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Federal Research Institute for Animal Health

HPAI H5N1

A short review of the situation in Europe and Germany

A. Globig, T. Harder, E. Starick, J. Teuffert, F. Conraths,
T. Mettenleiter, M. Beer

Situation of the recent years

- **Before 2005 HPAIV H5N1 Asia has never been detected in Europe**
  (exception: smuggled Hawk Eagles from Asia to Brussels)

Situation of the recent years

- Before 2005 HPAIV H5N1 Asia has never been detected in Europe
- **In 2005/2006 HPAI H5N1 emerged in 23 European countries,**
  mainly affecting wild birds
  with sporadic spill-overs to poultry

HP H5N1 Situation in 2006

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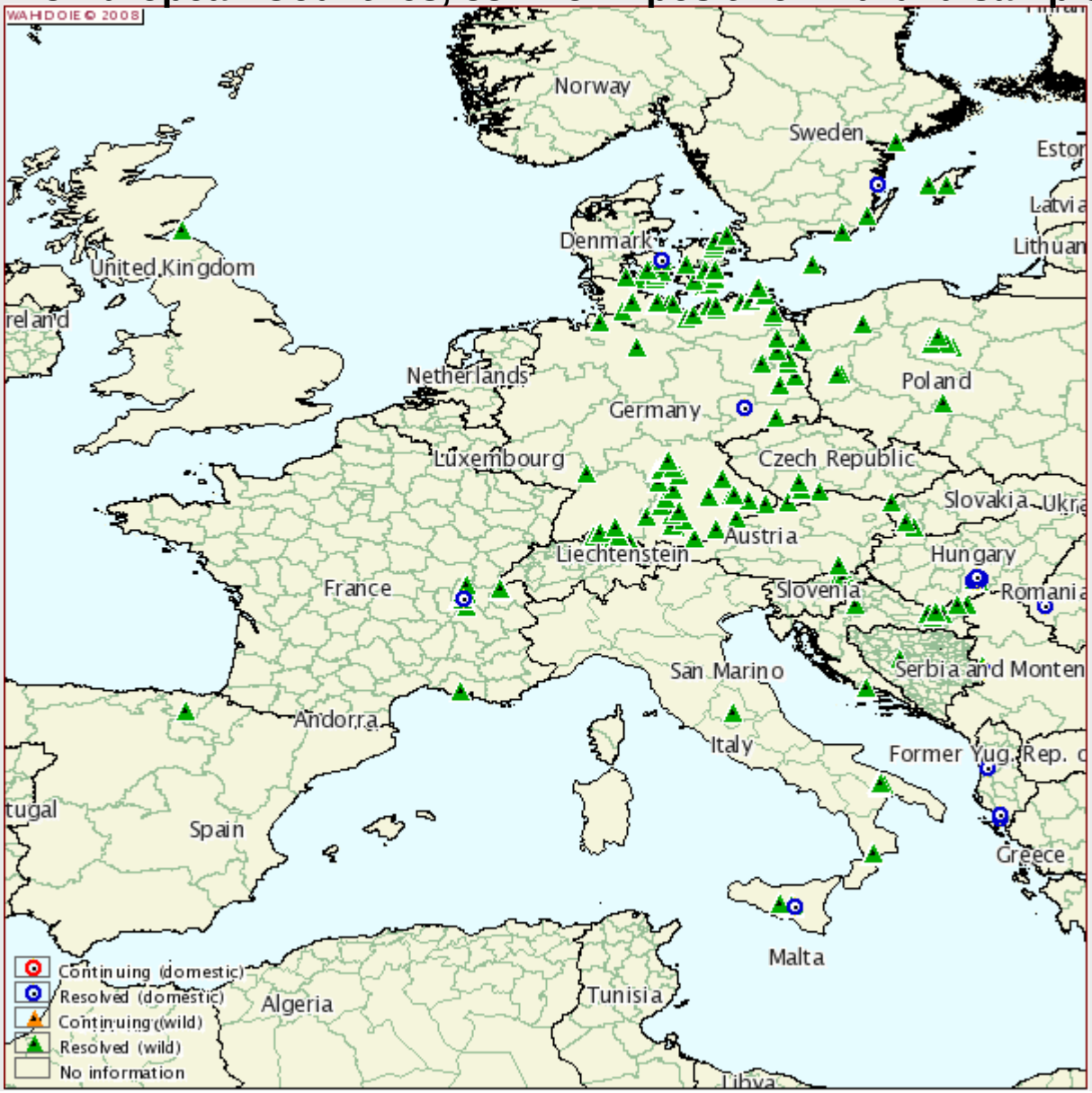
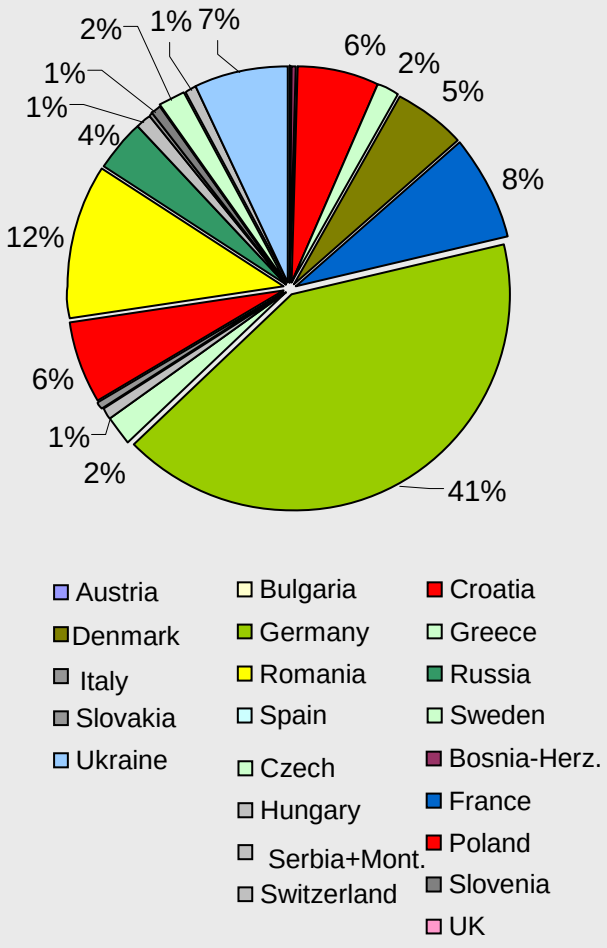
23 European Countries, 882 H5N1 positive wild bird samples



Dead and infected wild birds, rates per country



Data source: WAHID Interface 2006

HP H5N1 Situation in 2006

A.M.Kilpatrick et al., PNAS 103 (2006)19368-19373:

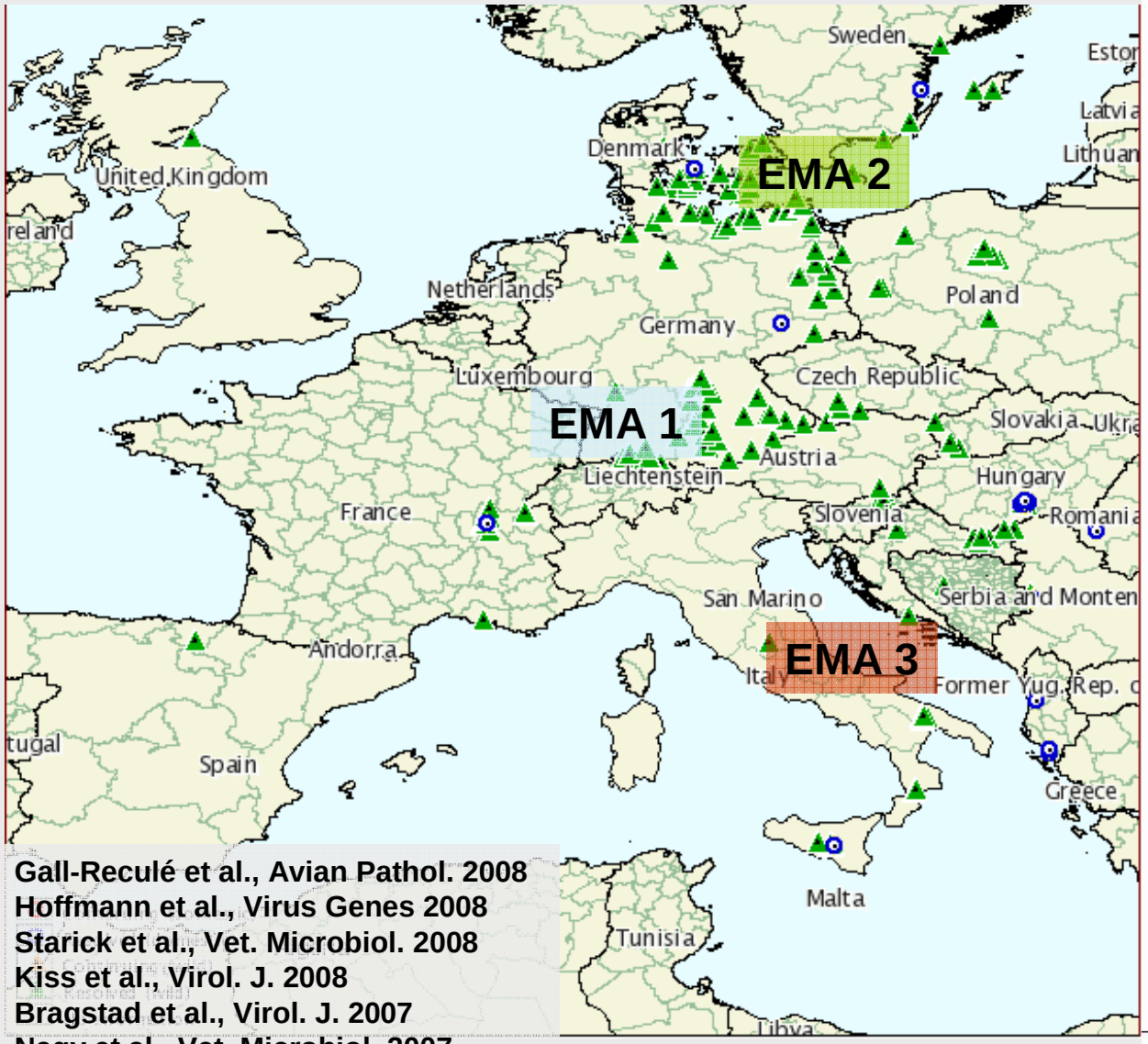
Combined data analysis on phylogenetic relationships of virus isolates & migratory bird movement & trade in poultry and wild birds

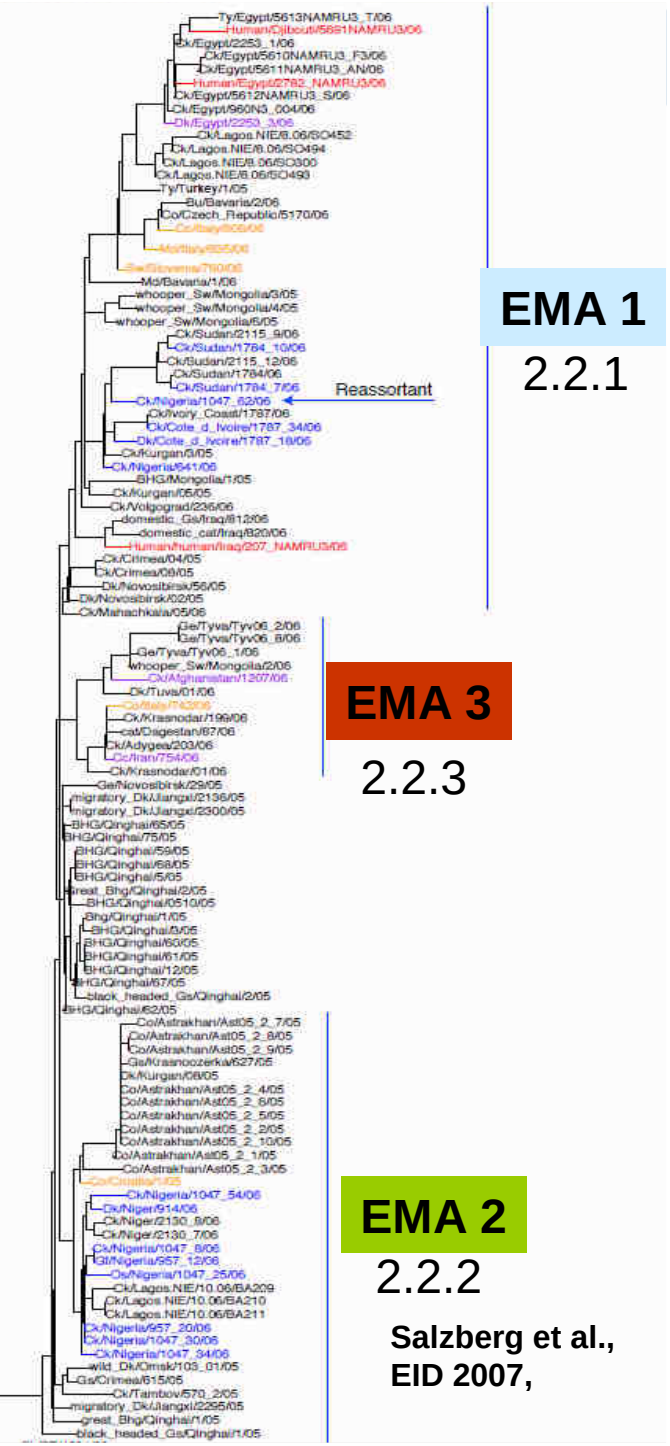
Migratory birds: most likely for H5N1 spread to 20 / 23 European countries in 2006 (3 exceptions: Romania, Albania, Spain)

