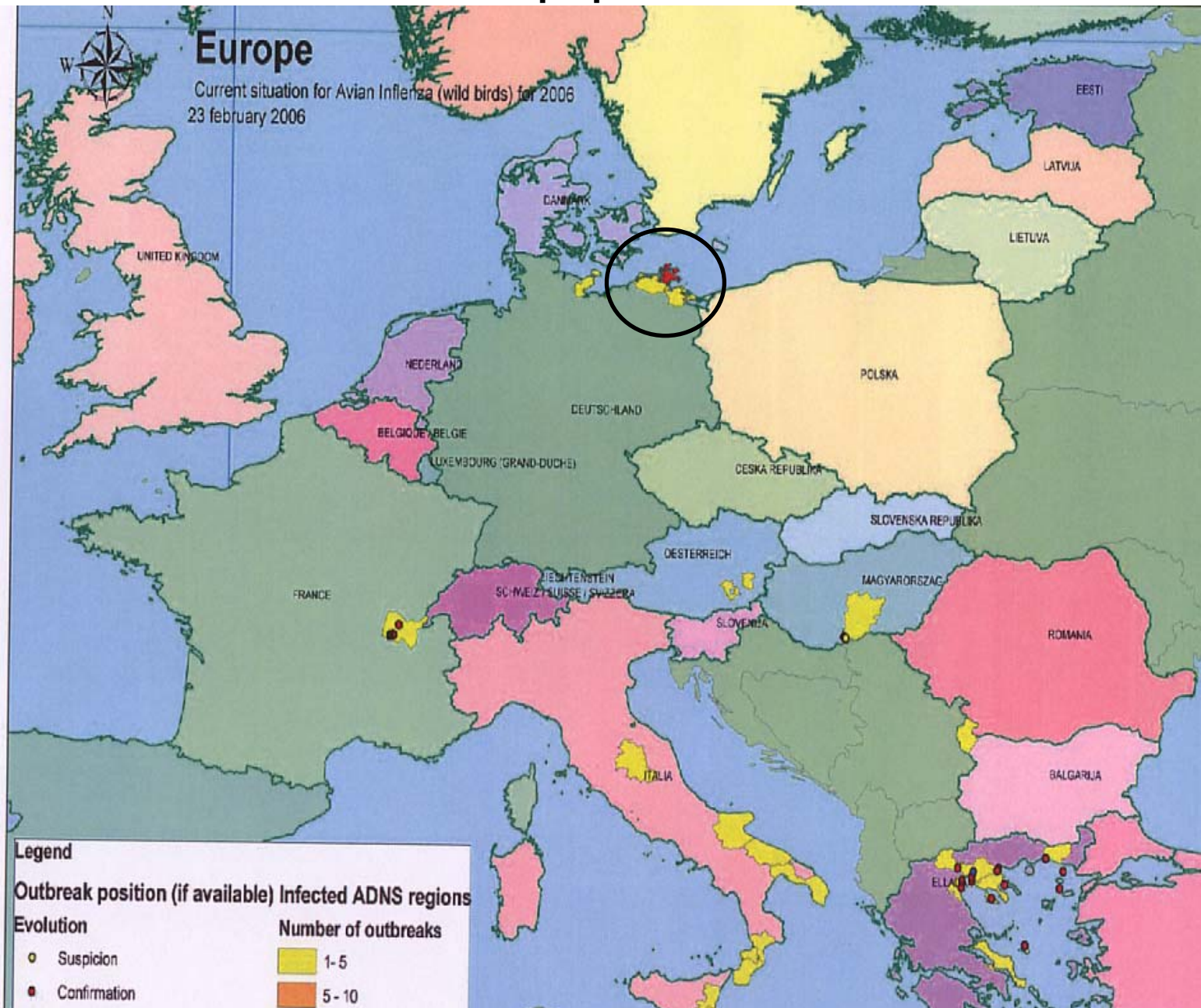


# **HPAI H5N1 infection of a Mute Swan flock in the city of Toruń in Poland**

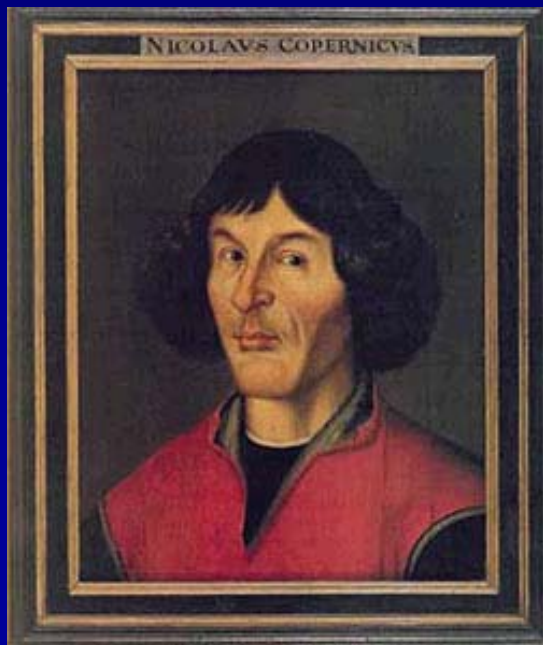
**Zenon Minta, Krzysztof Śmietanka, Katarzyna Domańska-Blicharz,  
Grzegorz Tomczyk, Tadeusz Wijaszka**

