H3 (33%), H4 (38%), H6 (12%)
Pennsylvania (Hinshaw et al, 1986)(n=171)	H3(20%), H4 (28%), H6 (50%)
Germany (Ottis and Bachman, 1983)(n=65)	H3(28%), H4 (27%), H11(21%)
Canada (Boudreault et al., 1980)(n=114)	H1(82%), H4 (10%), H6(5%)
Ohio (Slemons et al., 1991)(n=55)	H3(26%), H6(11%), H11(22%)
Louisiana (Stallknecht et al, 1991)(n=27)	H4(41%), H6(15%), H11(11%)
Minnesota (Hanson et al, 2003)(n=154)	H3 (14%), H4(27%), H6(14%)

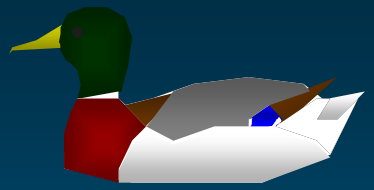
H5, H7, and H9 AIVs from ducks

Alberta (Kawaoka et al, 1988) (n=2599)	H5(0.2%),	H7(0.6%),	H9(0.3%)
New York (Diebel et al, 1985) (n=160)	H5(0.6%),	H7(0%),	H9(0%)
Pennsylvania (Hinshaw et al, 1986)(n=171)	H5(0.5%),	H7(0%),	H9(0%)
Germany (Ottis and Bachman, 1983)(n=65)	H5(0%),	H7(2%),	H9(0.%)
Canada (Boudreault et al., 1980)(n=114)	H5(1%),	H7(0%),	H9(0%)
Ohio (Slemons et al., 1991)(n=55)	H5(7%),	H7(2%),	H9(0%)
Louisiana (Stallknecht et al., 1991)(n=27)	H5(0%),	H7(0%),	H9(0%)
Minnesota (Hanson et al., 2003) (n=154)	H5(7%),	H7(3%),	H9(10%)

Based on 3,109 isolates
H5 0.25%
H7 0.61%
H9 0.22% of

Prevalence of these subtypes will vary with location/time.

When: Is there a temporal pattern?

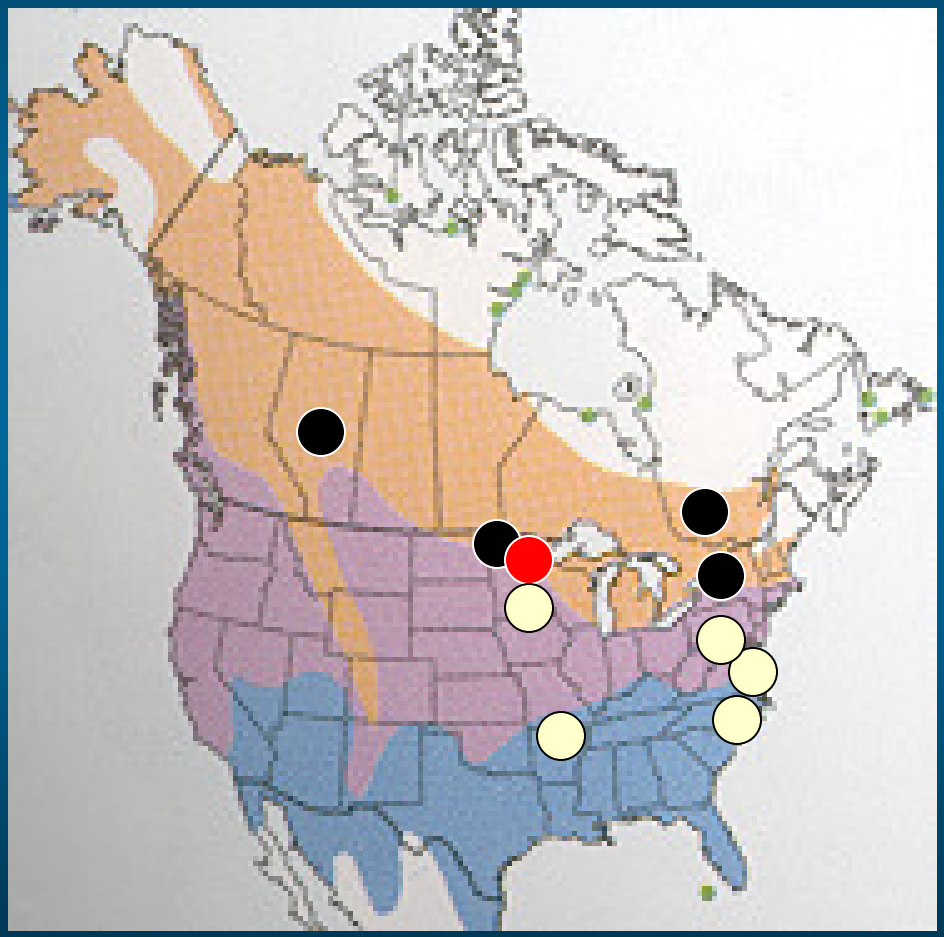


SEASONAL AND GEOGRAPHIC VARIATION in North American Mallards

Sample period	Location	% Positive
July-August	Quebec	37%
August	Alberta	30%
August	New York	42%
Aug.-Sept.	Minnesota	36%
September	Minnesota	13%
September	Minnesota	12%
Oct.-Nov	Minnesota	2%
November	Maryland	1%
Nov-Jan.	Arkansas	1%
Nov.-May	Pennsylvania	0%

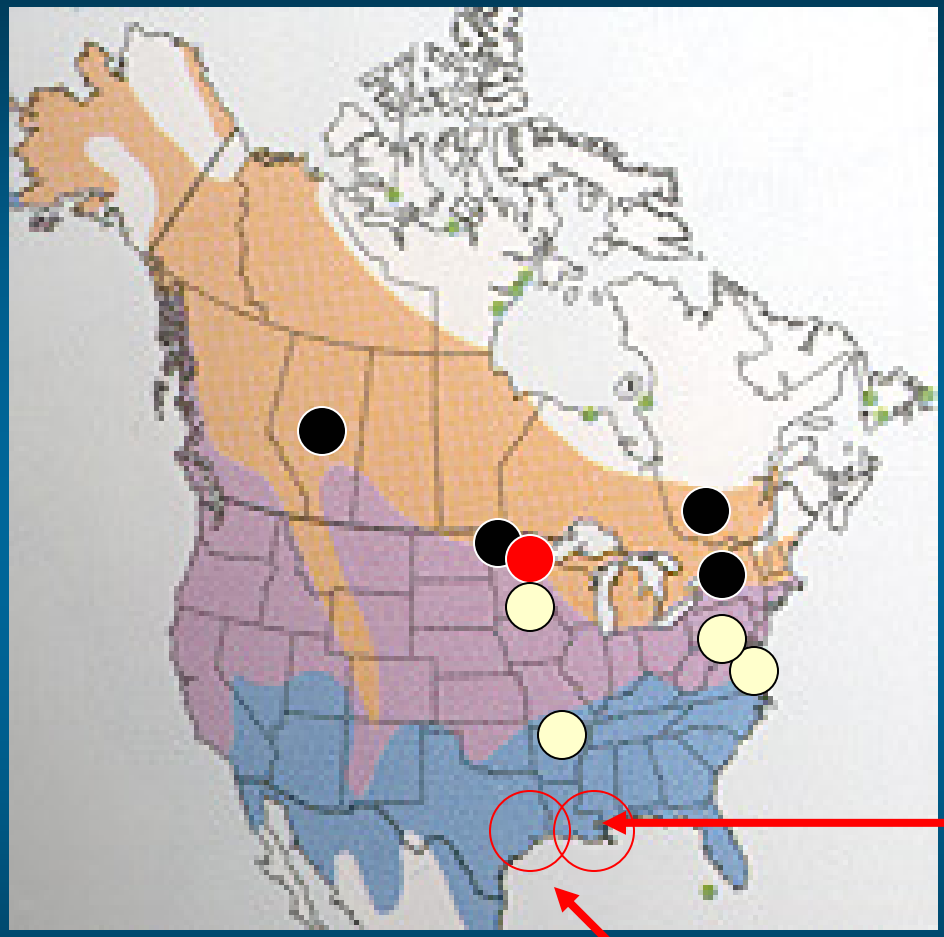
From: Boudreault et al., 1980; Hinshaw et al., 1980; Diebel et al.,1985; Karunakaran et al.,1983; Bahl et al.,1977; Nettles et al.,1985; Webster et al., 1986, Hanson et al, 2003

Where: Is there a spatial pattern?



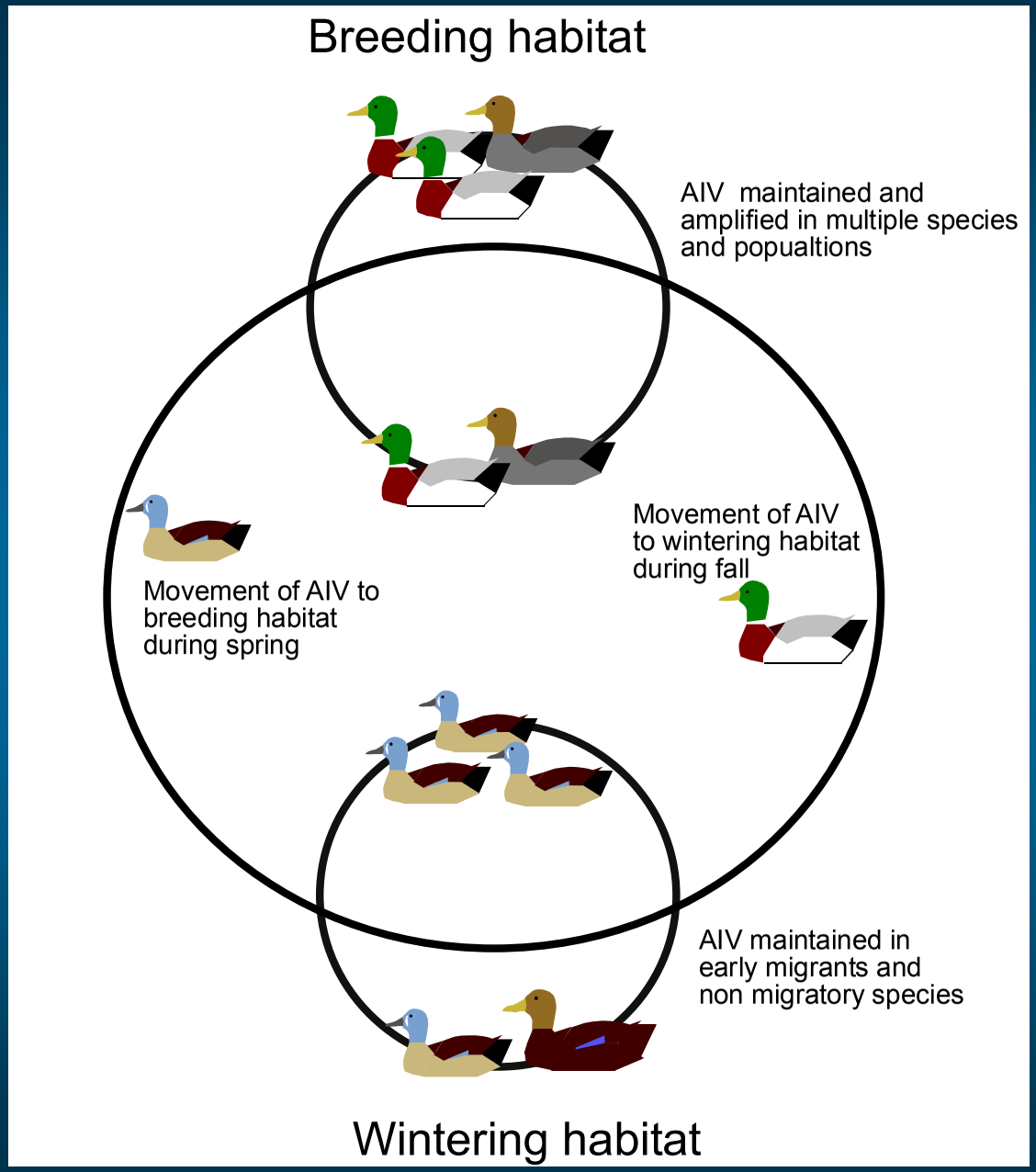
- 30%
- 5-30%
- <5%

AIV transmission on the wintering grounds



Early migrating teal 8%, transmission on wintering grounds as determined by isolations from mottled ducks