Italy, France, Germany: introduction by cold weather-induced movement of wild birds further West was more likely than by trade in wild birds and poultry

HP H5N1 Situation in 2006

Circulation of multiple Qinghai-like H5N1 genotypes in Western Europe





Gall-Reculé et al., Avian Pathol. 2008
Hoffmann et al., Virus Genes 2008
Starick et al., Vet. Microbiol. 2008
Kiss et al., Virol. J. 2008
Bragstad et al., Virol. J. 2007
Nagy et al., Vet. Microbiol. 2007

Salzberg et al., EID 2007,

HP H5N1 Situation in 2006 - Germany

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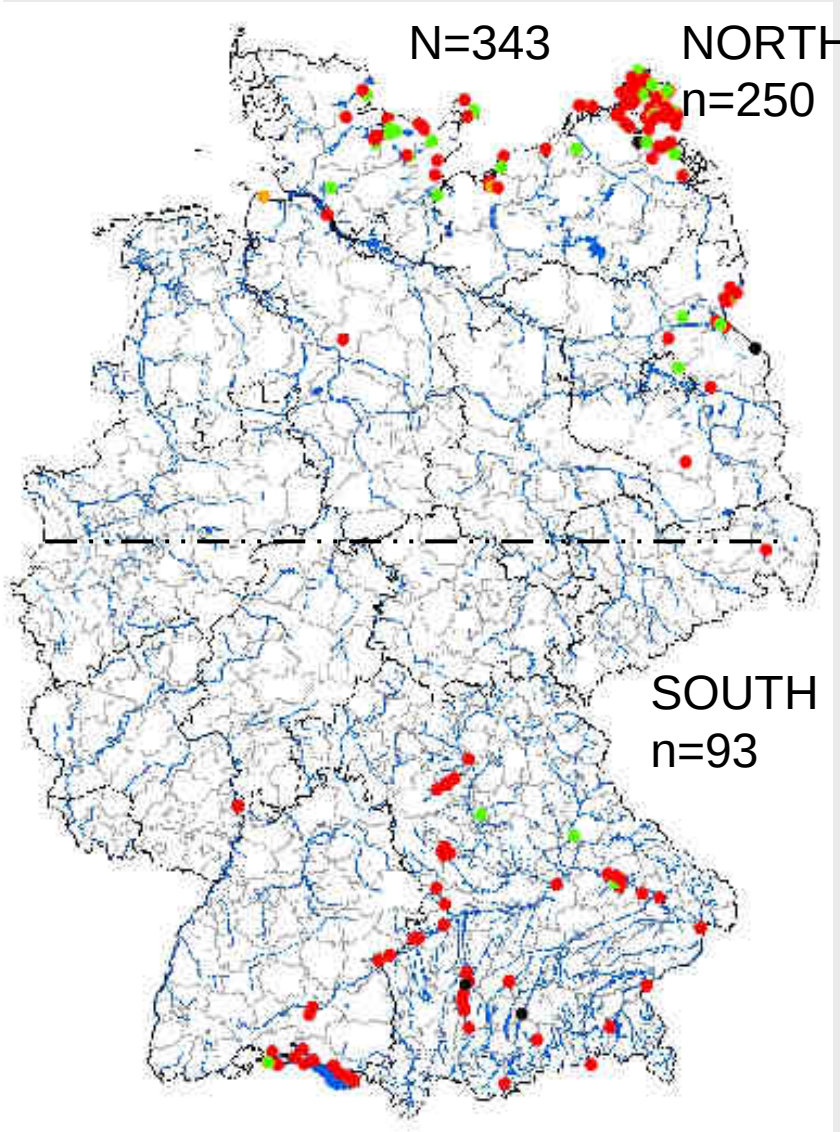
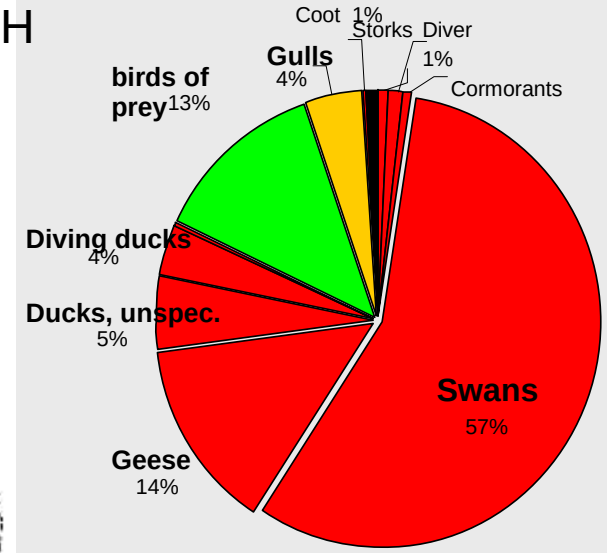
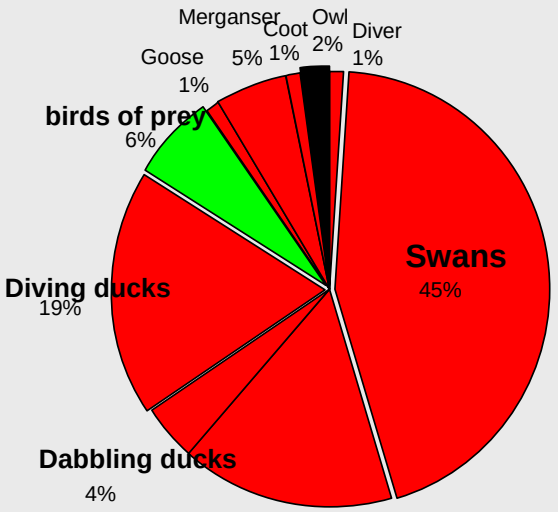