***National Reference Laboratory for AI and ND  
Department of Poultry Diseases  
National Veterinary Research Institute  
Pulawy, Poland***

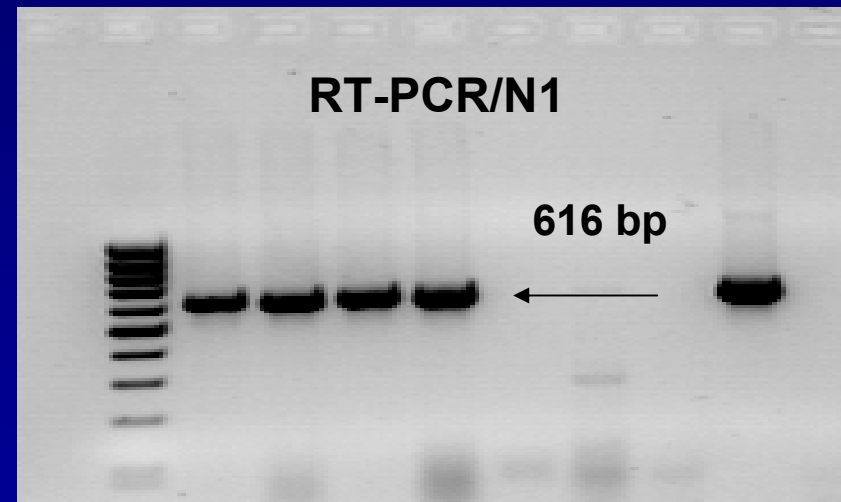
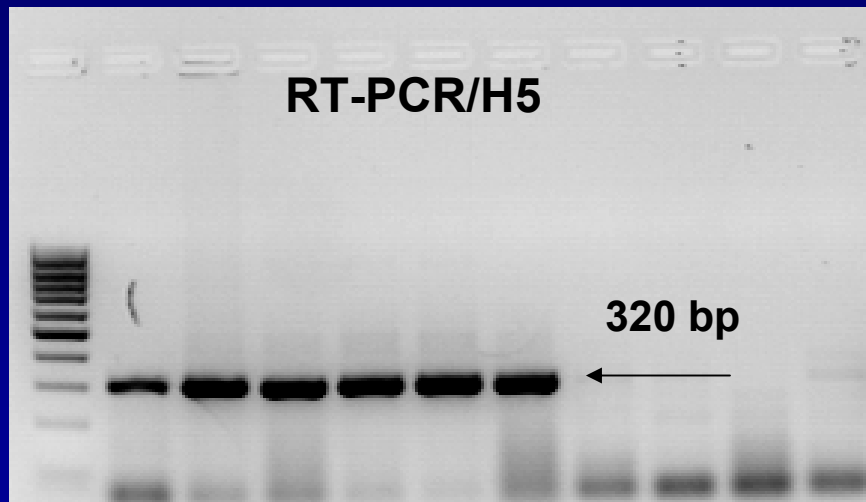
# HPAI/H5N1 situation in Europe prior to the 1st outbreak in Poland