10% infection late winter/early spring H2, H7, H8



Maintenance of AIV in wild waterfowl populations: species/population interactions may be critical

May also involve other waterbirds especially gulls/shorebirds and environmental persistence

Water and the transmission of AIV

- Transmission of AIV through fecal/oral route via contaminated water
- Large amounts of virus shed through feces: Muscovey ducks shed an estimated $1x10^{10}$ EID_{50} per day (Webster et. al 1978)
- Extended duration of shedding (>28 days) by Pekin ducks (Hinshaw et al. 1980)
- Isolations from surface water
- Long-term persistence in water (pH, salinity, temperature dependent

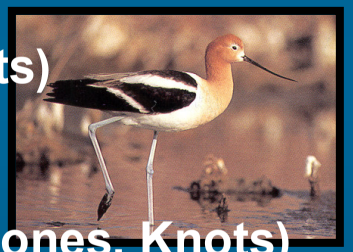
Charadriiformes: Who's Who

Family: Charadriidae (Plovers)

Family: Haematopodidae (Oystercatchers)

Family: Jacanidae (Jacanas)

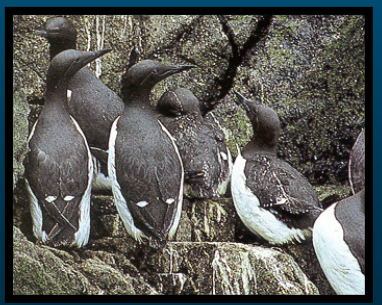
Family: Recurvirostridae (Avocets and Stilts)



Family: Scolopacidae: (Sandpipers, Turnstones, Knots)

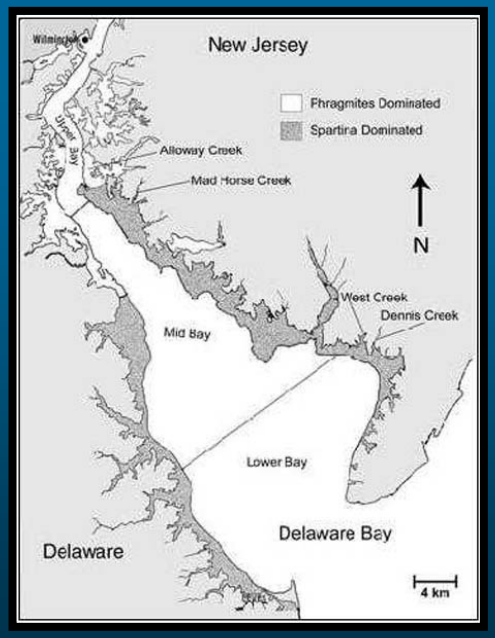
Family: Laridae: (Gulls, Terns, and Skimmers)

Family: Alcidae: (Murres, Auklets and Puffins)



Avian influenza and Charadriiformes:

- First isolation of an AIV from a free-living bird was associated with an outbreak of HPAI H5N3 in common terns in South Africa in 1961.



- Most isolations of AIV from Chardriiformes are reported from species in two families: Scolopacidae and Laridae
- Genetic variation exists between AIVs isolated from Charadriiformes and Anseriformes (based on sequence and subtype (such as H13 and H16) diversity.
- Some variation in host susceptibility (Not clear)
- If present, the prevalence of AIV in these populations is generally very low (Delaware Bay the exception)

Who: What species are infected?

Family: Scolopacidae (Snipe, woodcock, sandpipers, turnstones)

Eurasian woodcock, Eastern dunlin, Red knot, Timmicks' stint,

Sanderling, Ruddy turnstone, Least sandpiper, Semipalmated sandpiper, Spur-winged lapwing

Family: Laridae (Gulls, terns)

Lesser noody tern, Sooty tern, Arctic tern, White-winged

black tern, Sandwich tern, Common tern, Black-tailed gull,

Franklin's gull, Greater black-backed gull, Herring gull,

Ringed -billed gull

Family: Alcidae (Auks, murres, puffins)

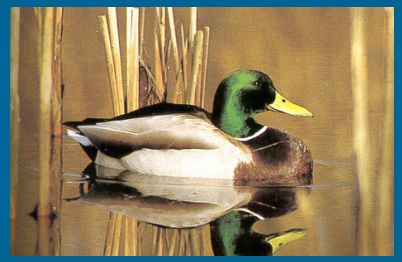
Common murre

Olsen et al. Global patterns of Influenza A virus in wild birds. Science 312:384-388. (limited to studies where species were identified)

10 species 21/2,637 (0.8%)

9 species 199/14,505 (1.4%)

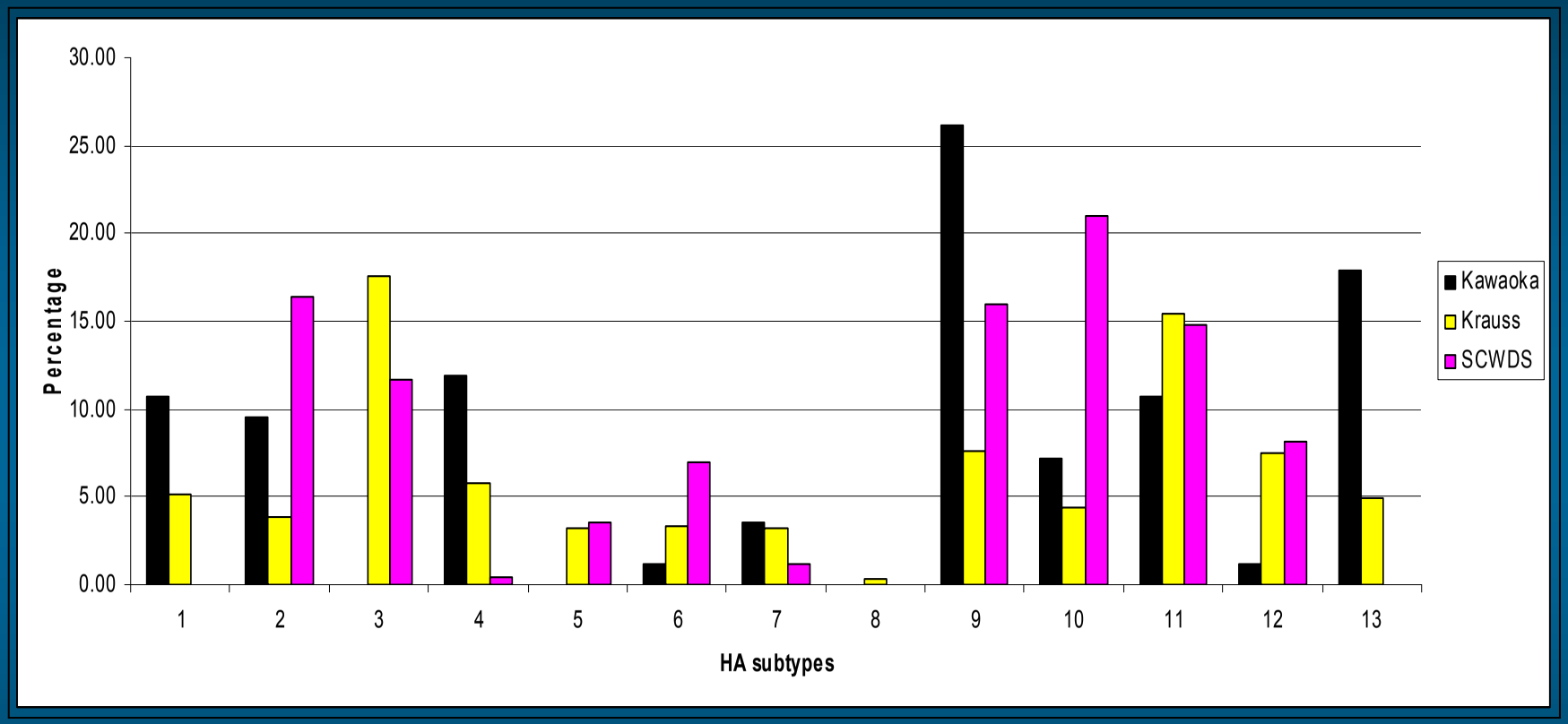
9 species 24/2,521 (0.9%)



3,275/34,503 (9.5%)

Mallards: 12.9%

What: What viruses are they infected with?



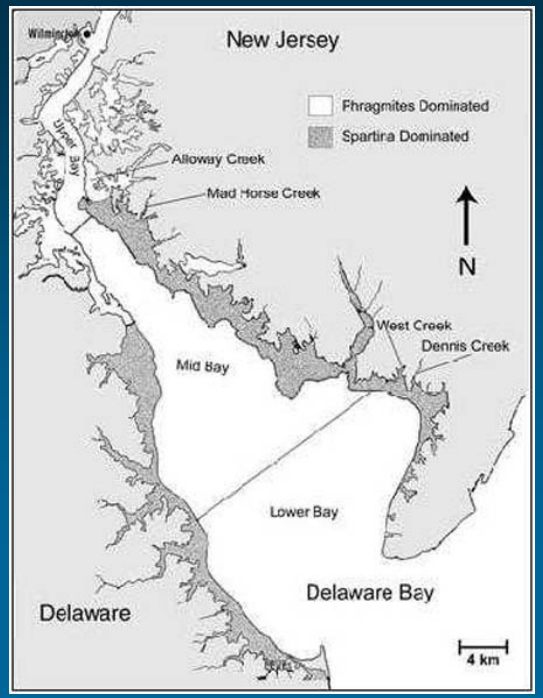
Kawaoka et al. 1988. Virology 163:247.
Krauss et al. 2004. Vector Borne and Zoonotic Diseases 4:177

ARE SHOREBIRDS A RESERVOIR FOR AVIAN INFLUENZA VIRUSES?