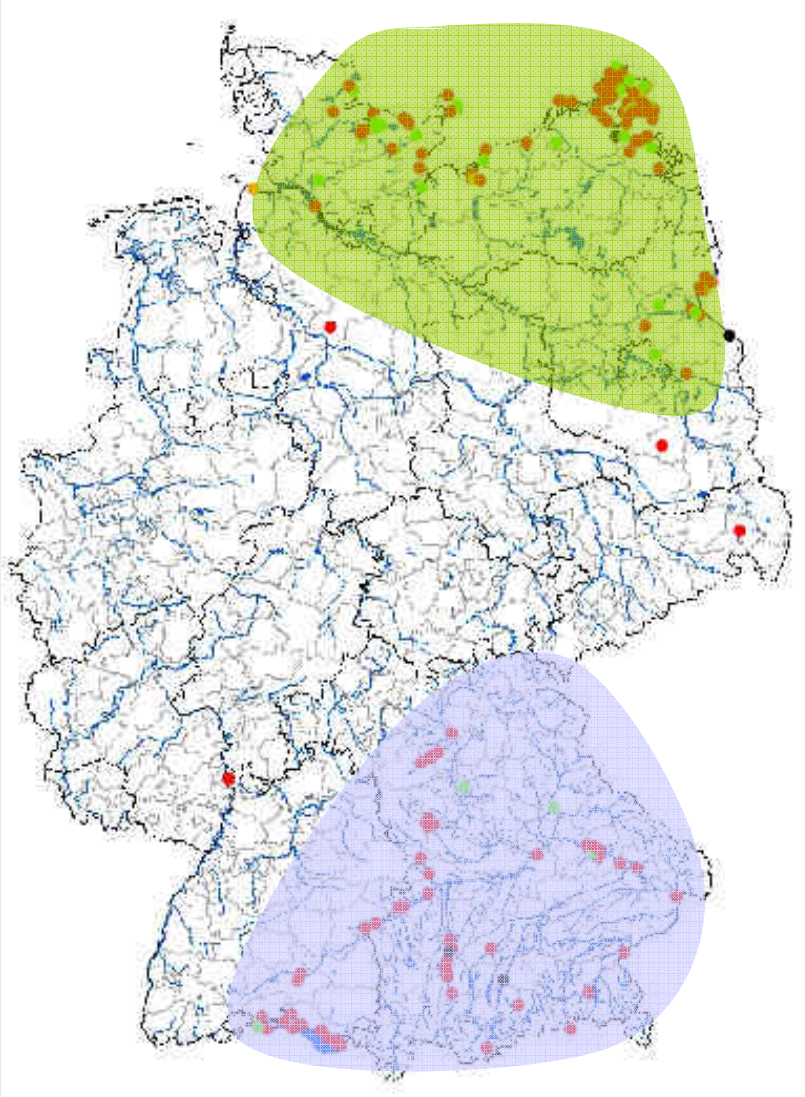


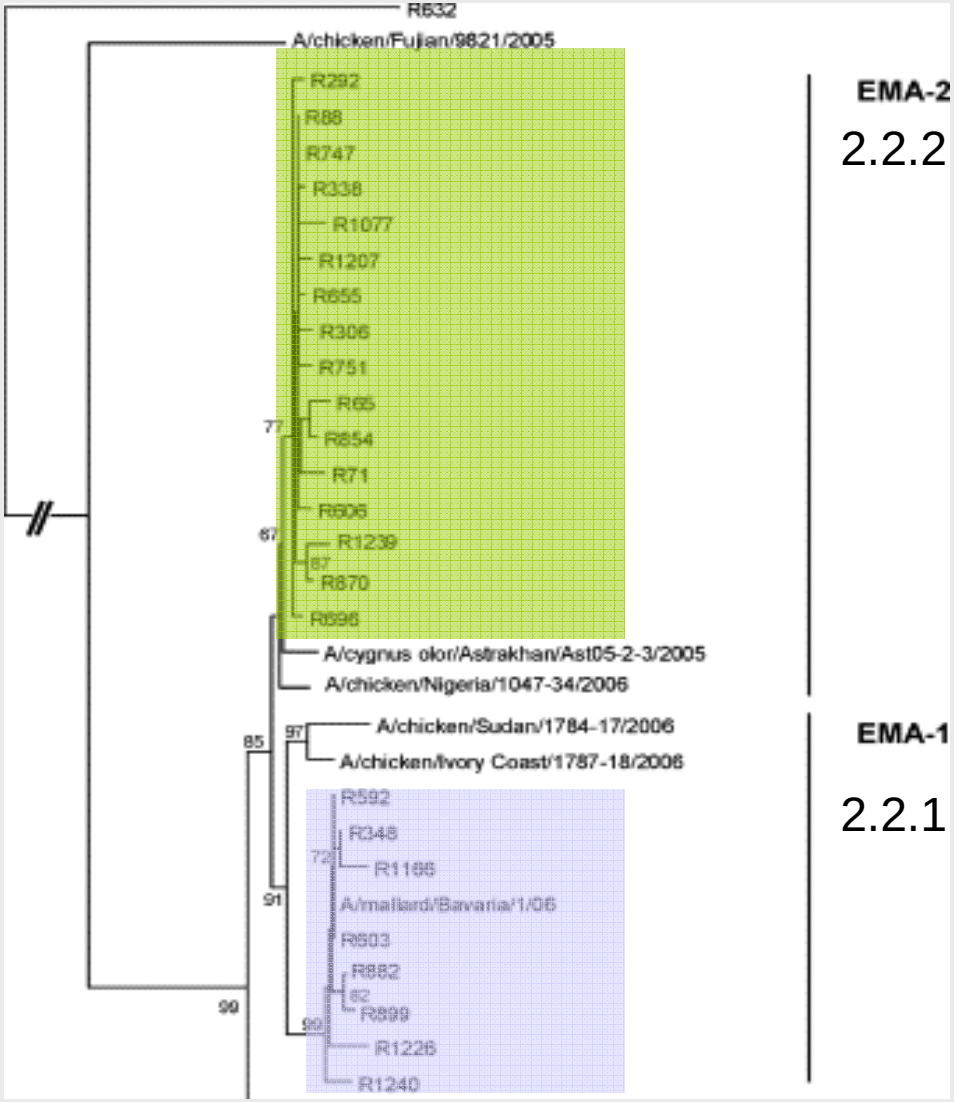


Local dispersal of the same virus after introduction

Globig et al., TBED in press

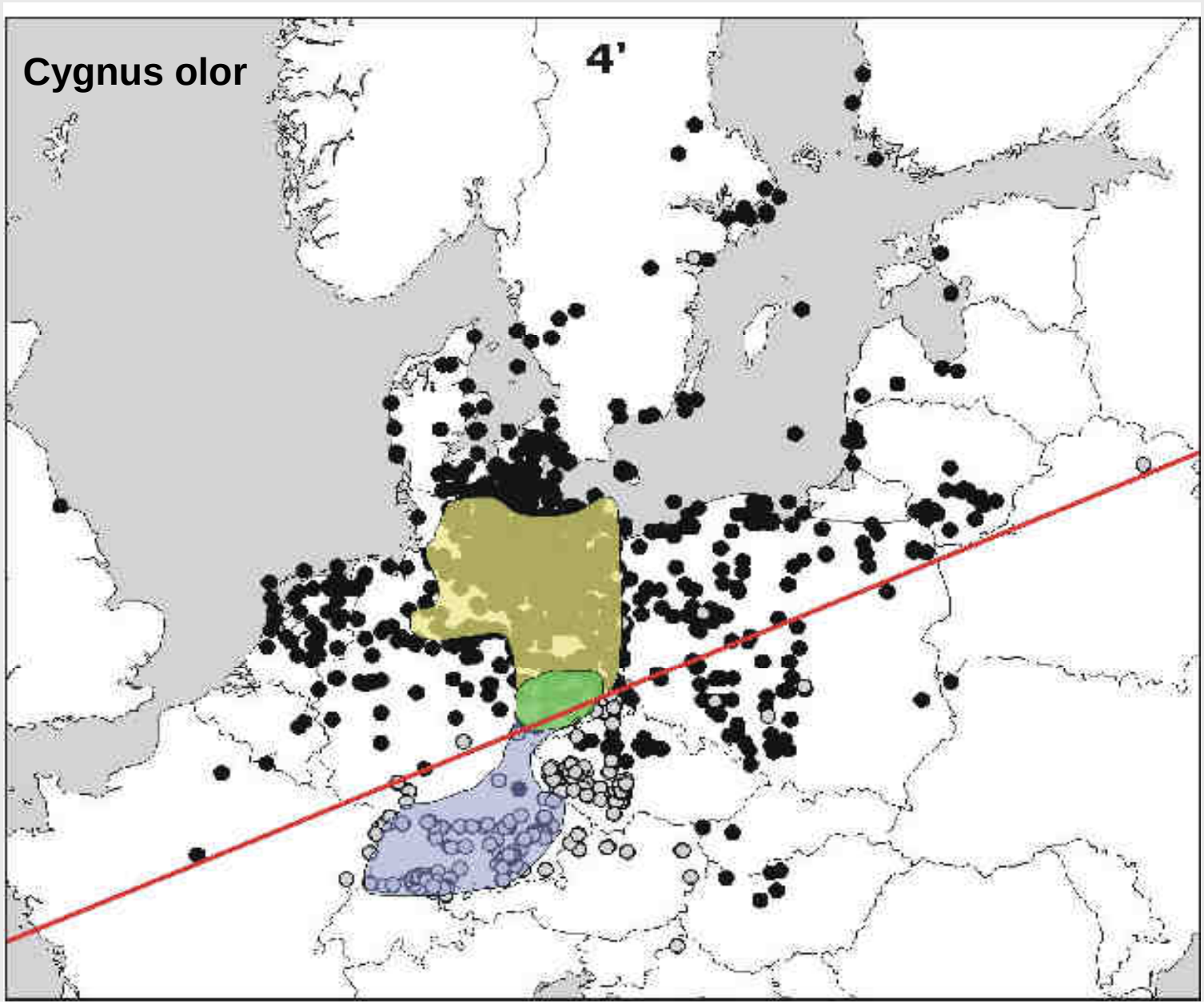
HP H5N1 Situation in 2006 - Germany





Starick et al. Vet. Microbiol. 2008

Data on ringed waterbirds North vers. South

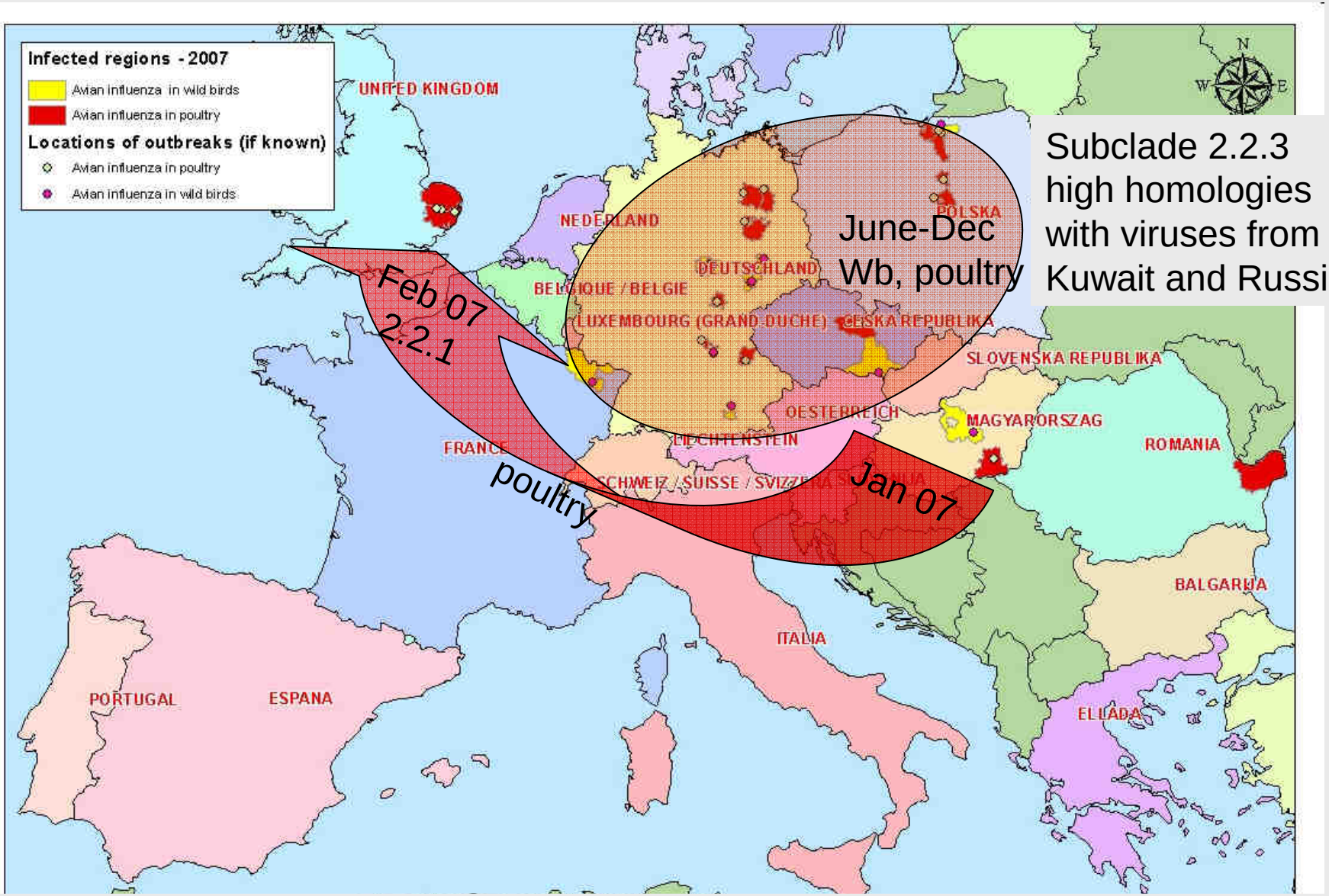


Situation of the recent years

- Before 2005 HPAIV H5N1 Asia has never been detected in the European Union (EU)
- In 2006 HPAI H5N1 emerged in 23 European Countries, mainly affecting wild birds with sporadic spill-overs to poultry

- **In 2007 HPAI H5N1 occurred in Russia, Hungary and Great Britain; in Czech Republic, France, Germany and Russia; in Romania and Poland - affecting poultry and wild birds on large scale**

HP H5N1 Situation in 2007 - Europe



http://ec.europa.eu/food/animal/diseases/adns/adns_maps_poul_wb_2007.jpg

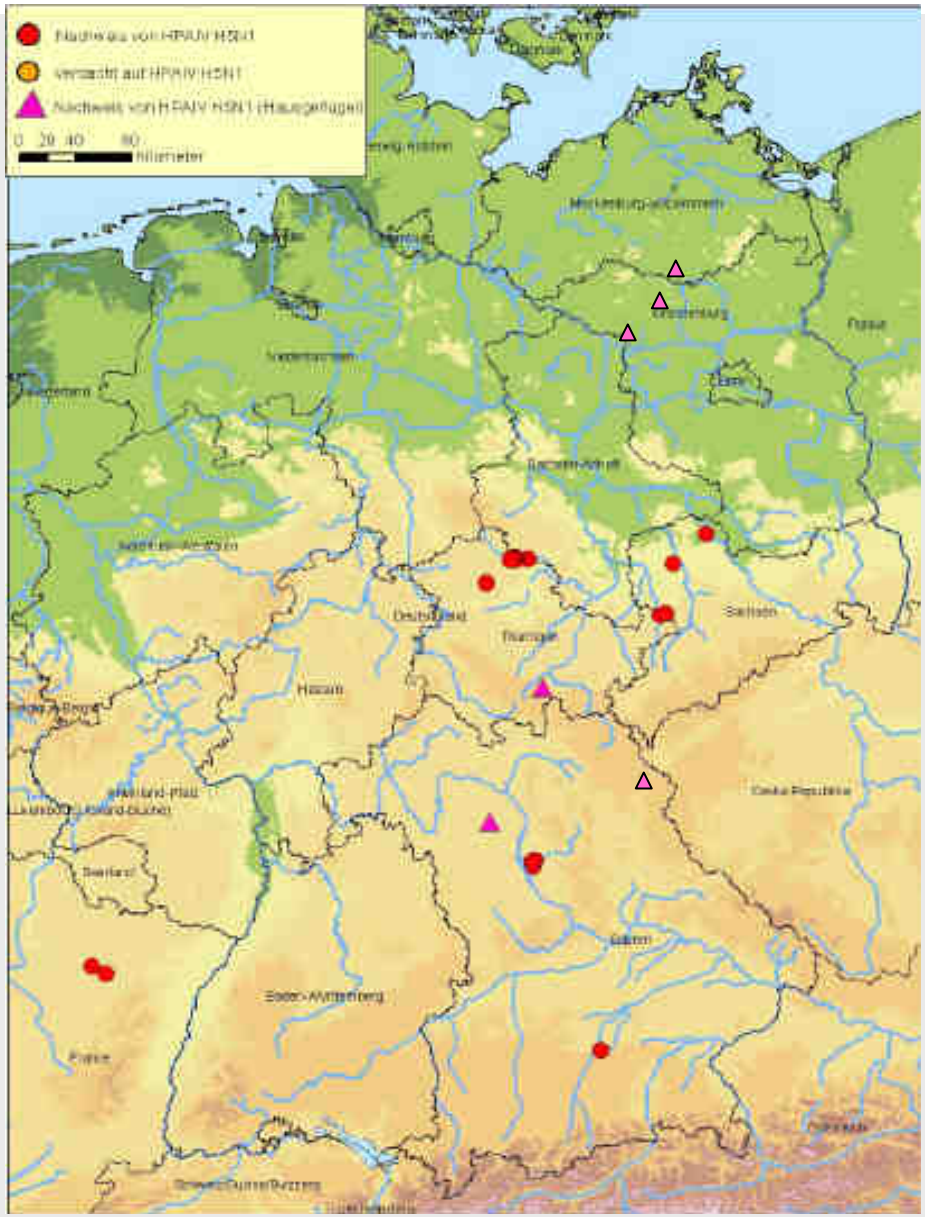
HP H5N1 Situation in 2007 - Europe

- 14 outbreaks in domestic poultry have been reported in six Member States

- Although three countries (Hungary, Germany and the Czech Republic) have also reported cases in wild birds, the timing and location of cases in domestic poultry offered no obvious epidemiologcial link to wild bird infection in the majority of cases.