# First cases of HPAI H5N1 in wild birds in Poland - chronology of events



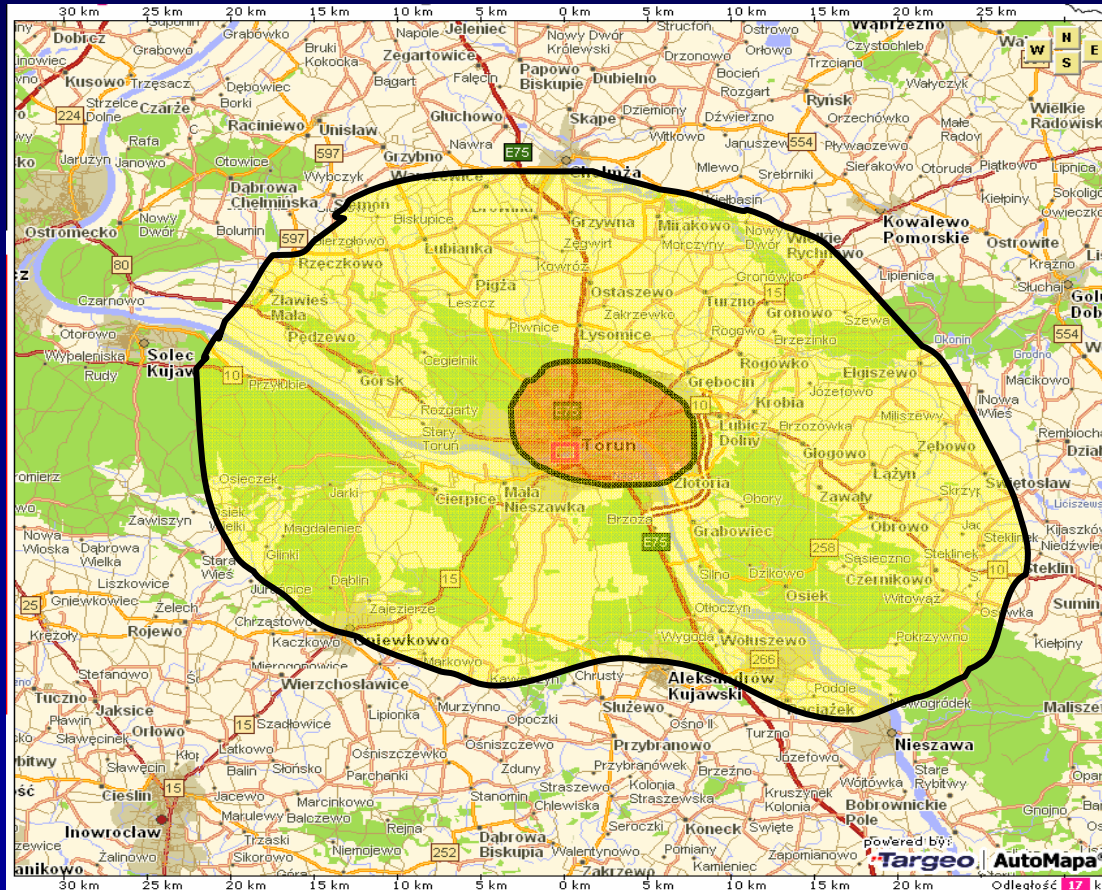
- **March 2, 2006 – 2 dead mute swans found on the bank of the Vistula River in the city center; another one found on March 4**
- **March 5 – RT-PCR/H5 positive**
- **March 6 – RT-PCR/N1 positive**



- **March 7 - virus isolation on SPF embryos positive, HI identification: H5, isolates dispatched to CRL Weybridge**
- **March 9 - sequencing: PQGERRRKKRGLF —→HPAI**
- **March 10 - official confirmation by CRL Weybridge: HPAI H5N1**



- **March 5 - establishment of protection and surveillance zone (Commission Decision 2006/115/EC)**



**Protection zone**

**Surveillance zone**

**March 10 - 113 swans locked up in a aviary located on the river bank**

**> cloacal swabs sampled from 25 birds: 6 positive in RT-PCR/H5**

**March 15 - 1 dead swan found in the aviary positive for H5N1**



**March 20**

- **meeting held in the Ministry of the Environment**

- **options discussed:**

- **euthanasia of all birds (?)**

- **replacement of birds to restricted area and observation/surveillance (?)**

- **setting free (???)**

**Provisional decision was made to clinically examine birds on-site and collect samples for further testing**



**March 26 – swans ringed**

**March 28 - samples of blood, tracheal and cloacal swabs taken**

**Initial tests:**

- **RT-PCR/H5**
- **HI-H5N2**

**Further tests:**

- **virus isolation on SPF embryos**
- **Real Time RT-PCR/Matrix**
- **AGID**
- **C-ELISA (in house, using commercial MAb)**
- **HI/H5N1, H7N1, H1N1, H3N2, H6N8, H9N2**