Britta Hanson, Dennis Senne, Page Luttrell, Ginger Goekjian, David Swayne, and David Stallknecht

Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine, The University of Georgia, Athens, GA
Southeast Poultry Research Laboratory, ARS, USDA, Athens, GA
National Veterinary Services Laboratories, Veterinary Services, Animal and Plant Health Inspection Service, USDA, Ames, IA

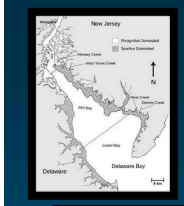
AIV isolations DE Bay 2000-2005



Dunlin	1/377	(<1%)
Red Knot	15/1,993	(<1%)
Sanderling	8/745	(1%)
Semipalmated	4/439	(1%)
Ruddy turnstone	262/2,358	(11%)
S-B dowitcher	1/157	(<1%)
Laughing gull	1/78	(1%)

292 AIV isolates

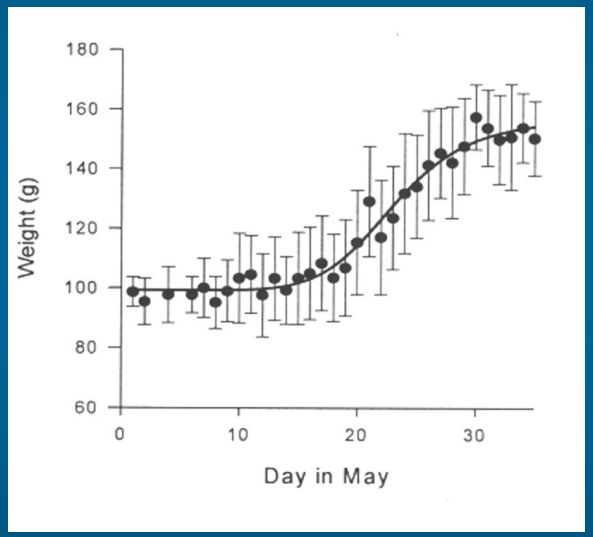
HA subtype	Number isolations	%
H1	1	<1%
H2	43	15%
H3	30	11%
H4	3	1%
H5	11	4%
H6	17	6%
H7	3	1%
H9	40	14%
H10	77	27%
H11	38	13%
H12	22	8%

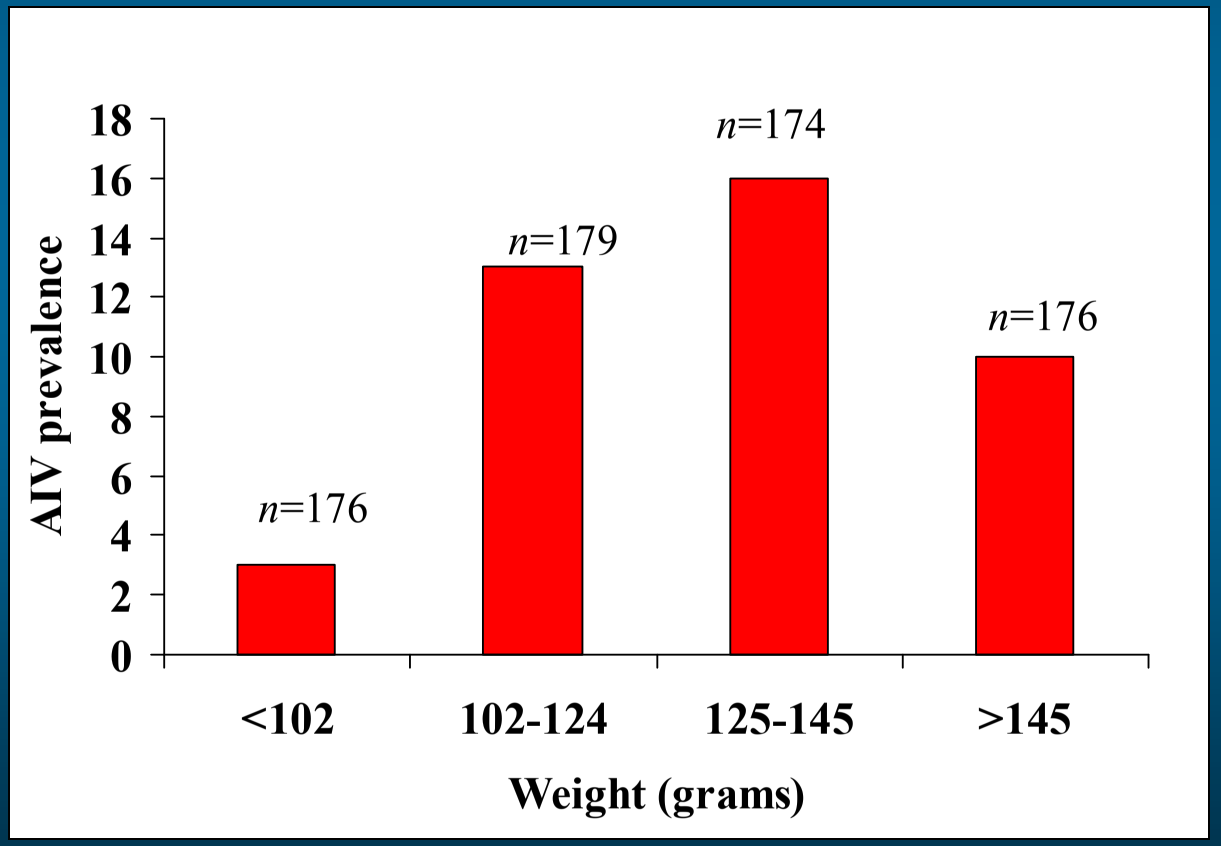


Subtype diversity: Shorebirds DE Bay

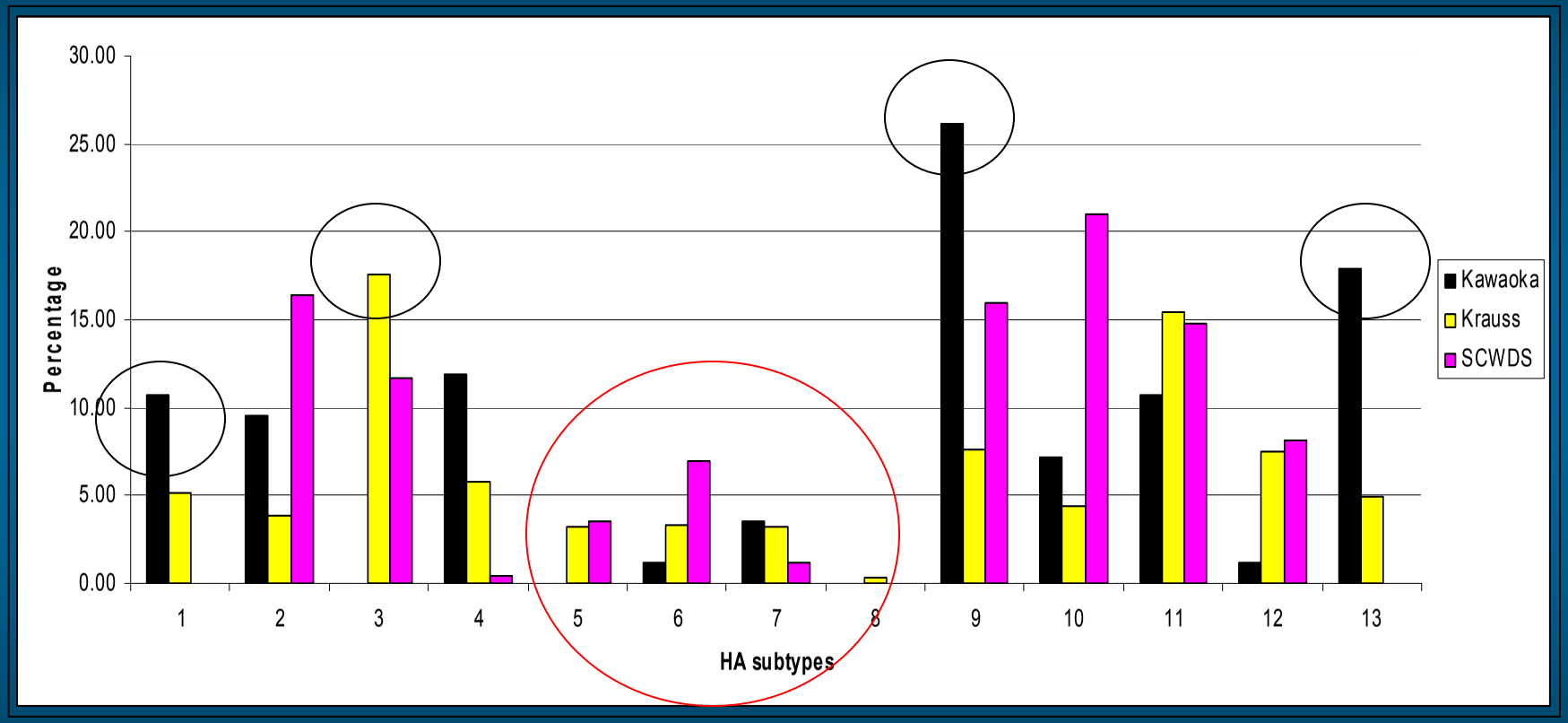
2000	2001	2002	2003	2004	2005
		H1N9			
		H2N3, H2N4, H2N9, H2N?	H2N6,H2N9		
					H3N6, H3N8
	H4N6	H4N?			
H5N3	H5N2, H5N7, H5N8		H5N2,H5N9	H5N7	
H6N1, H6N4	H6N2, H6N5	H6N4, H6N8	H6N8	H6N8, H6N4,8	
H7N9		H7N3			
		H9N4,H9N5, H9N9	H9N2,H9N5,H9N7, H9N8,H9N9, H9N1,4		
H10N7	H10N7	H10N7		H10N7	
H11N6	H11N2,H11N7	H11N2,H11N3 H11N4,H11N9			H11N8,H11N6
H12N4, H12N5	H12N5	H12N5	H12N4,H12N5, H12N9.		

Avian influenza prevalence is dependent on ruddy turnstone weight gains at Delaware Bay (P = 0.001)





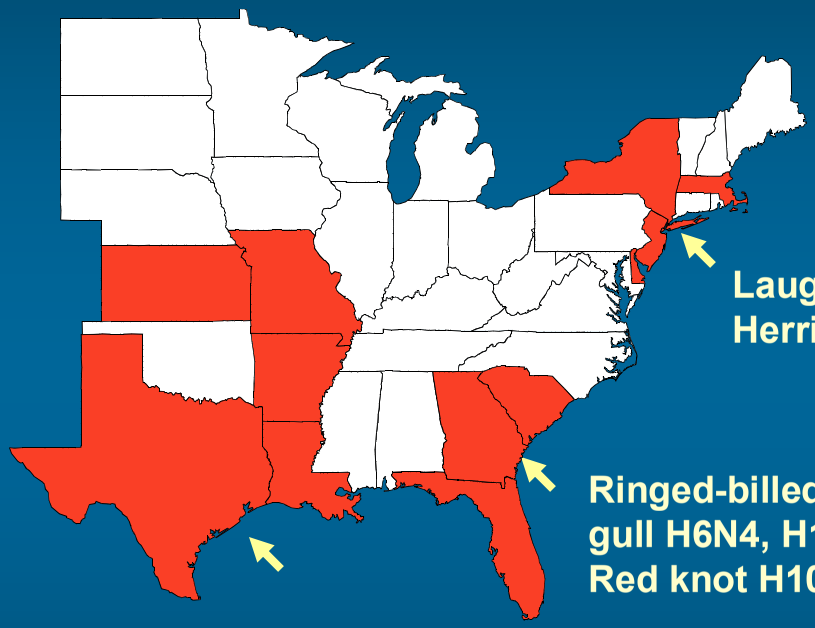
Temporal variation and study duration. Why long-term studies are essential



Kawaoka et al. 1988. Virology 163:247. (Short-term)
Krauss et al. 2004. Vector Borne and Zoonotic Diseases 4:177 (Long-term)

Is there a spatial pattern?