-> entry into domestic poultry most probably through trade in poultry products, or via fomites (esp. Hungary-United Kingdom), spill-over to wild birds

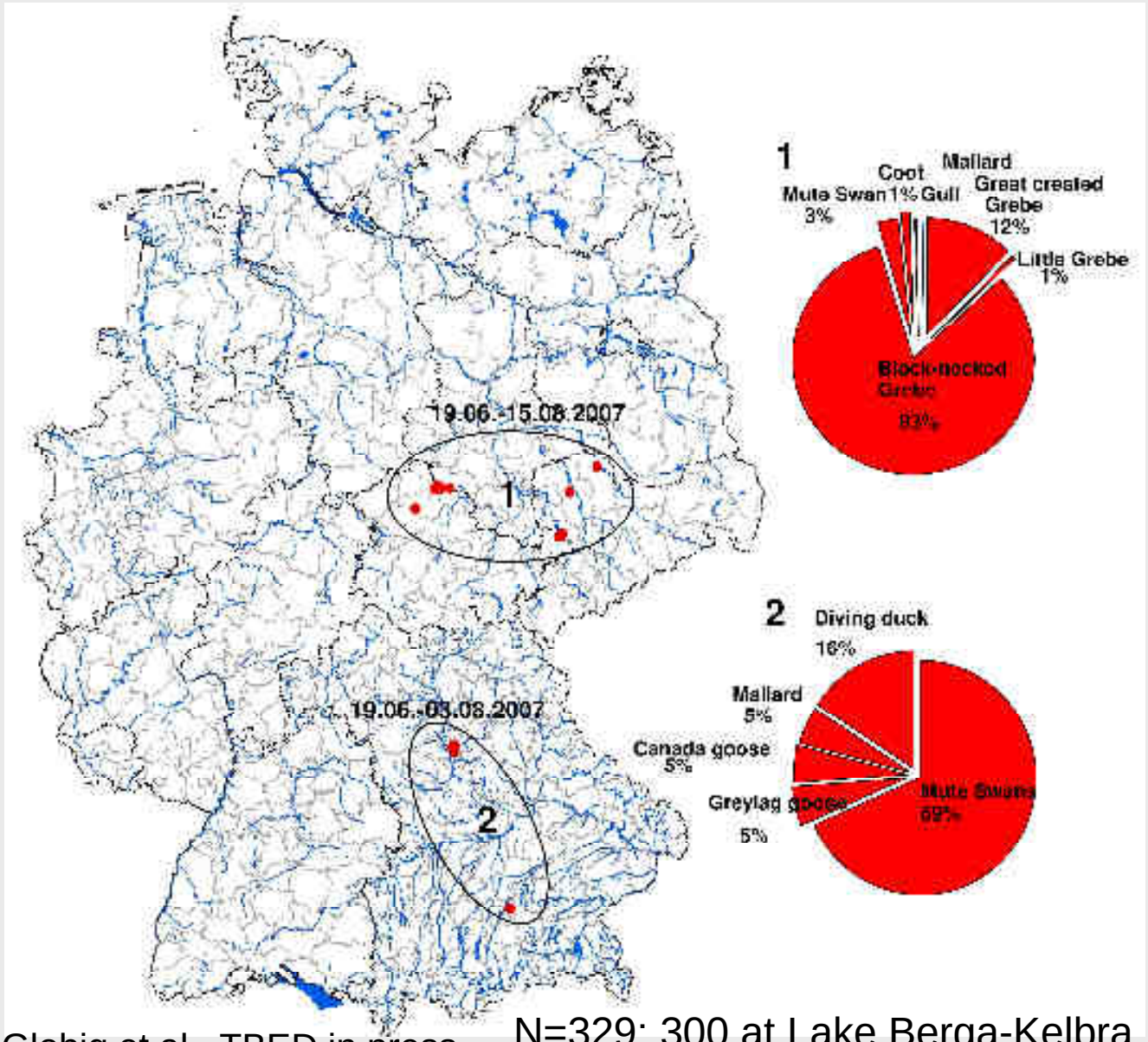
HPAIV H5N1 in Germany, 2007



Two outbreak phases:

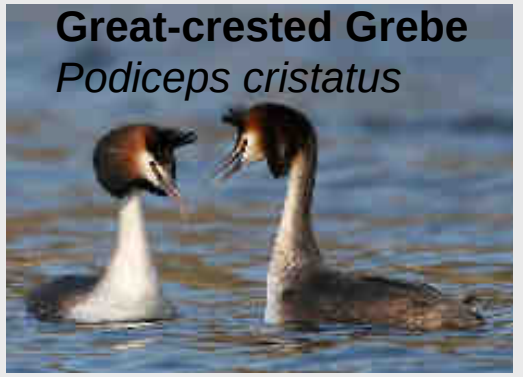
- **June-August 2007 (mainly wild birds, 2 industrial duck holdings, 1 backyard)**
- **December 2007 (3 back-yard chicken holdings)**

HPAI H5N1 in wild birds in Germany, 2007



Globig et al., TBED in press

N=329; 300 at Lake Berga-Kelbra



HPAI H5N1 in poultry in Germany, 2007

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	Date of detection		Federeal state	County	Poultry	Size of holding	died
1	06.07.		TH	Saalfeld-Rudolstadt	Ducks, geese	10	1
2	25.08.	A	BY	Erlangen-Höchstadt	Fattening ducks	169.857	4.000
3	10.09.	B	BY	Hofing/Schwandorf	Fattening ducks	170.856	0
4	15.12.		BB	Oberhavel	Chickens	11	10
5	21.12.		BB	Potsdam Mittelmark	Chickens	30	19
6	25.12.		BB	Ostprignitz	Chickens	15	11

Clinical signs

- **Ducks: None, if aged > 10 days; mortality only in association with opportunistic bacterial/parasitic infections, daily mortality rates below 1,5%**
- **Geese: Neurologic symptoms**
- **Chickens: Apoplectiform deaths, severe depression**

Phylogenetic analysis (HA gene)

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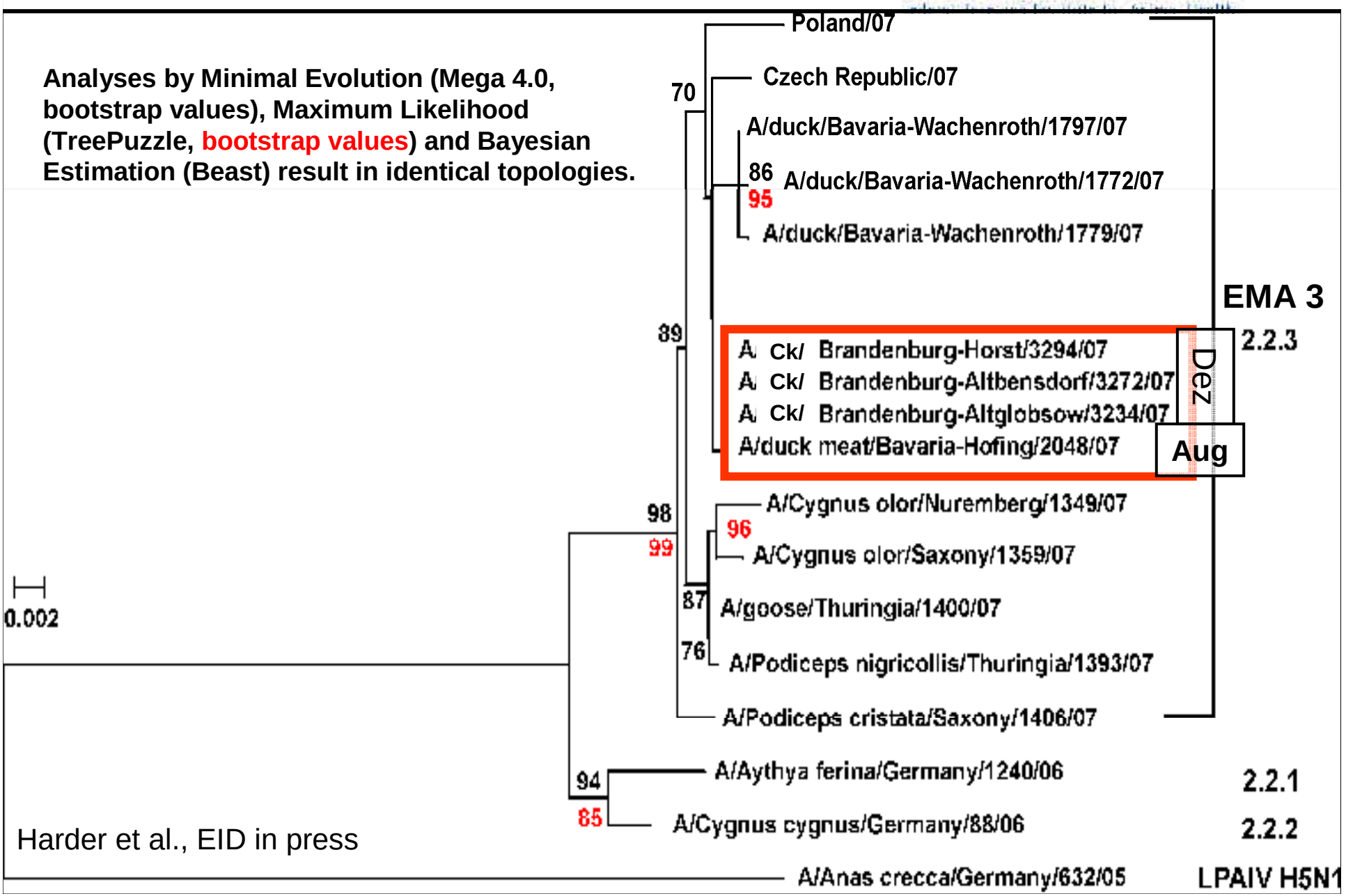
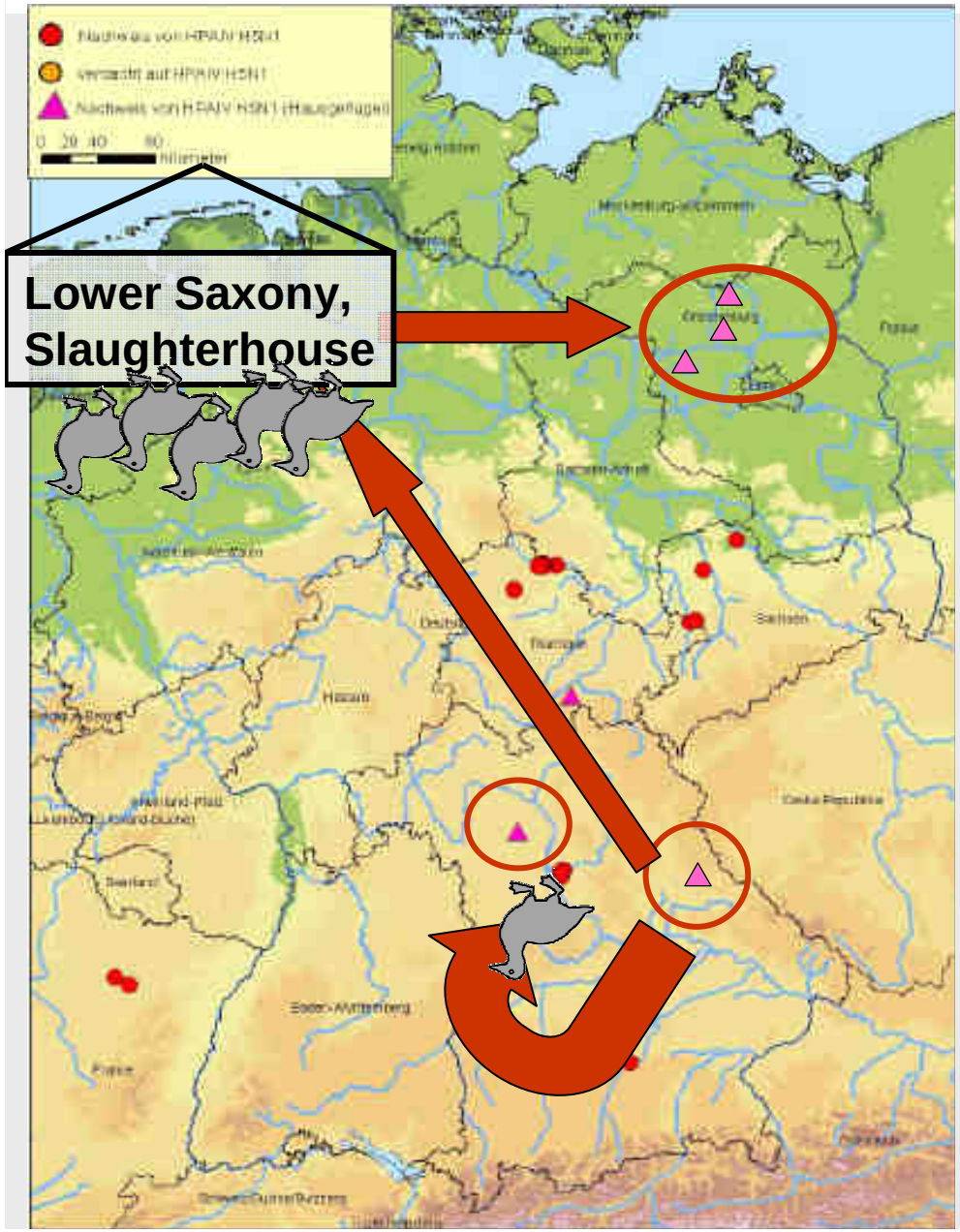
Analyses by Minimal Evolution (Mega 4.0, bootstrap values), Maximum Likelihood (TreePuzzle, bootstrap values) and Bayesian Estimation (Beast) result in identical topologies.