## Results of initial tests:

**RT-PCR/H5: 32 swans (28,6%) positive - weak PCR signal  
(14: T+C, 13 – C, 5-T)**



**HI-H5N2: 83 swans (75%) positive**

**20 birds (24,1%) positive by RT-PCR and HI**

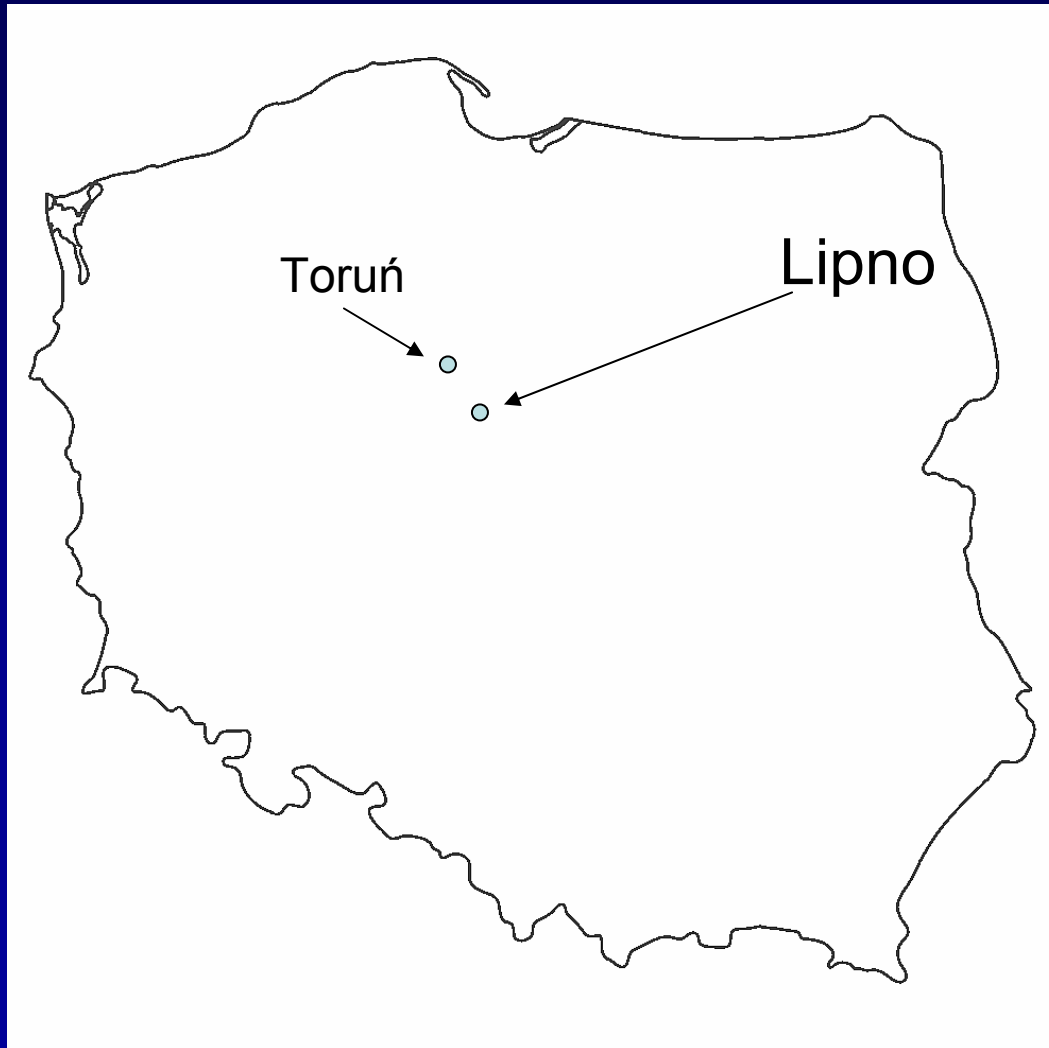
**Flood on Vistula river** —————> **CVO Decision:**