With all of the work on AIV in shorebirds to date, a high prevalence of infection has been identified in only one species at only one area at only one time of year.



>600 shorebirds (predominantly red knots) sampled in Argentina Bermuda and Chile-all tested negative for AIV

Species tested

Delaware Bay: 292 isolations from 6,340 birds (4.6%)

Ruddy turnstones: 11.1%

Other species (7): 0.7%

Outside of Delaware Bay: 8 isolations from 2780 (0.3%) birds

Scolopacidae: (17 species) 2/1849 (0.1%) AIV positive

Red knot (938), Semipalmated sandpiper (309), Least sandpiper (155), white-rumped sandpiper (144) No isolations from 21 ruddy turnstones.

Laridae: (9 species) 6/840 (0.7%)

Laughing gull (529), Ringed-billed gull (156), Common tern (105), Herring gull (35)

Haematopodidae (American oystercatcher: *n*=83)
Charadriidae (4 species *n*=41)
Recurvirostridae (Black-winged stilt, *n=6)*

AIV in Charadriiformes-Who knows?

- AIV prevalence in species within Charadriiformes is generally very low and is species and location dependent

- High prevalence in ruddy turnstones at Delaware Bay reflects site specific amplification (from the turnstone's perspective: a new emerging disease every year)

- Why ruddy turnstones? Probably related to behavior (feeding behavior and habitat selection)

- AIV subtype diversity in ruddy turnstones at this site is inconsistent and may reflect introductions from several sources

- Outside of Delaware Bay, gulls appear to be a more "reliable" source of AIV isolates than shorebirds and these may represent the true reservoir within Charadriiformes.

The Unnatural History of Avian Influenza: HPAI H5N1 1997 Hong Kong-

- Human infection and mortality
- Spillover to wild birds (with mortality and transmission)

Experimental Infection of wild birds with HPAI H5N1 (a taste of things to come)

Perkins and Swayne, 2001;2002;2003

A/chicken/Hong Kong/220/97 (H5N1)

- **Multiple avian species susceptible (intranasal challenge)- variable among species even in the same order**
- **Gallinaceous birds most susceptible**
- **Also zebra finches, house finches, budgerigars**
- **Emus and Embden geese- moderate susceptibility**
- **House sparrows, starlings, pigeons, laughing gulls, Pekin ducks- refractory**
- **Respiratory rather than cloacal shedding**

Experimental infection of mallards with HPAI (H5N1)

Sturm-Ramirez et al. 2004

- **Challenge with 12 H5N1 Hong Kong viruses (human, chicken, wild/captive duck**
- **High mortality associated with Kowloon and Penfold waterfowl isolates**
- **Some mortality associated with Pheasant (2001) and Teal (2002) isolates**
- **ALL viruses replicated in mallards**
- **ALL but one transmitted to contact birds**
- **Viral titers highest in tracheal swabs rather than cloacal swabs**
- **Viral titers much reduced at PID 5**
- **Virus isolated from drinking water**

Tracheal swabs PID 3 (3-5.7 EID_{50}), PID 5 (<5 EID_{50})
Cloacal swabs PID 3 (<4.4 EID_{50}), PID 5 (<3.5 EID_{50})

HInshaw et al. 1980 **A/duck/Alberta/35/76**

Fecal samples Pekin ducks 2.5-6.3 EID_{50}, PID 4-30, 80% of birds positive to PID28

Field reports of wild bird mortality

- Hong Kong (2002-2003) Black-headed gull, little egret, grey heron, greater flamingo, pigeon, tree sparrow, various waterfowl
- Hong Kong (2003 and 2004) Peregrine falcon, Grey Heron
- Cambodia (2004) (Zoo collection) raptors and psittacines
- Japan and Korea (2004) Crows and magpies
- Thailand (2004) Pigeons, open-billed storks, little cormorant, red collared dove, scaly-breasted munia, black drongo
- China (2004) grey heron and Chinese pond heron
- China (2005) Bar-headed geese
- Mongolia (2005) Bar-headed goose and whooper swan
- Russia (Siberia) (2005) Wild birds?
- Kazakhstan (2005) Wild birds?

- Turkey, Romania, Croatia (2005)
- Europe/Africa (2005-2006)

Susceptibility North American Birds to HPAI H5N1

Potential reservoir of dead end?

- Species related differences?
- Environmental persistence?
- Dose response?

Designed for field relevance—species selection and age of birds.

Two HPAI H5N1 strains investigated:

Anyang—Korean Duck meat (subclinical infection in ducks)

Mongolia—Associated with wild bird mortality

Funded by the United States Poultry and Egg Association, the Morris Animal Foundation, and ARS, USDA

Study Objective

- To determine whether North American avian species in the Orders Anseriformes and Charadriiformes are susceptible to infection with HPAI H5N1 Asian viruses

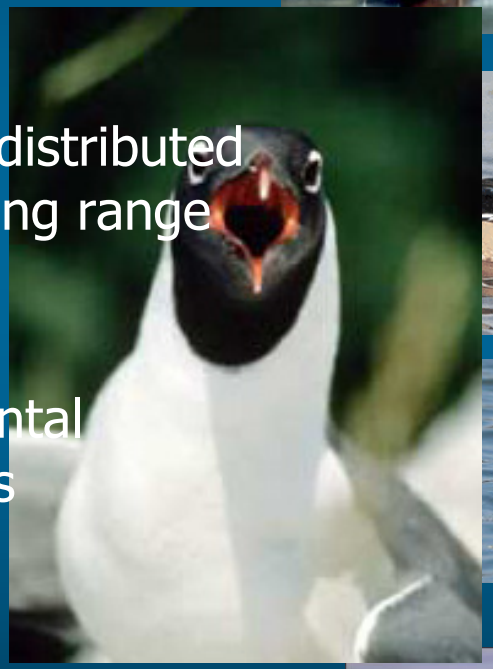
- Study designed for field relevance
  - Species
  - Age

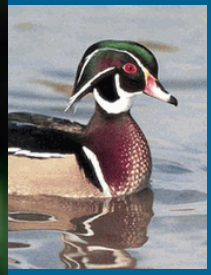
Anseriformes

Bay duck (diving duck)

Charadriiformes

Widely distributed breeding range





Transcontinental migrants

Most common duck species in North America

Methods

- 9 birds from each species were evenly divided into 3 groups:
  - A/Whooper Swan/Mongolia/244/05 (H5N1)
  - A/Duck Meat/Anyang/01 (H5N1)
  - Sham-inoculated controls
- Oral and cloacal swabs and serum were collected from each bird prior to inoculation with virus
- AIV-inoculated birds were inoculated IN with 10^6 EID_{50}/ml

Results

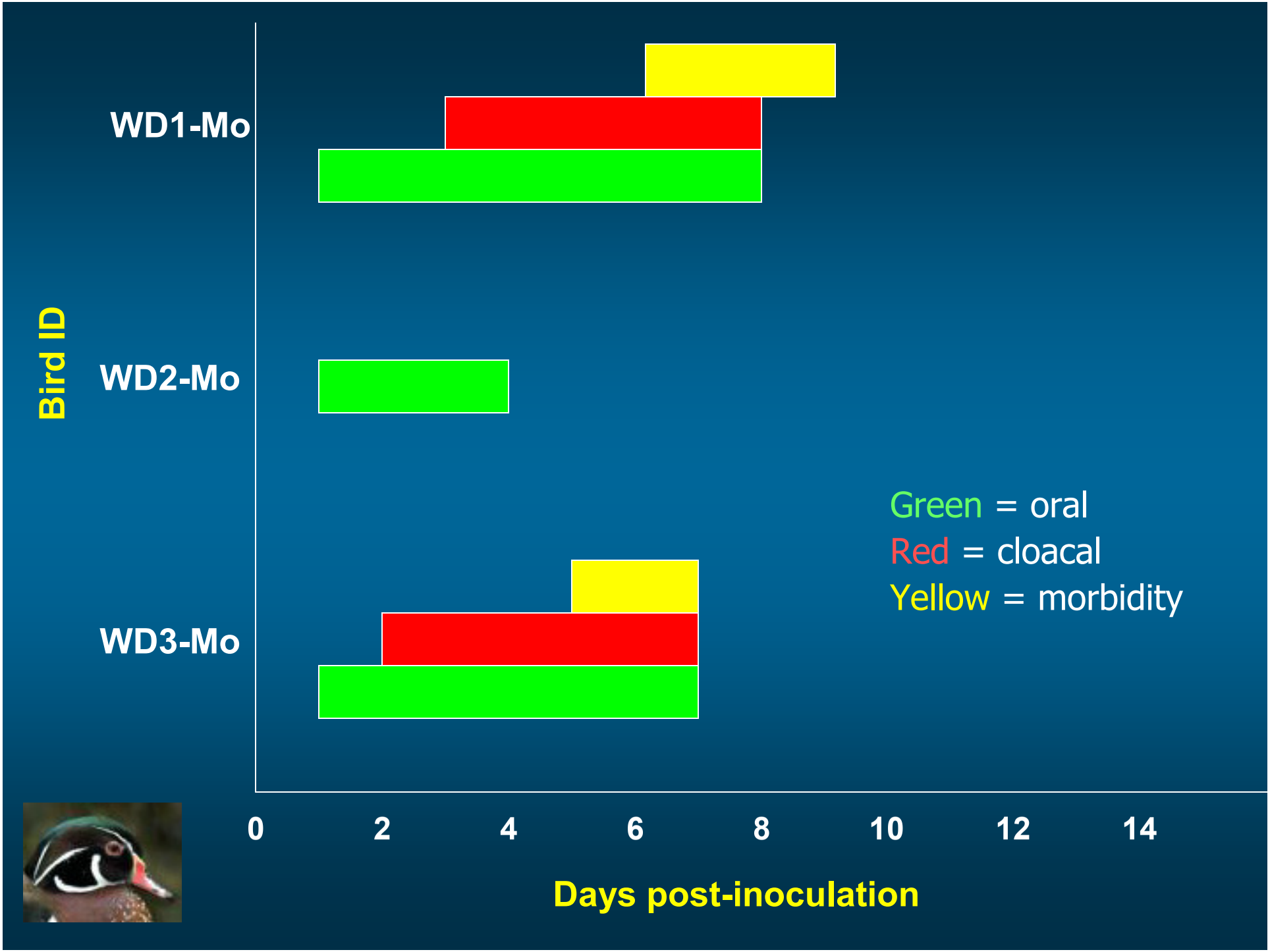
- Only wood ducks and laughing gulls exhibited morbidity and/or mortality
  - The other 4 duck species remained clinically normal

Wood Ducks

- Mongolia/05 virus
  - 2/3 morbidity
  - 2/3 mortality
- Anyang/01 virus
  - 2/3 morbidity
  - 1/3 mortality
- Clinical signs were similar with both HPAI H5N1 viruses
- Cloudy eyes, weakness, incoordination, tremors, and seizures

Laughing Gulls

- Mongolia/05 virus
  - 3/3 morbidity
  - 2/3 mortality
- Anyang/01 virus
  - 3/3 morbidity
  - 2/3 mortality
- Clinical signs were similar with both HPAI H5N1 viruses
- Cloudy eyes, ruffled feathers, weakness, incoordination, and torticollis


WD1-Mo
WD2-Mo
WD3-Mo
Bird ID
Green = oral
Red = cloacal
Yellow = morbidity
0
2
4
6
8
10
12
14
Days post-inoculation

Average Mean Titer (log_{10} EID_{50}/ml)

	Oropharyngeal	Cloacal
Wood duck-Mo	4.6	3.8
Wood duck-An	5.0	2.8
Laughing gull-Mo	4.2	2.6
Laughing gull-An	5.0	2.0
Blue-winged teal-Mo	3.8	1.0
Blue-winged teal-An	2.0	---
Redhead-Mo	2.8	1.2
Redhead-An	4.0	---
Mallard-Mo	3.1	1.0
Mallard-An	2.1	1.0
N. Pintail-Mo	1.5	1.0
N. Pintail-An	1.1	---

Are the Eurasian HPAI H5N1 as fit as “wild type” AIVs for long-term environmental persistence?