Harder et al., EID in press

Reconstruction of HPAIV H5N1 poultry cases



Brandenburg

„Feeding“ offal of christmas ducks to chickens

Bavaria-B

Incursion in June??
Silent spread, no mortality

Bavaria-A, Slaughterhouse

Clinically „overt“ infections in August, retain samples of slaughter slots from B tested positive for HP H5N1

Harder et al., EID in press

Plea for regular, virological duck monitoring

- **High risk of silent circulation in ducks**
- **High risk of spread to gallinaceous poultry where disease becomes visible**
- **Increased risk of spread to workers in duck poultry holdings**
- **Risk of incursion into the food chain**

- **No syndrome surveillance possible (cumulative mortality, opportunistic infections)**
- **Sentinel placement difficult**

Situation of the recent years

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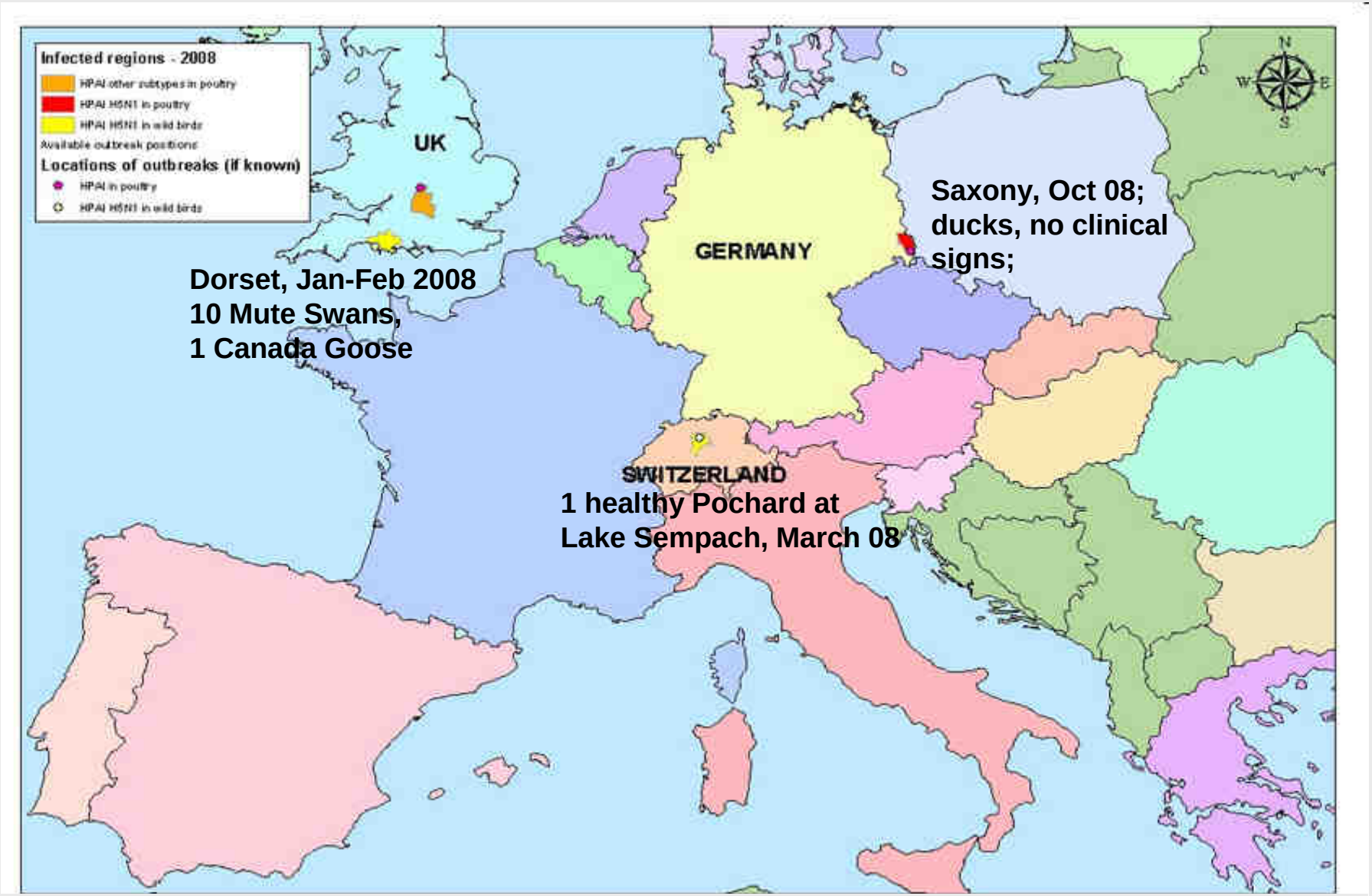
- In 2006 HPAI H5N1 emerged in 23 European Countries
- In 2007 HPAI H5N1 occurred in Russia, Hungary and Great Britain; in Czech Republic, France, Germany; in Romania and Poland – affecting poultry and wild birds on large scale

- **In 2008 HPAIV H5N1 was only sporadically detected from samples from wild birds in GB (Jan/Feb, n=11) and Switzerland (n=1) and in one mixed poultry holding in Germany**

HP H5N1 Situation in 2008



http://ec.europa.eu/food/animal/diseases/adns/adns_maps_poul_wb_2008.jpg

HP H5N1 Situation in Germany, 2008

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02/10/2008: Monitoring: 30 ducks (1x hp H5N1 positive), 30 geese (negative)
Subsequent slaughter of all ducks (24 hp H5N1 positive)

800 geese*
550 ducks*
60 chicken*
245 turkeys

106 cattle
4 sheep
8 pigs
1 pony
1 donkey

* free range holding

Only ducks were found positive for HPAIV H5N1, no mortality
Source of introduction remains unclear (no cases in radius of 2000 km)

Summary

The 2007/2008 HPAI H5N1 outbreaks represent new incursions of virus into Europe, however:

a) The source remains unknown.
b) No wild bird reservoir of the virus has been identified.
c) It is possible that once introduced, wild birds may have spread the virus over short distances.

Conclusions

- The re-emergence of genetically similar viruses in widely distant geographic locations (e.g. Kuwait-Germany-Russia) indicates that clade 2.2 - H5N1 viruses may have become endemic in Central or Eastern Asia (including Siberia, Russia)

- From here they have been repeatedly introduced to Europe, the Middle East and Africa

- Continuous investigation of both, wild birds and poultry will give more insights of the prevalence of HPAIV H5N1 clade 2.2 in Europe


J´aimerais vous remercier
pour votre aimable attention

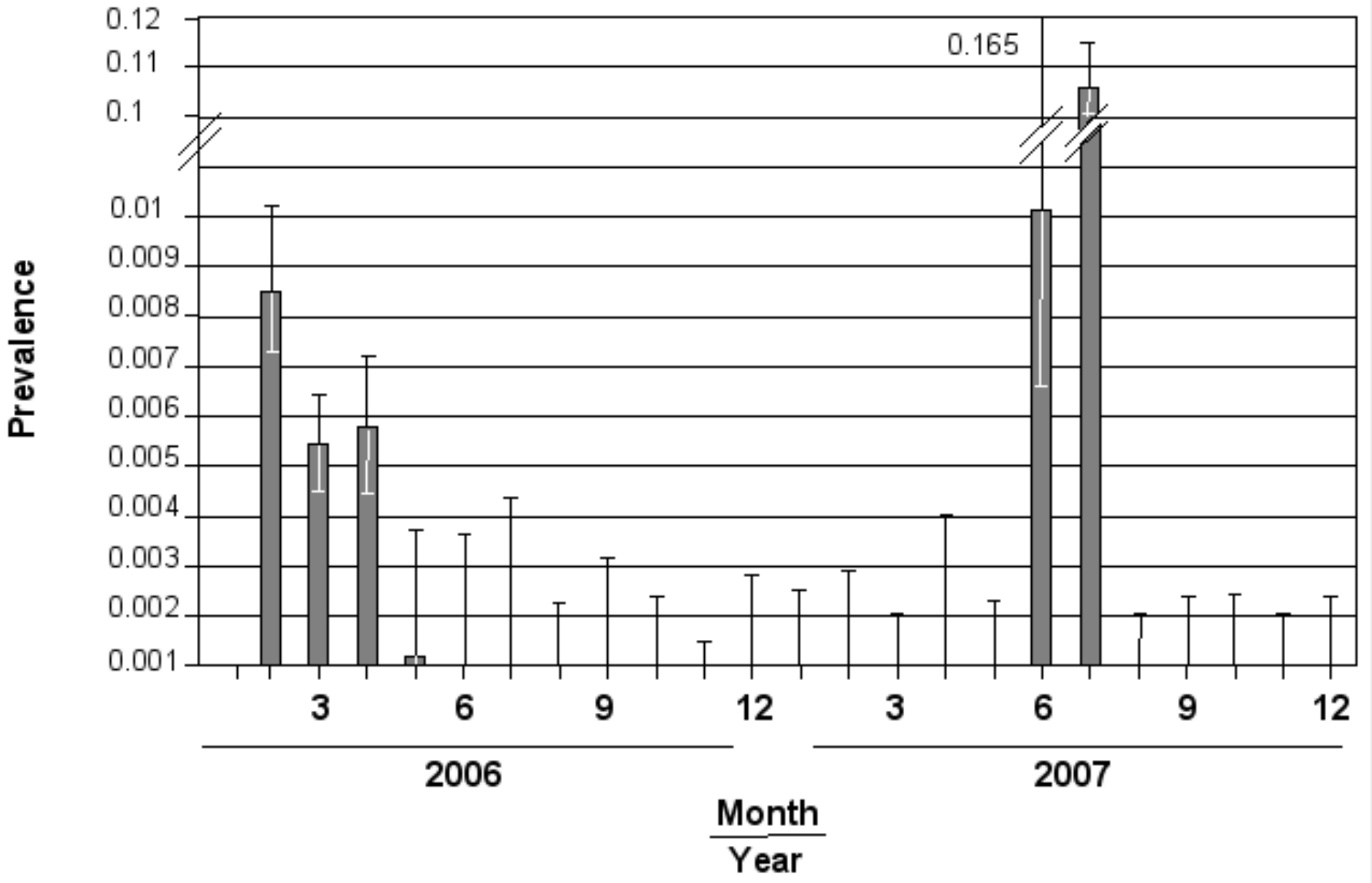
Recommendation of Duck Monitoring

- **Virologic monitoring (rRT-PCR) of all duck holdings > 1000 animals:**

  Oropharyngeal/cloacal swabs of 60 dead ducks, taken during a period of three weeks

- **Continuous serologic screening during slaughter-course:**

  40 blood samples (slaughter-batch) with cELISA as screening-test.


0.12
0.11
0.1
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.165
Prevalence
3
6
9
12
3
6
9
12
2006
2007
Month
Year

Mortality rates fattening ducks (Bavaria 2)

Hatched 20.06.2007 (house 5, slaughtered 01.08.2007):
2 HPAIV H5N1-positive samples (isolates available) detected in frozen retain samples.

Mortality rates fattening ducks (Bavaria 2)

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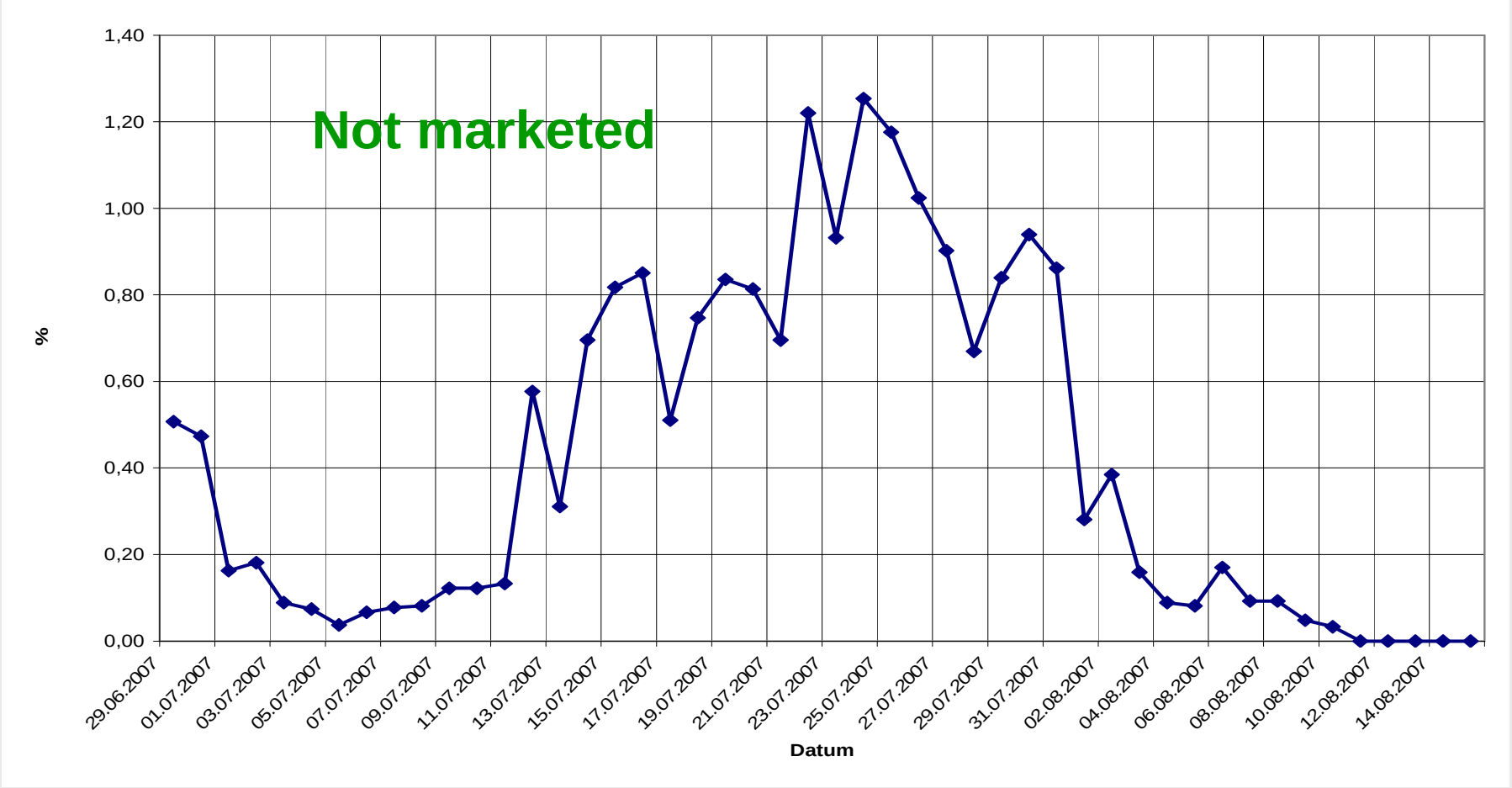
Hatched 29.06.2007 (house 1, slaughtered 11.08.2007):
1 HPAIV H5N1-indiscriminate samples (H5N1 ct 36 non-typable) detected in frozen retain samples.



Mortality rates fattening ducks (Bavaria 2)

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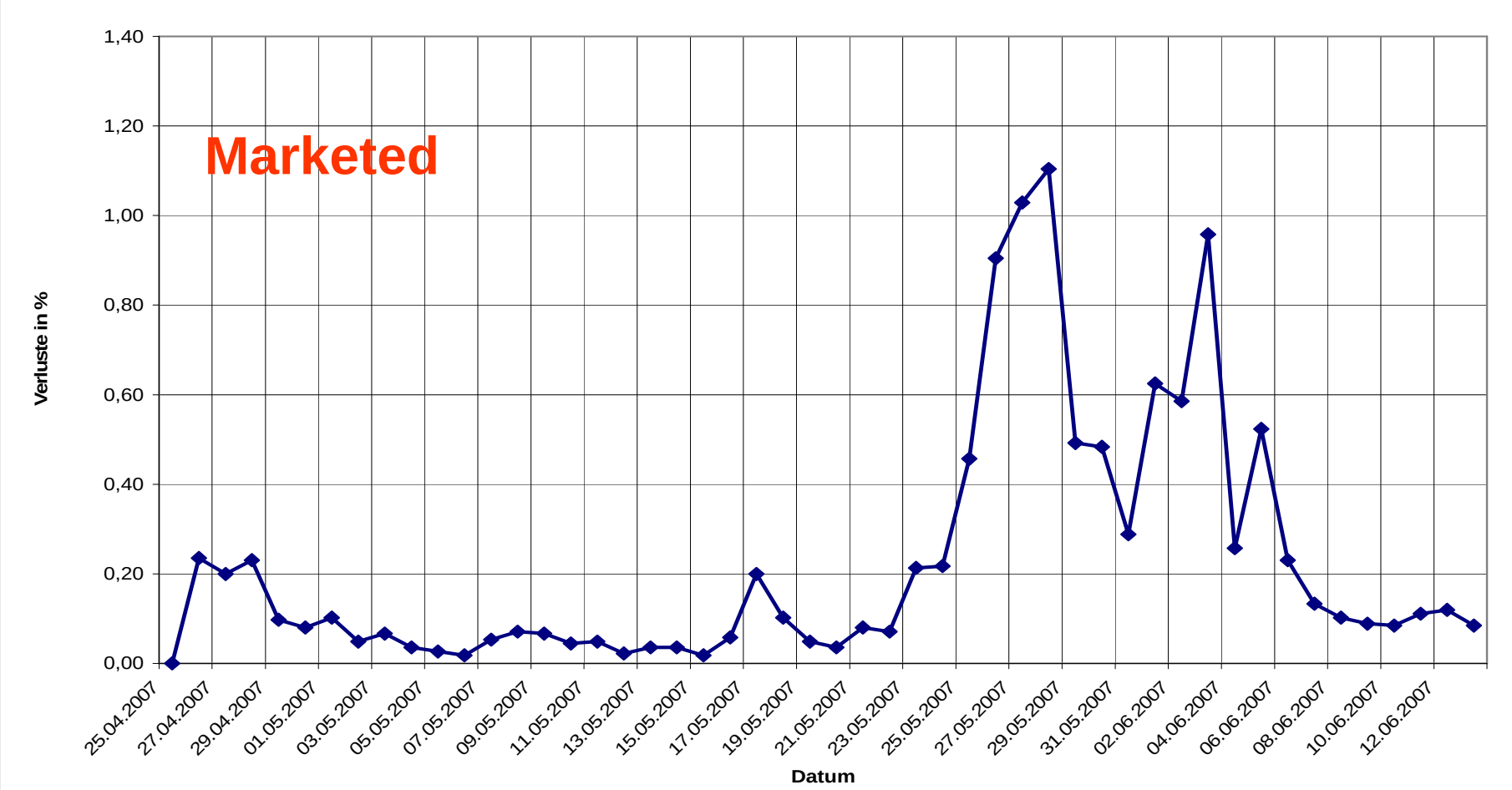
Hatched 25.04.2007 (house 5, slaughtered 14.06.2007): No retain material available.



HP H5N1 Situation in 2006

Most of the 33 domestic poultry outbreaks reported in five affected Member States (Hungary (29), Sweden (1), Germany (1), France (1), Denmark (1)) were preceded by the positive identification of virus in wild birds in the vicinity of the index case

-> strong epidemiological link to the source of infection (spill-over from wild birds)

HPAI H5N1 outbreaks July-Oct 05

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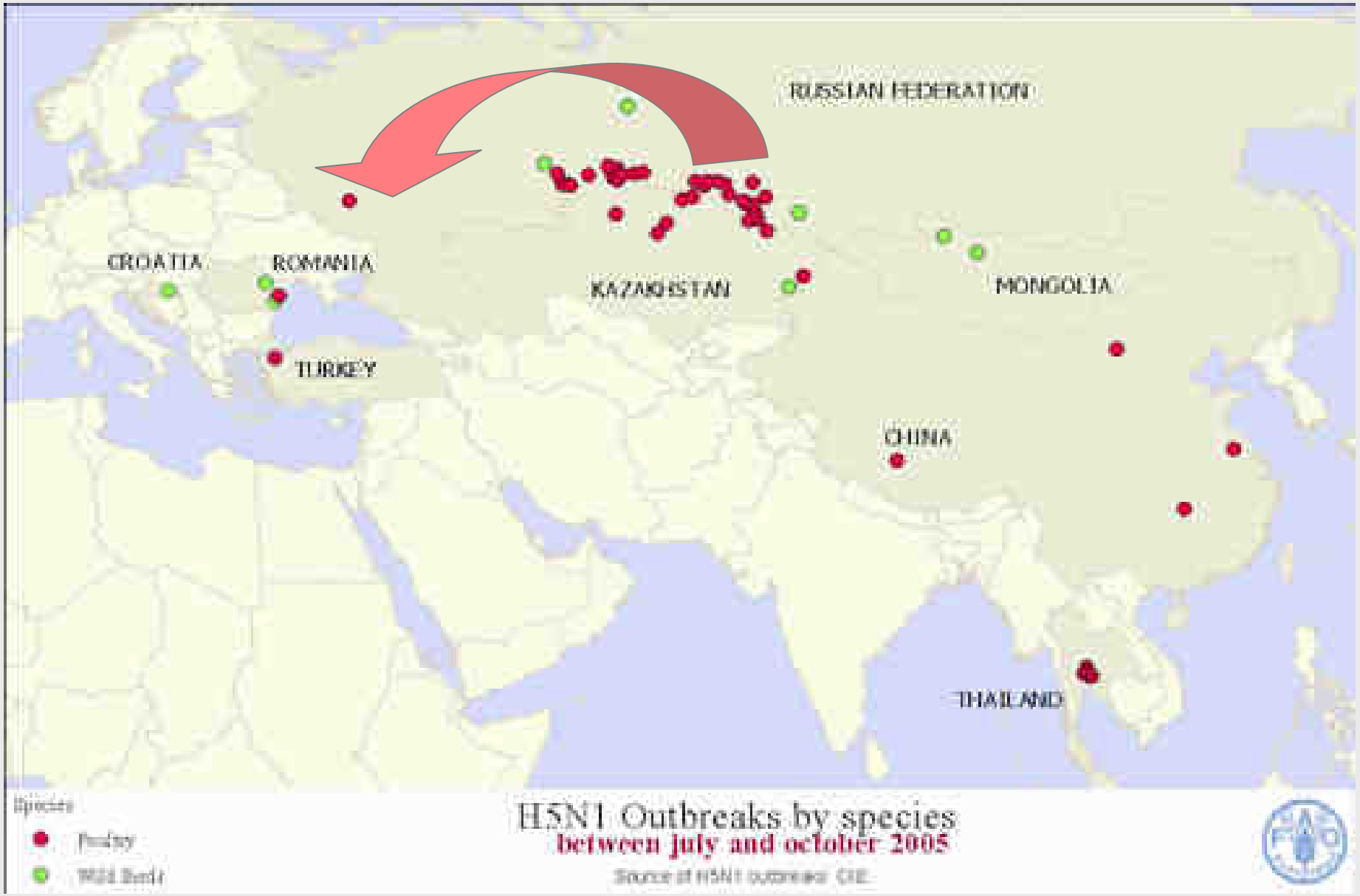

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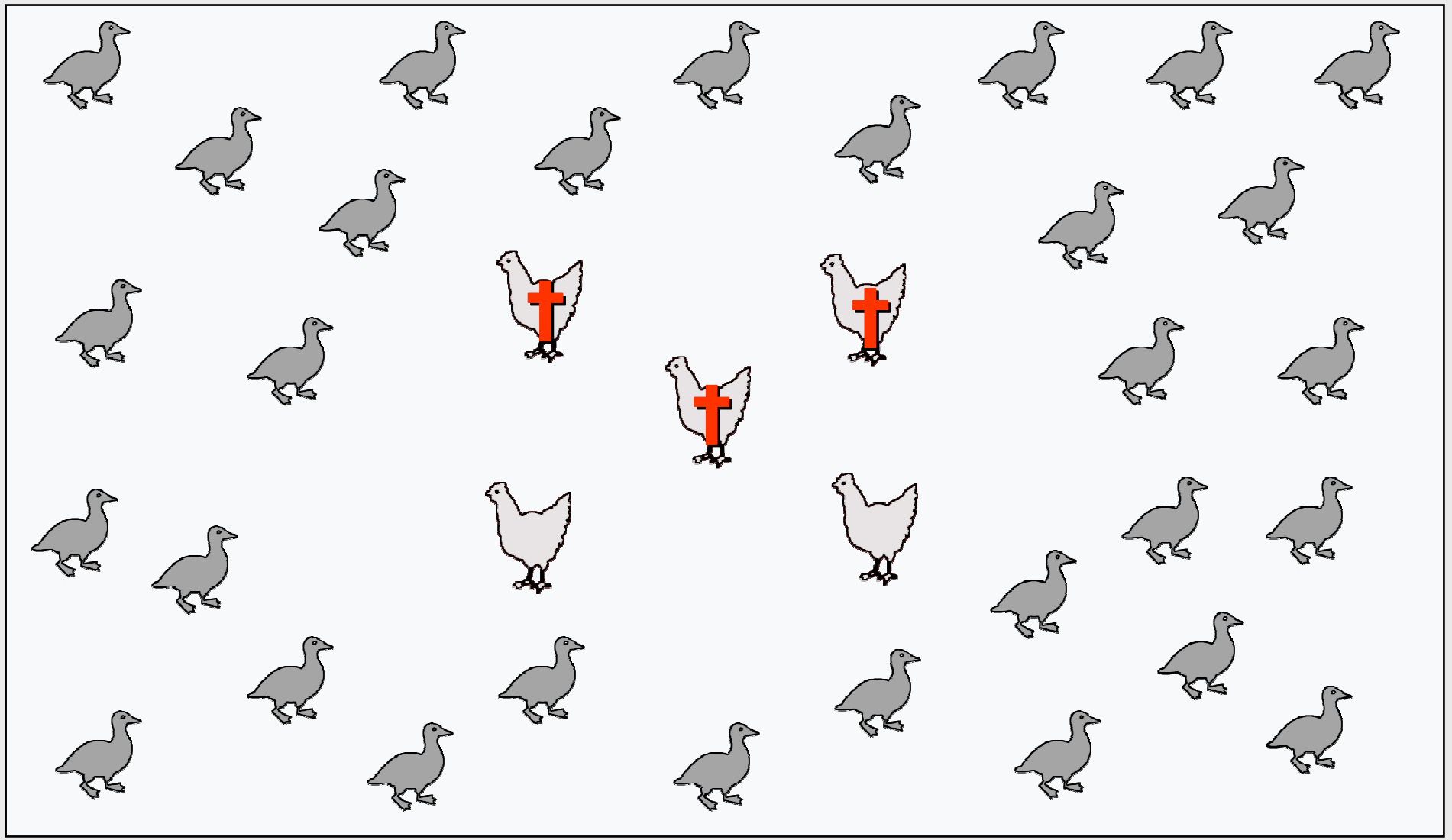
Pekingenten 28 Tage, R1959/07 10^3 EID_{50}
HPAIV H5N1 oro-nasale Infektion



Free ranging sentinel chicken 1 day p.i.