Wild type H5s

A/mallard/MN/182742/98 (H5N2)
A/mallard/MN/355790/00 (H5N3)

A/RUTU/NJ/828227/01 (H5N8)
A/RUTU/NJ/828219/01 (H5N7)

Wild type H7s

A/mallard/MN/182761/98 (H7N3)
A/blue-winged teal/TX/578597/02 (H7N4)

A/RUTU/DE/650635/02 (H7N3)
A/Laughing gull/DE/AI00-2455 (H7N3)

HPAI H5N1

A/whooper swan/Mongolia/244/05 (H5N1)
A/duck meat/Anyang/01 (H5N1)

Results: Persistence of H5 and H7 AIV at 17C
Estimated infectivity of 10^6 $TCID_{50}$ in days

Virus	0 ppt	15 ppt	30 ppt
Mallard/MN/98 (H5N2)	429	128	63
Mallard/MN/00 (H5N3)	316	333	113
RUTU/NJ/01 (H5N7)	231	375	85
RUTU/NJ/01 (H5N8)	286	125	58
Mallard/MN/98 (H7N3)	214	133	92
BWT/TX/02 (H7N4)	176	133	107
RUTU/DE/00 (H7N3)	667	143	105
LGull/DE/02 (H7N3)	194	140	171
WSwan/Mongolia/05 (H5N1)	158 **(10)**	83 **(10)**	82 **(8)**
Duck Meat/Anyang/01 (H5N1)	94 **(10)**	182 **(3)**	115 **(2)**

Stallknecht et al., 1990 Louisiana duck AIVs in water at 17C/ 0ppt salinity: H3N8 (194), H4N6 (207), H6N2 (176), H10N7 (146), H12N5 (126)

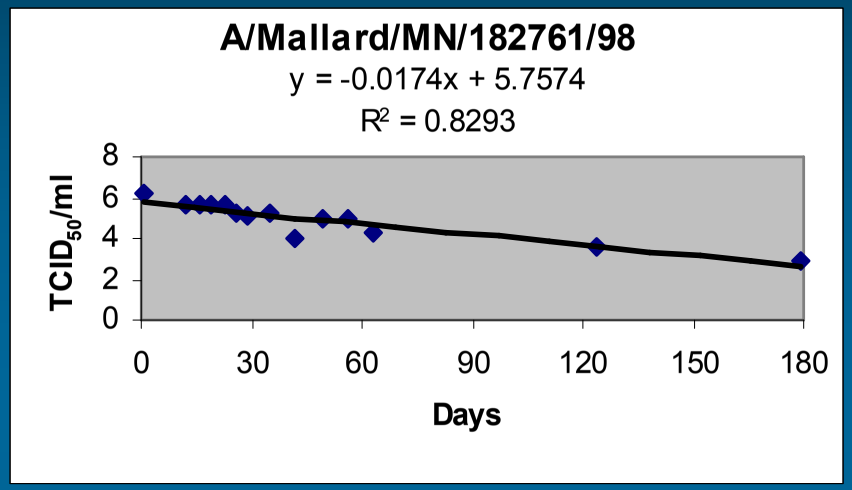
Results: Persistence of H5 and H7 AIV at 28C
Estimated infectivity of 10^6 $TCID_{50}$ in days

Virus	0 ppt	15 ppt	30 ppt
Mallard/MN/98 (H5N2)	118	61	28
Mallard/MN/00 (H5N3)	85	56	22
RUTU/NJ/01 (H5N7)	53	47	26
RUTU/NJ/01 (H5N8)	36	42	42
Mallard/MN/98 (H7N3)	74	29	32
BWT/TX/02 (H7N4)	60	19	29
RUTU/DE/00 (H7N3)	67	26	22
LGull/DE/02 (H7N3)	24	25	26
WSwan/Mongolia/05 (H5N1)	26 **(9)**	28 **(7)**	18 **(9)**
Duck Meat/Anyang/01 (H5N1)	30 **(8)**	28 **(7)**	17 **(10)**

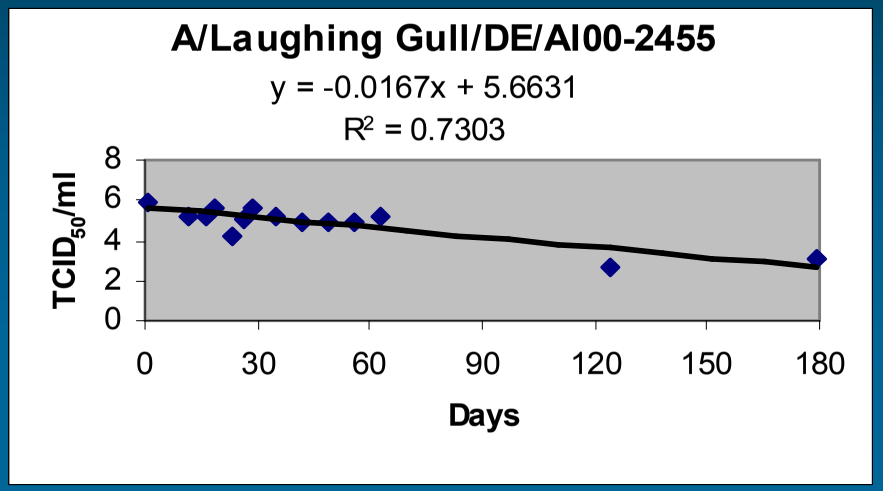
Stallknecht et al., 1990. Louisiana duck AIVs in water at 28C/0ppt salinity
H3N8 (66), H4N6 (80), H6N2 (98), H10N7 (102), H12N5 (30)

Results: Validation of linear models over 180 days

H7N3



H7N3



- Linear regression models continued to appropriately describe the data from viruses that were assayed at 120 and 180 days
  - Slopes from the 2-month-data and the 6-month-data did not vary more than 0.01 for any of three viruses
- Coefficients of determination for the 6-month-data were higher for all three viruses than the 2-month-data

Conclusions

- North American duck species differ in their susceptibility to these viruses and their potential to transmit them
- Of all viruses tested to date, the HPAI H5N1 Asian viruses are the least persistent in freshwater (0 ppt) -- Freshwater habitats are important in the biology of waterfowl
- Based on unpublished data from wood ducks, the infective dose is very low

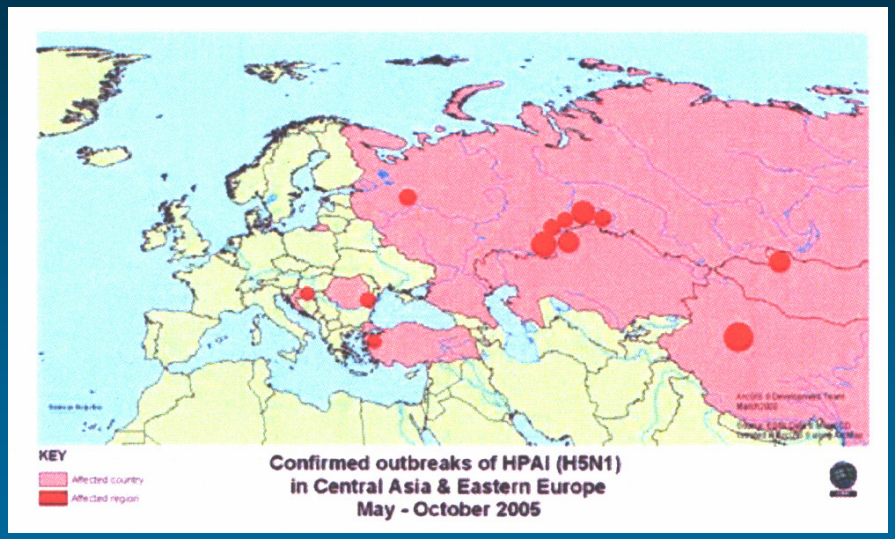
Outbreaks of HPAI H5N1 virus in Europe during 2005/2006

Department for Environment Food and Rural Affairs (UK)

International Animal Health Division

International Disease Monitoring Unit

Qualitative risk Assessments

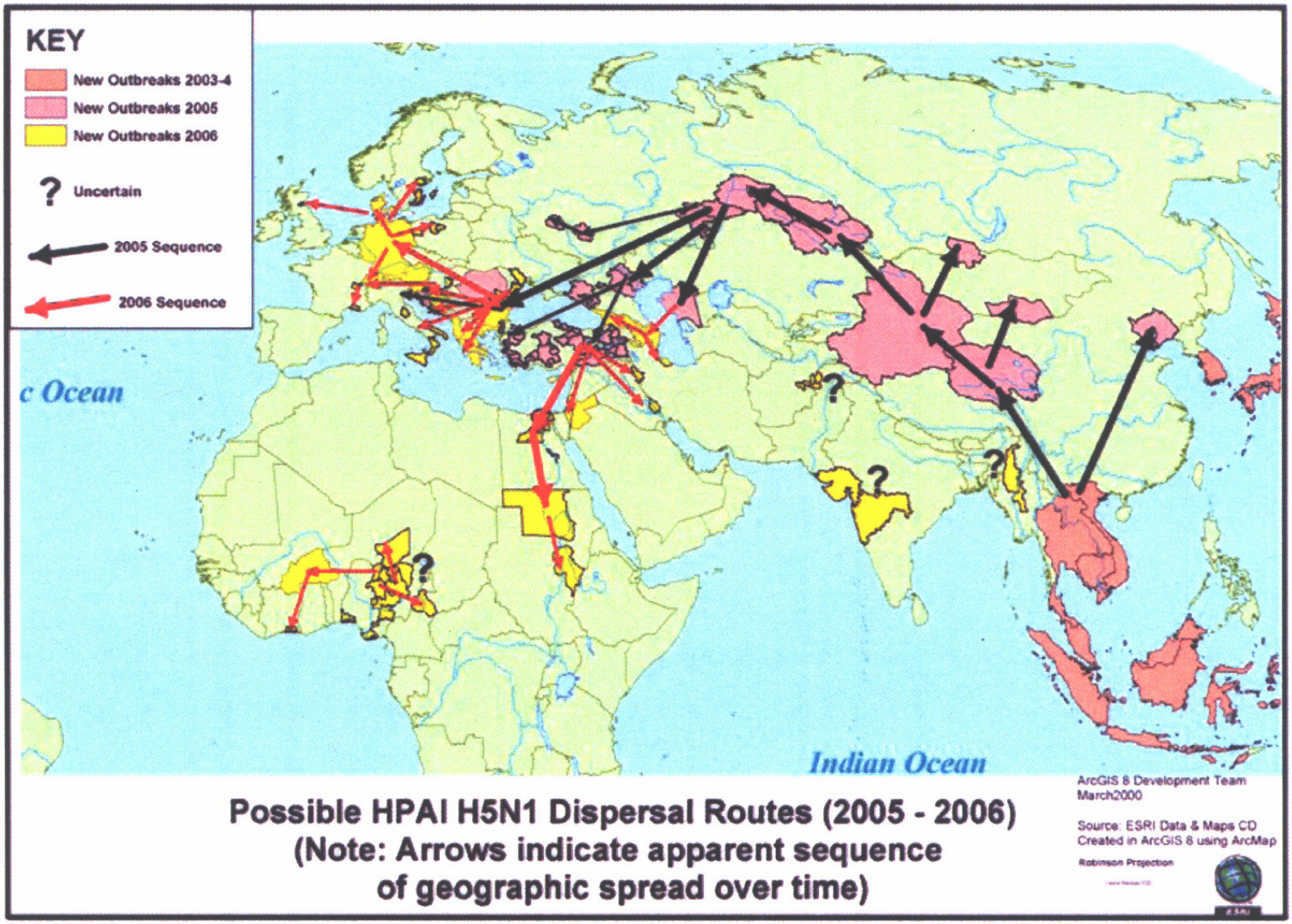


Movement of HPAI H5N1 during 2005

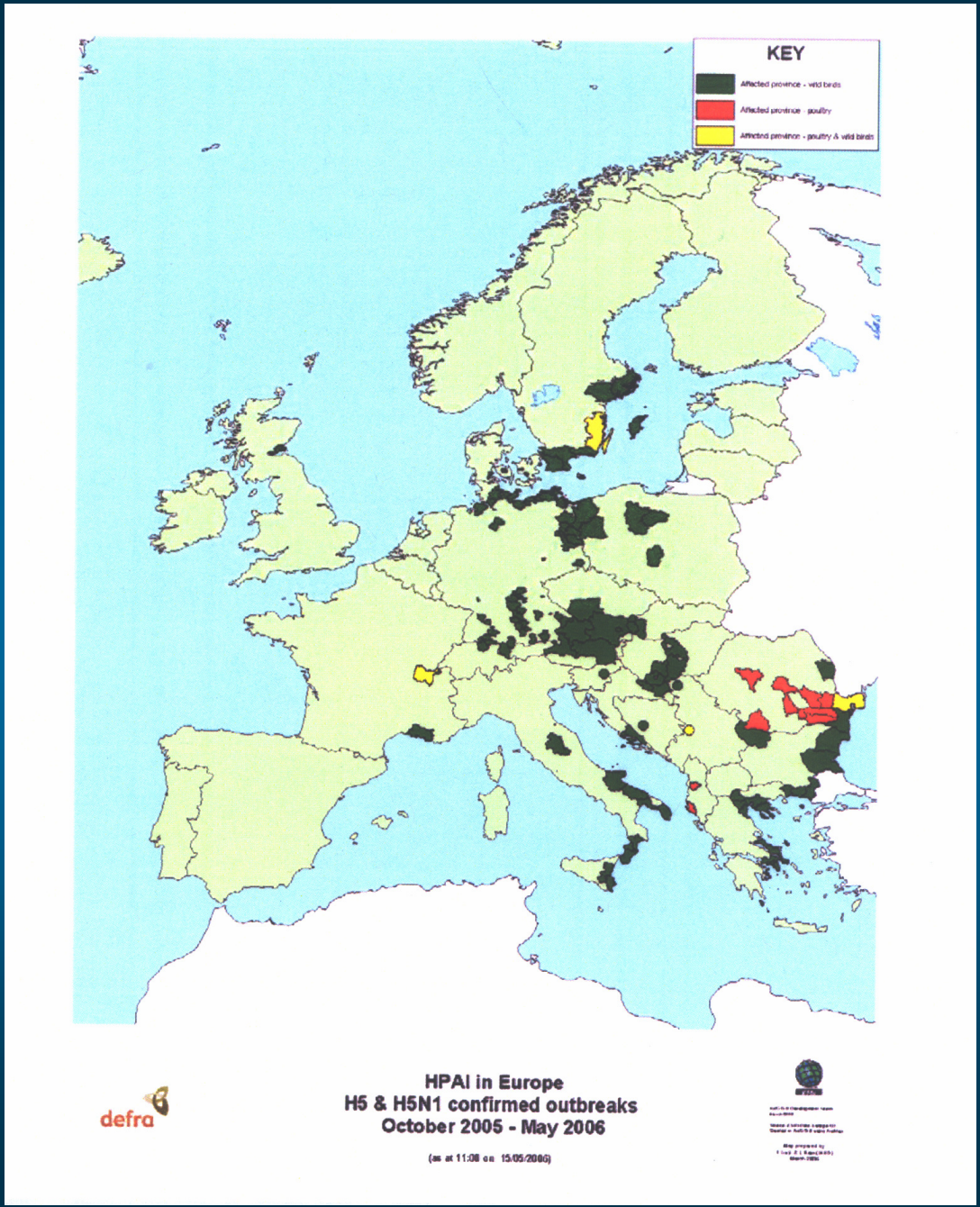
May 2005 China

August 2005 Mongolia/Russia

Turkey October 2005

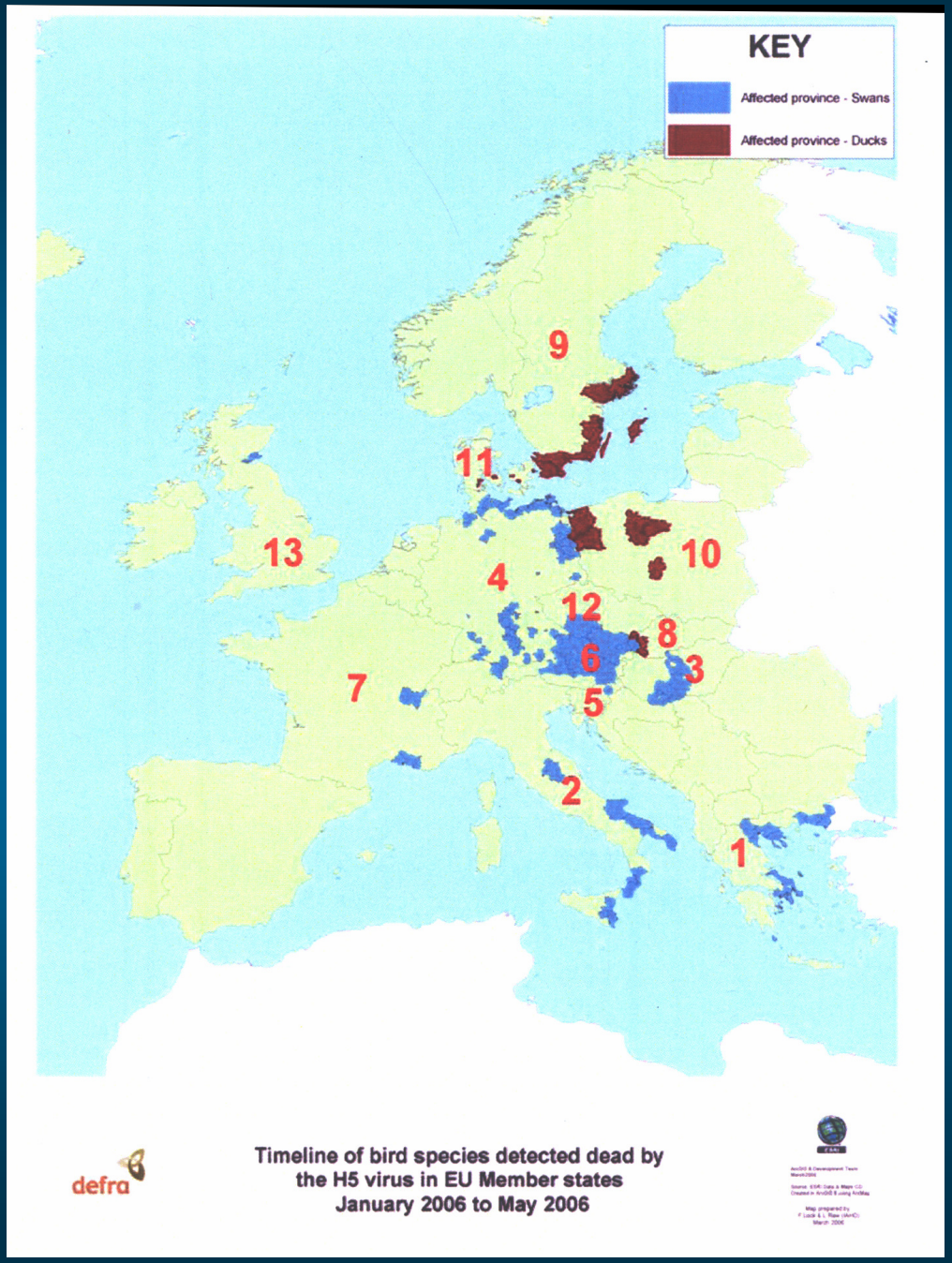


indicate the apparent sequence of spatial (geographic) spread during the years 2005 and 2006.



HPAI outbreaks in Europe

- Most involved wild bird mortality
- Few cases of domestic poultry infections
- All stamped out



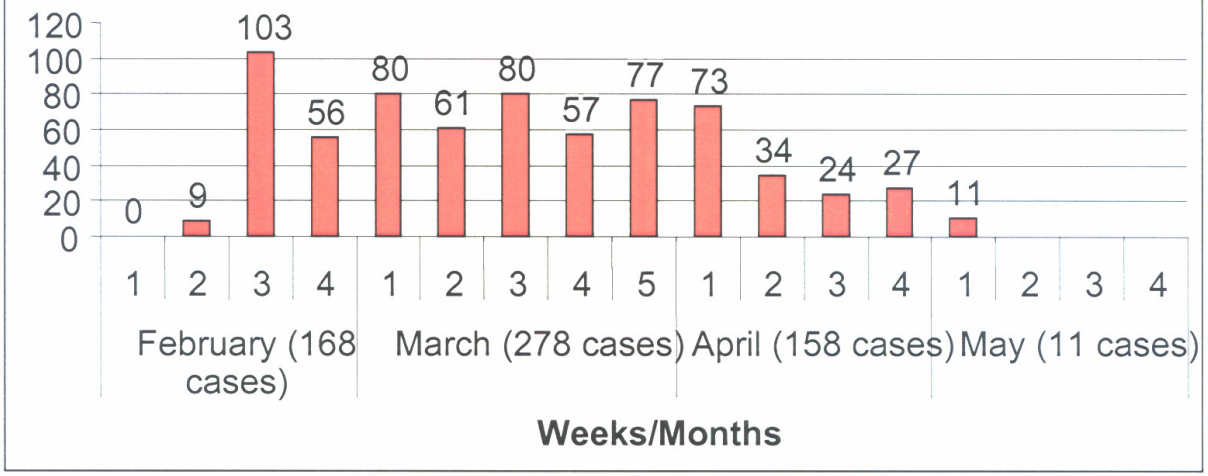
HPAI in wild birds

- Most positive birds are species of Anseriformes
- Two primary species: mute swans and tufted ducks
- All positive associated with mortality