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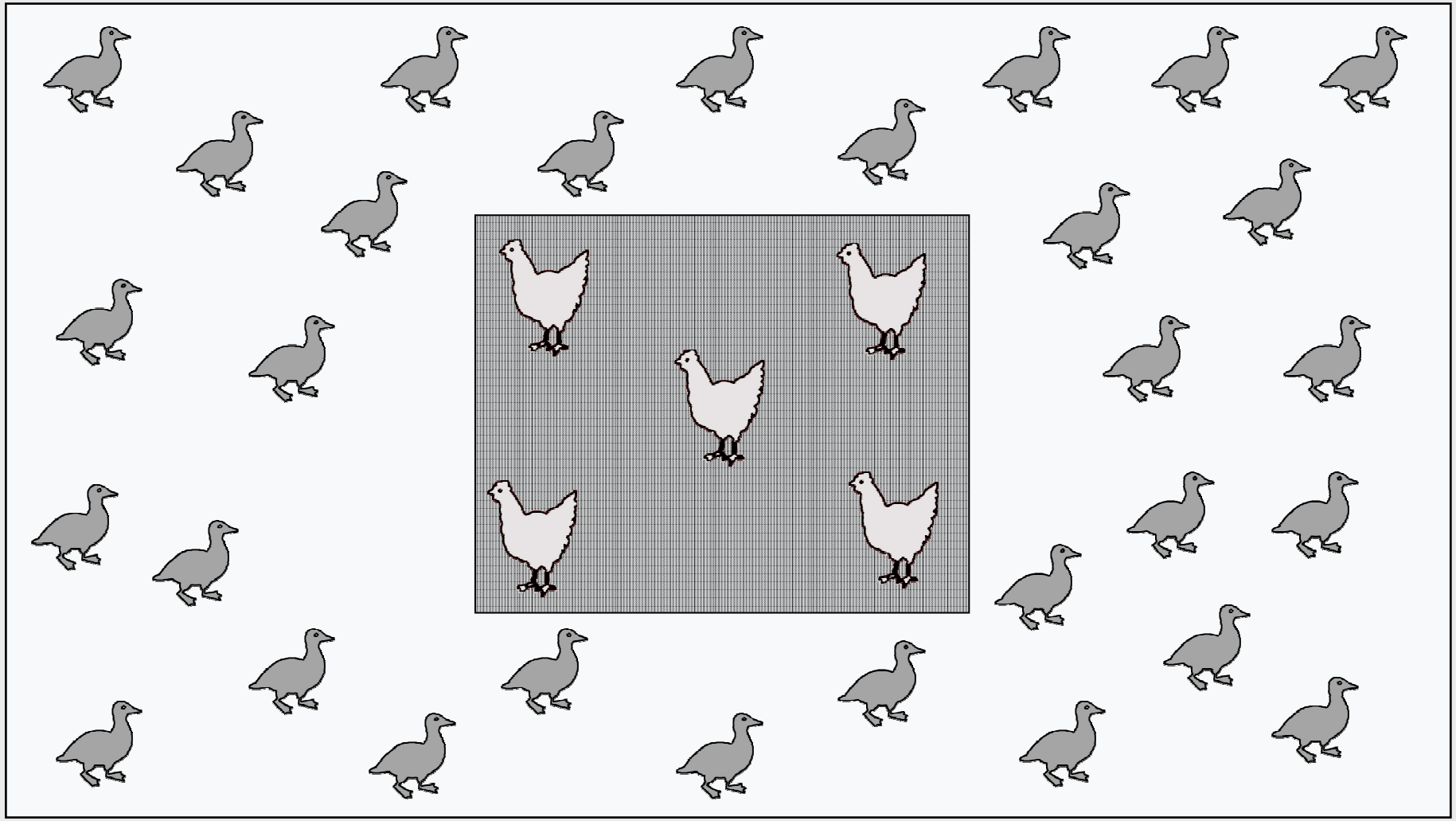
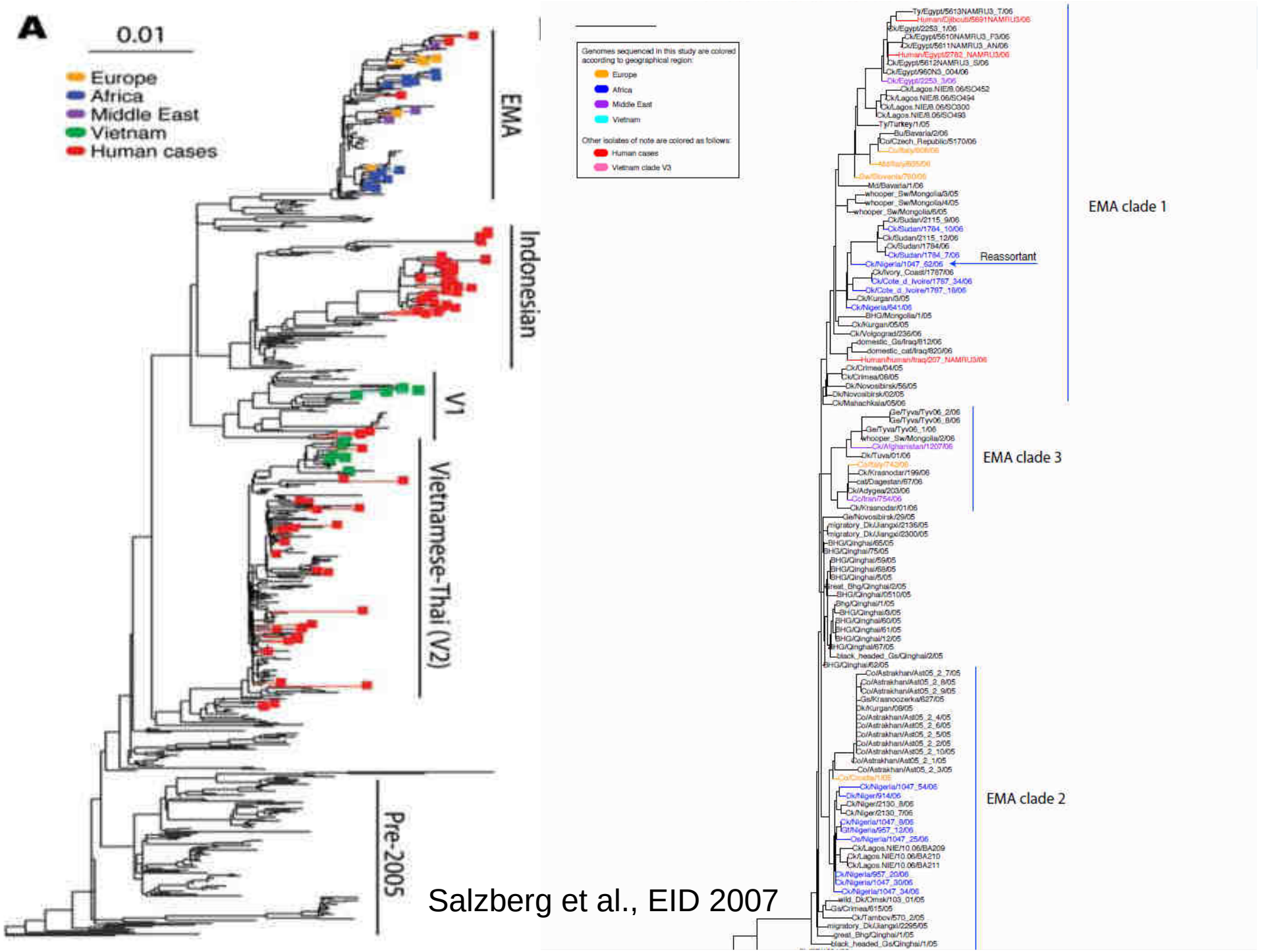
Pekingenten 28 Tage, R1959/07 10^6 EID_{50} HPAIV H5N1 oro-nasale Infektion





Salzberg et al., EID 2007

Rekonstruktion des H5N1Ausbruchs Markersdorf

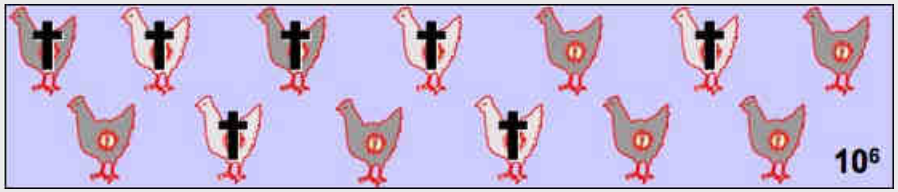
- Saisonmästerei (Herbst), handelt nur geschlachtete Tiere
- Zukauf im August, mehrfache Umstallungen,
  kaum Verluste
- begrenzter Personenverkehr (im Stallbereich) trotz
  Vermietung von Ferienwohnungen
- Praktikantin aus Tschechien
- Krähen an Futterautomaten im Entenauslauf

- Keine Hinweise auf H5N1 in Restriktionsgebieten oder
  Kontakt- (Herkunfts-) betrieben
- Kein Virusvorkommen in Hausgeflügel- und Wildvögeln
  im Umkreis von 2000 km

Layers – Challenge experiments

10 dpi

Trial #1 after 1st vaccination

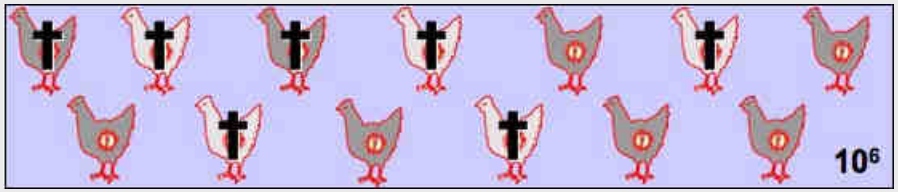


Contact birds die;
2 of 8 vaccinated layers die;
massive virus excretion by Vaccinated birds

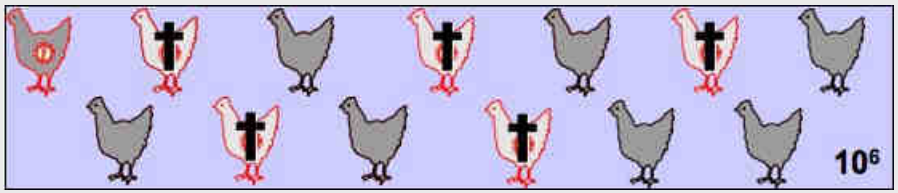
Layers – Challenge experiments

10 dpi

Trial #1 after 1st vaccination



Trial #2 after basic vaccination

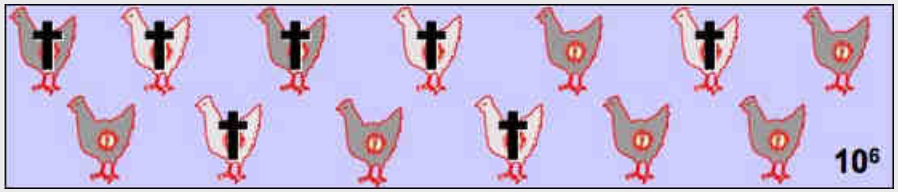


Contact birds die; vaccinated birds survive; sporadic virus excretion by vaccinated birds

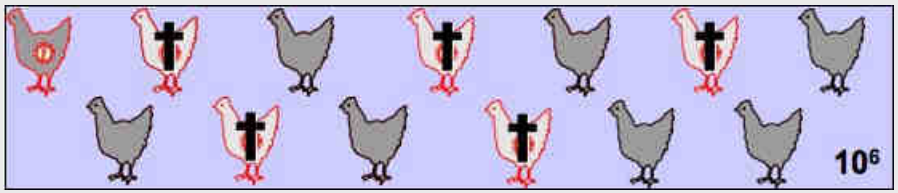
Layers – Challenge experiments

10 dpi

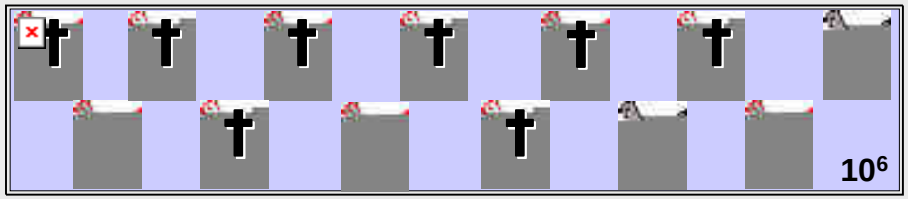
Trial #1 after 1st vaccination



Trial #2 after basic vaccination



Trial #3 6 months after 2nd boost



E.coli in 5 of 8 birds

Contact birds die;
3 of 8 vaccinated layers die;
opport. bacterial infections;
moderate virus excretion
By vaccinated birds

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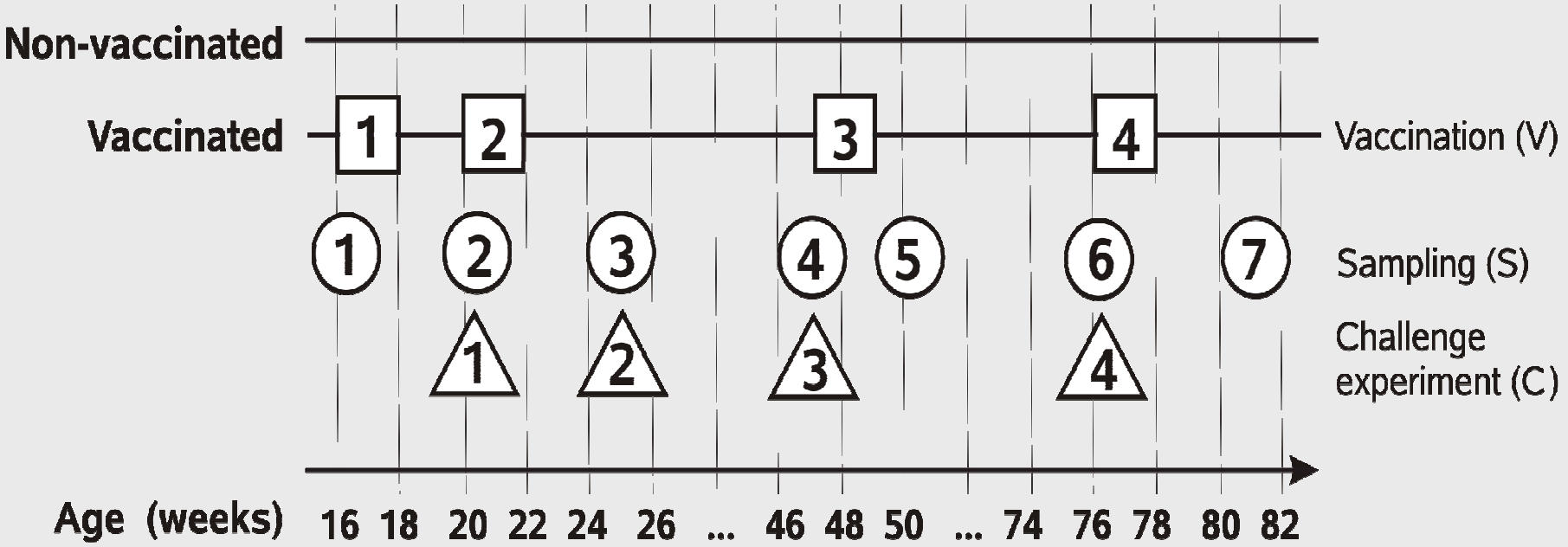
Layers – Challenge experiments

Status	Dead contact birds		Dead vaccinees (n=8)	Virus excretion by vaccinees until day pi	Excretion of infectivitiy	
	Non-vaccina-ted (n=5)	Vaccina-ted (n=5)			Contact birds	Vaccinees
1x vaccinated (21 dpv)	5		2	10	Yes	Yes
2x vaccinated (21 dpv)	5		0	10	Yes	No
2x vaccinated (6 Mo. pv)	5		3	10	Yes	No
2x vaccinated (12 Mo. pv)		0	4	8	No	No
1x re-vaccina-ted (21 dpv)		0	1	8	No	No
2x vaccinated (18 Mo. pv)		2	5	10	Yes	Yes
1x re-vaccina-ted (12 Mo. pv)		0	0	10	No	Yes
2x re-vaccina-ted (6 Mo. pv)		0	1 (n=7)	10	No	No

Geese parents – study plan



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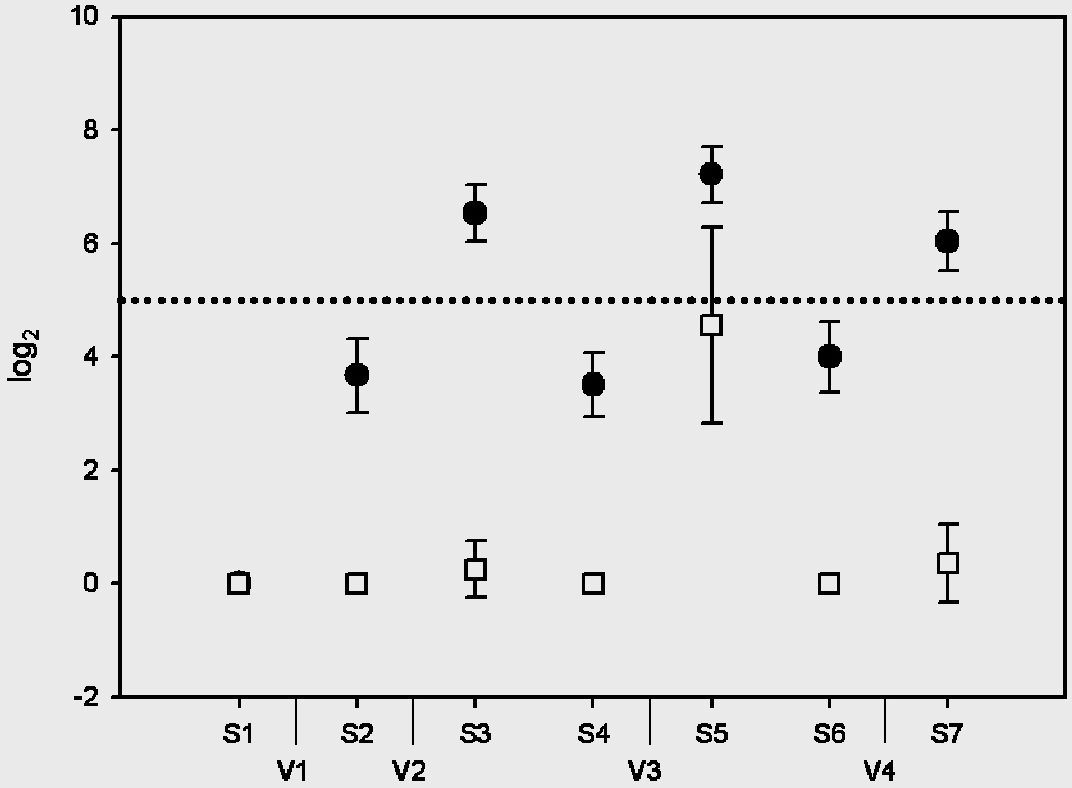
Geese parents - Serology



HAH Titer gegen Impfvirus H5N2

HAH Titer gegen Challengevirus H5N1

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Geese parents – Challenge experiments

Status	Dead contact birds		Dead vaccinees (n=8)	Virus excretion by vaccinees until day pi	Excretion of infectivity	
	Non-vacc. (n=5)	Vacc. (n=5)			Contact birds	Vaccinees
1x vaccinated (21 dpv)	5		0	9	Yes	No
2x vaccinated (21 dpv)	2		0	2	Yes	No
2x vacc. (6 Mo. pv)	1		0	10	Yes	No
2x vacc. (12 Mo. pv)		0	1	10	No	Yes

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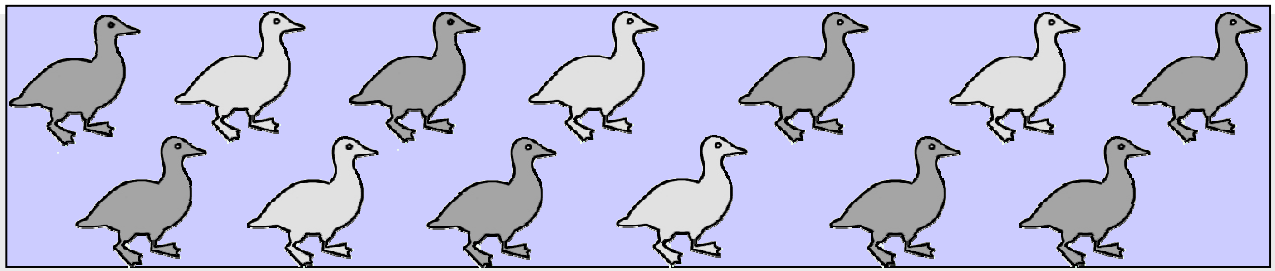
Fattening ducks

Fattening ducks – Challenge experiments

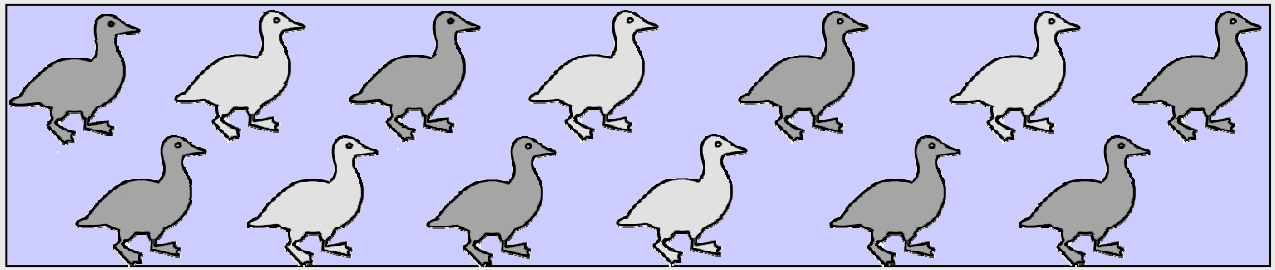
Trial #1 after 1st vaccination



10 dpi

None died or got sick, 4 vaccinated ducks shed virus

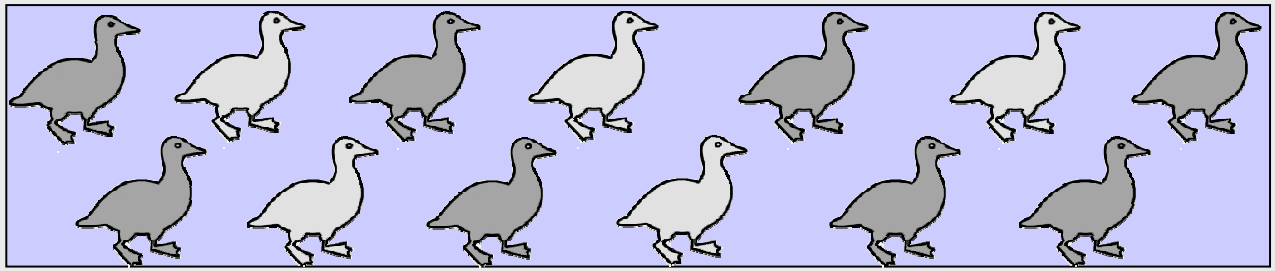
Trial #2 after 2nd vaccination



10 dpi

None died or got sick, 2 vaccinated ducks shed virus

Trial #3 6 months after 2nd vaccination



10 dpi

None died or got sick, 1 vaccinated duck shed virus

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AI vaccination in the field

- **Basic immunization** required for full clinical protection (layers)
- **Clinical protection** can be compromised by bacterial co-infections (layers)
- **Reduction of virus excretion** did not prevent transmission of infection of susceptible contacts(layers, geese), but effectively did so for vaccinated contacts (geese)
- **Reduced susceptibility** of semiadult Pekin ducks against challenge strain
- Possible protective effects of **heterologous AI immunity** (H10N4) not clear

AI vaccination in the field

- AI **vaccination recommendable for geese and ducks** in case of high pressure of infection (aids in reduction of virus spread)
- **AI vaccination of layers critical**

  (Loss of syndrome surveillance capabilities but no sterile immunity; however: reduction of virus loads aids in reduction of spread)
- High **logistic needs** (costs, time)
- Induction of **homogenous immunity** required