Table 1. Wild bird species reported to be affected in affected EU Member States

	Swans	Ducks	Geese	Gulls	Crested Grebe	Raven	Raptors	Herons	Date of First Report	Date of Last Report
Greece	X		X						01/02/2006	03/03/2006
Italy	X	X					X		21/02/2006	23/02/2006
Hungary	X	X		X					15/02/2006	13/03/2006
Germany	X	X	X	X		X	X	X	17/02/2006	
Slovenia	X	X						X	15/02/2006	10/03/2006
Austria	X	X			X				18/02/2006	on-going
France	X	X	X		X			X	18/02/2006	21/03/2006
Slovakia		X					X		23/02/2006	23/02/2006
Sweden	X	X							28/02/2006	30/03/2006
Poland	X	X					X	X	06/03/2006	
Denmark	X	X	X	X	X		X		16/03/2006	
Czech Rep	X								27/03/2006	07/05/2006
UK	X								31/03/2006	31/03/2006

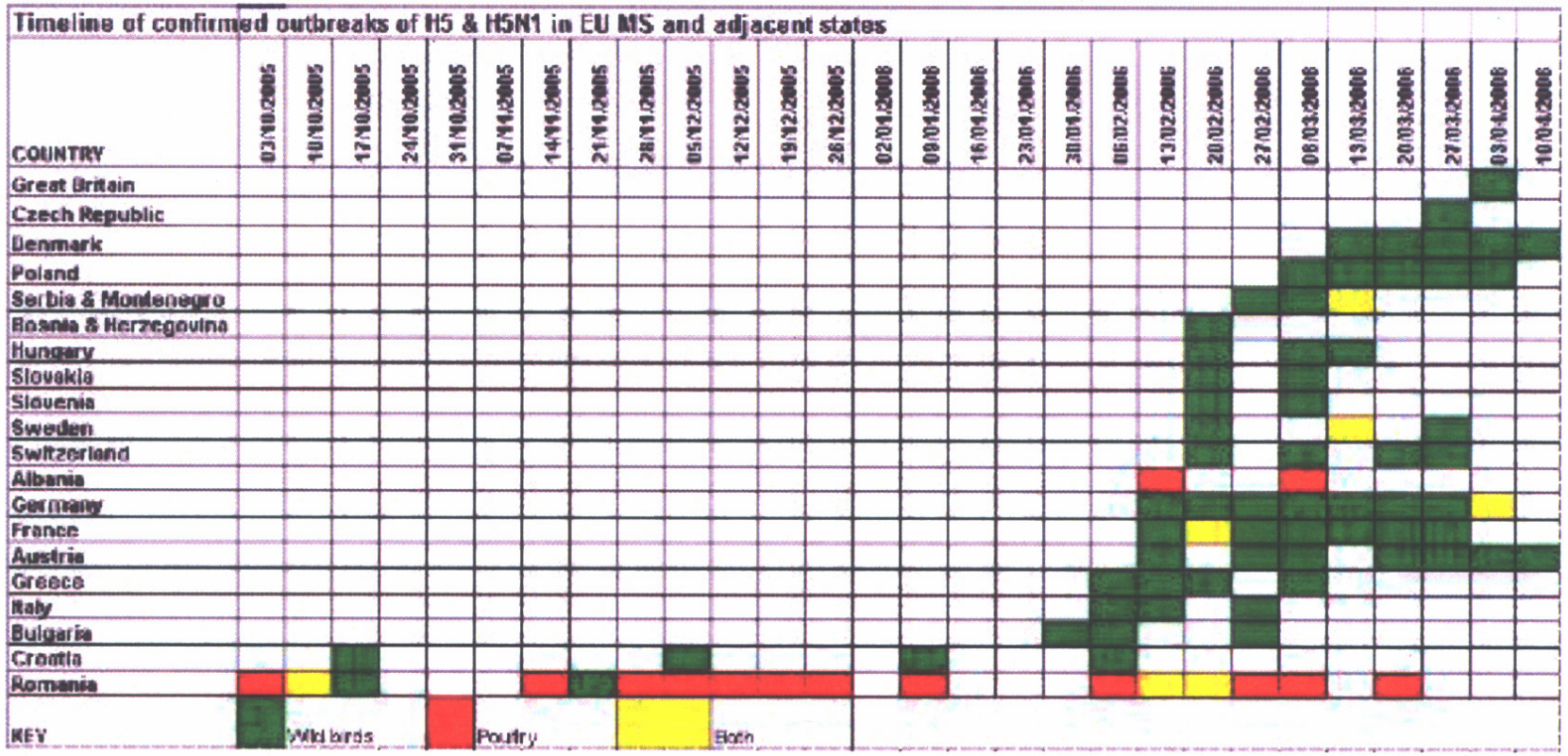
Fig. 3. HPAI cases in wild birds in the EU MS notified to ADNS from 1 February to 5 May 2006 (Total 615 cases)



Common threads

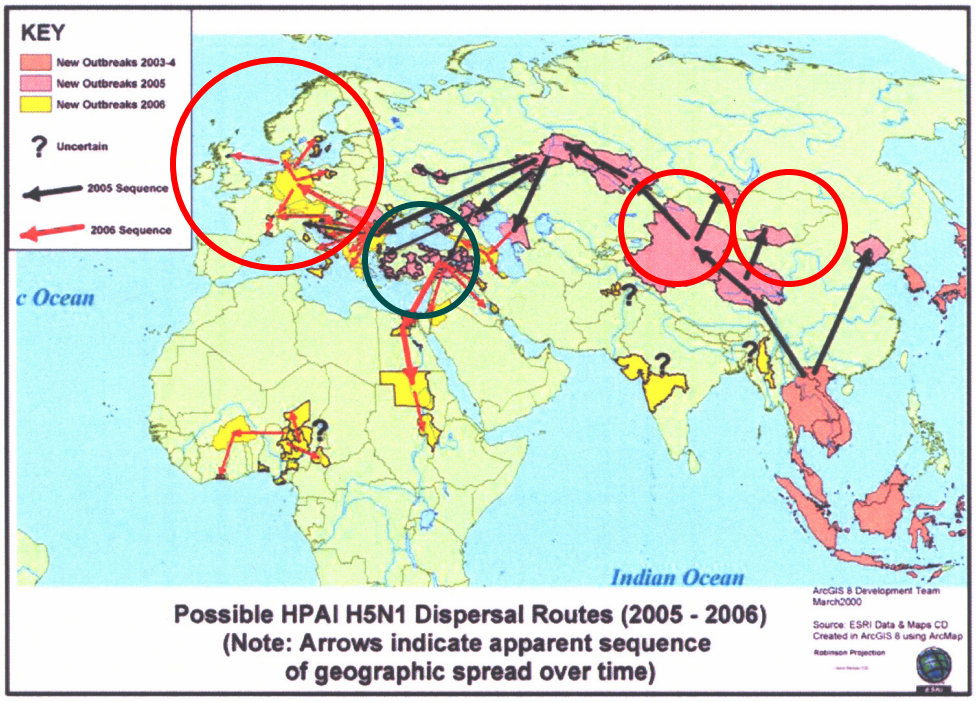
- Anseriformes
- Water birds
- Predators
- Scavengers

Fig.1. Affected EU Member States and the EU neighbouring countries



Primarily Poultry

Wild birds



indicate the apparent sequence of spatial (geographic) spread during the years 2005 and 2006.

- Wild bird viruses from China, Mongolia, and Europe are the same lineage

- The domestic poultry viruses of Croatia/Turkey have evolved

Fig. 2. Chart showing number of European countries affected each week since October 2005

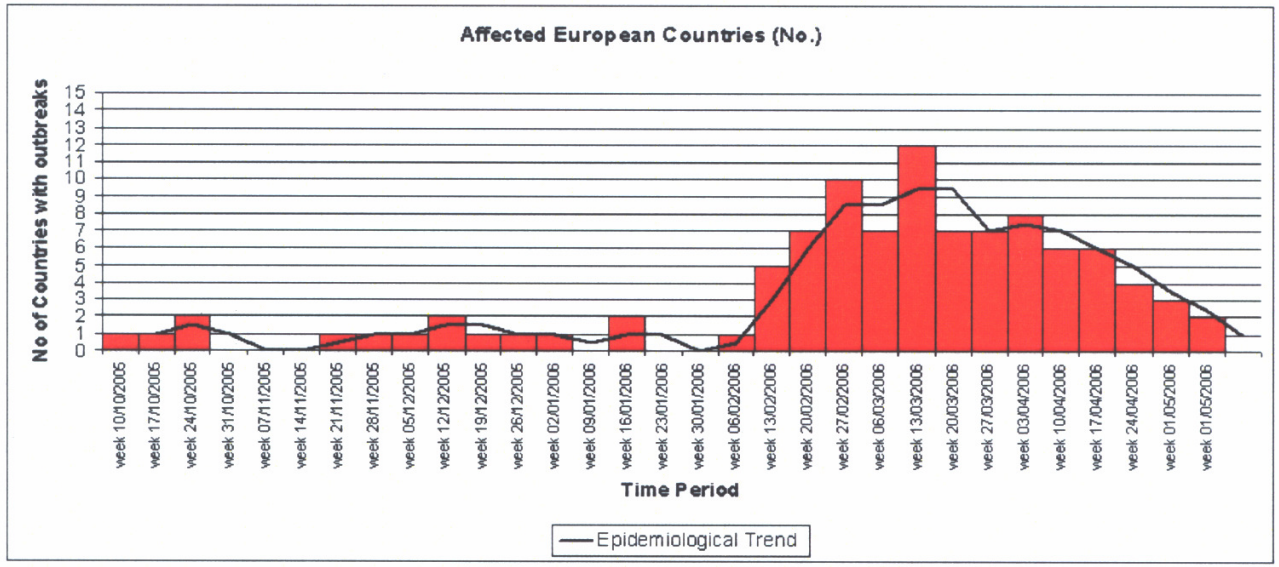
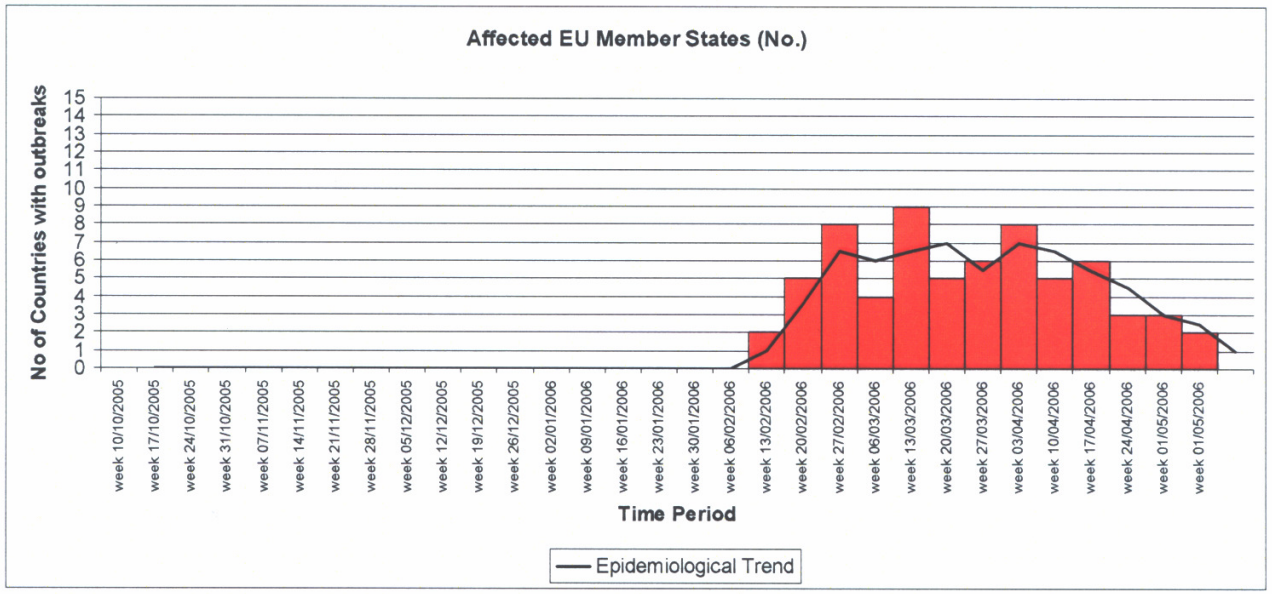


Fig. 3. Chart showing number of EU countries affected each week since February 2006



Epidemic curves

No new cases

Fig. 2. Chart showing number of European countries affected each week since October 2005

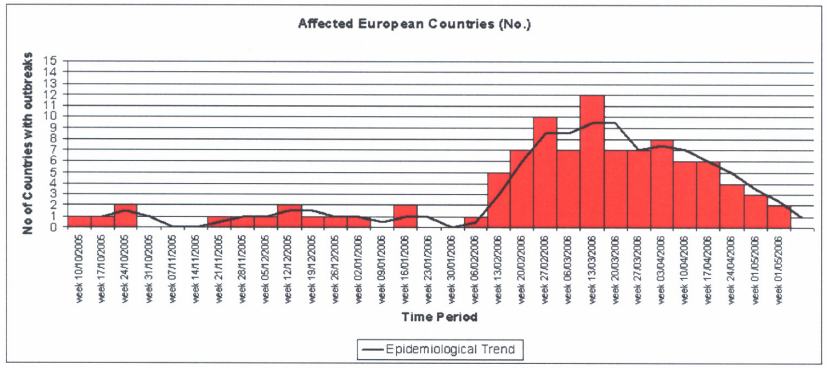
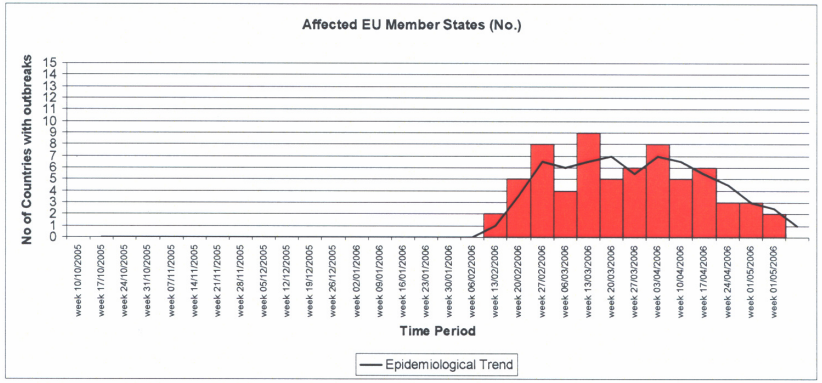


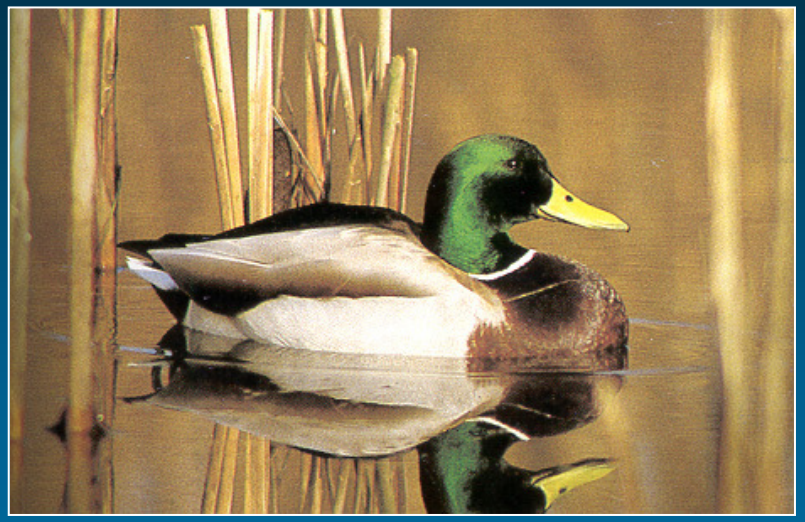
Fig. 3. Chart showing number of EU countries affected each week since February 2006



The movement of this virus into Europe probably resulted from the abnormal migration of birds associated with a very cold winter.

Maintained in highly susceptible species: mute swan and tufted ducks with transmission enhanced by climatic conditions.

Did it return to the breeding grounds with waterfowl?



Questions?

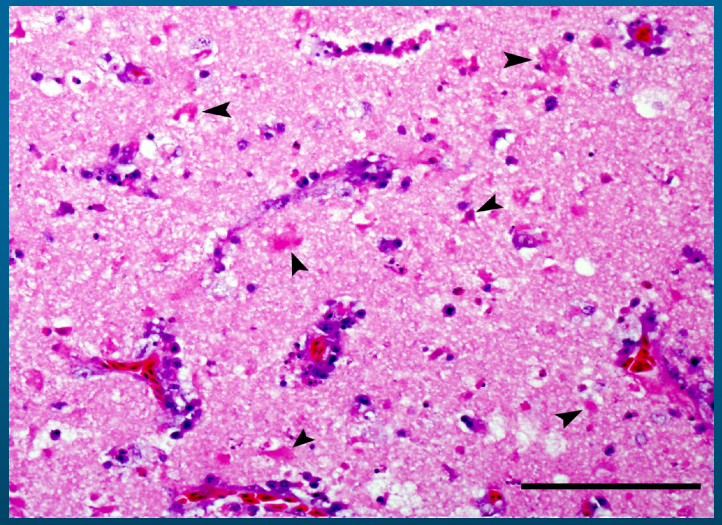
Gross Lesions

Gross lesions were only present in birds that died from AIV infection

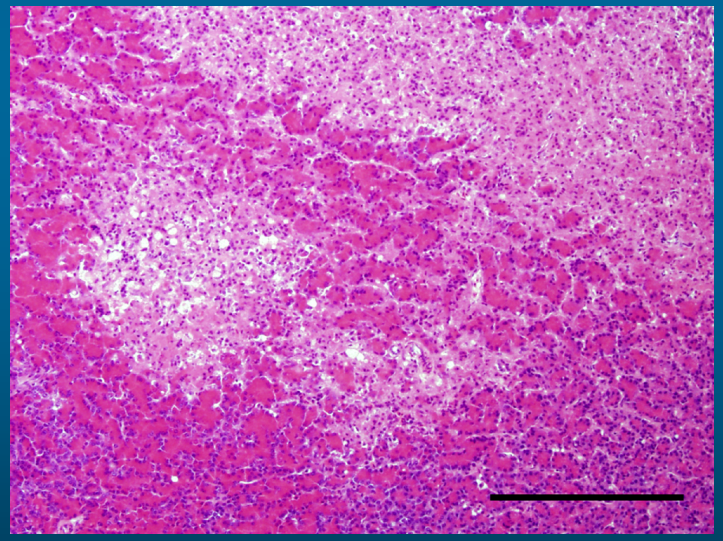
- Wood ducks
  - Petechial hemorrhage in the pancreas
- Laughing gulls
  - Hemorrhage in the ventriculus, apex of the heart, cerebrum, and pancreas

Microscopic Lesions

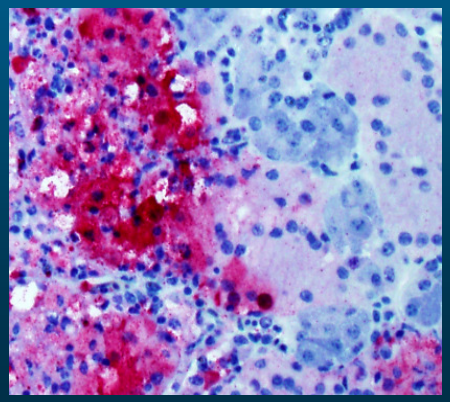
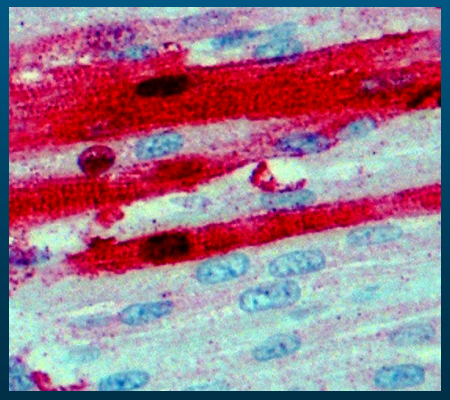
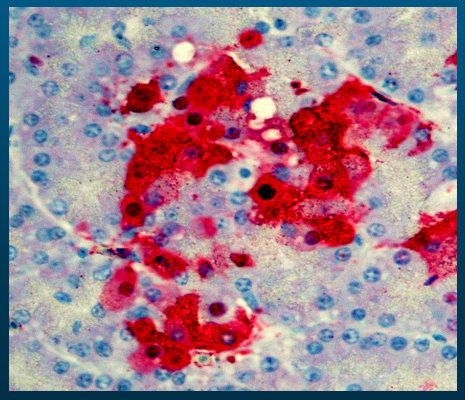
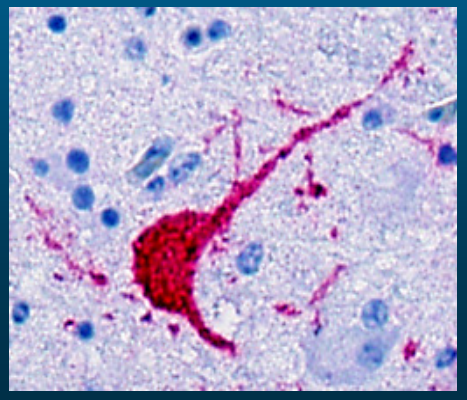
- Wood ducks
  - Neuronal necrosis
  - Myocardial necrosis
  - Necrotizing pancreatitis and adrenalitis



- Laughing gulls
  - Neuronal necrosis
  - Necrotizing pancreatitis and adrenalitis



In both species, histologic lesions were mild in birds that recovered and consisted of heterophilic pancreatitis and lymphoplasmacytic encephalitis

Viral Antigen

- Wood ducks
  - Primary sites
    - Brain, heart, adrenal glands, intestinal parasympathetic ganglia, and pancreas
  - Recovered birds
    - Brain
- Laughing gulls
  - Primary sites
    - Brain, pancreas, and adrenal glands
  - Recovered birds
    - Brain and pancreas