**April 1** 80 swans negative by RT-PCR released

**April 3** 32 swans positive by RT-PCR euthanased

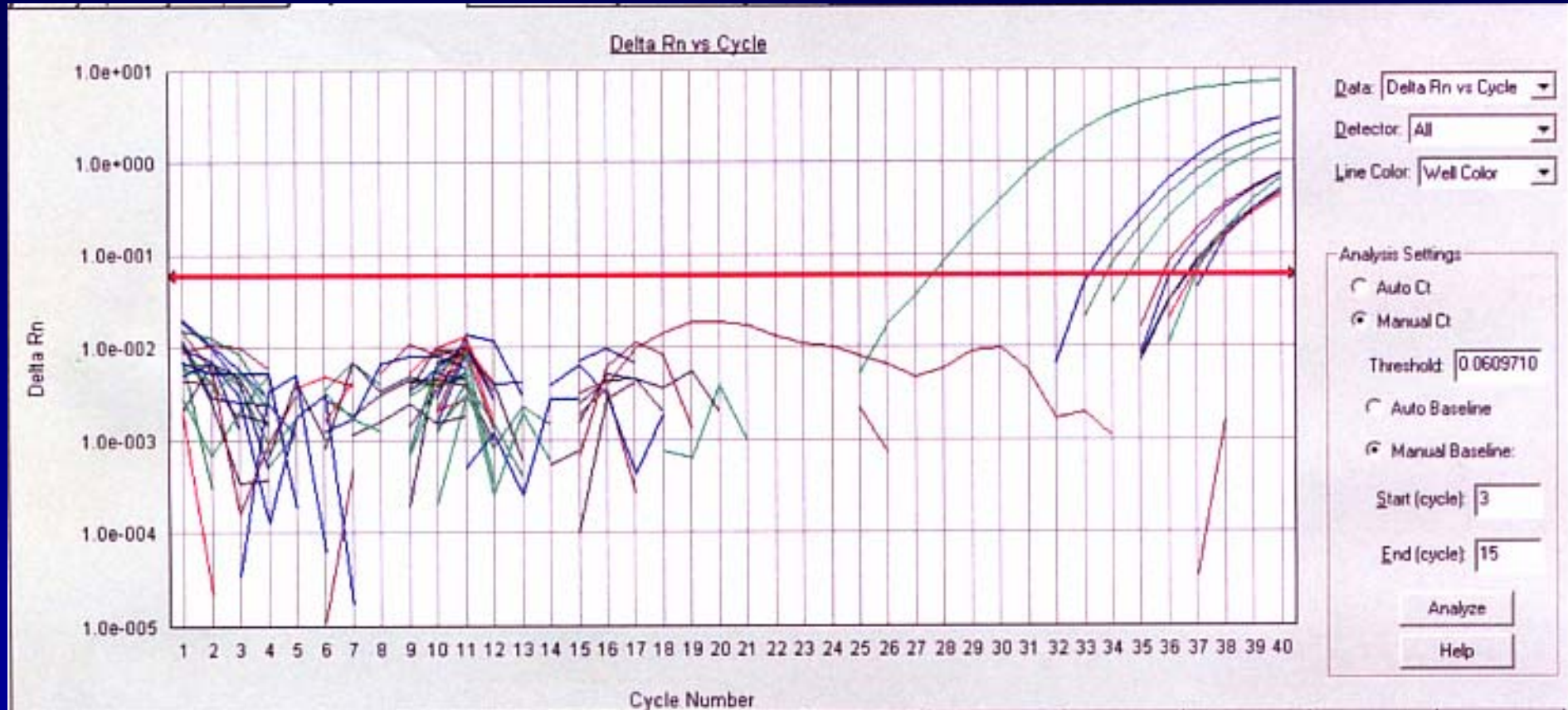


**April 7. A dead swan found in Lipno (approx 50 km from Toruń) and identified as having belonged to the Torun's flock —————> negative for AIV**



## Results of further tests:

- **Real Time RT-PCR/MATRIX: 10 birds positive (6 tracheal and 8 cloacal samples)**
- **results indicate low amount of viral RNA (Ct values 33-38)**



**Virus isolation – negative (following 2 passages) – all RT-PCR positive samples tested individually**

## Results of further tests : SEROLOGY

Tests	Positive/tested (%)	HI titre max.
AGID	34/112 (30,4)	
HI/H5N2 <sup>1</sup>	81/112 (72,3)	64
H5N1	80/112 (71,4)	≥ 512
H7N1	11/112 (9,8) <sup>2</sup>	32
H1N1	58/110 (52,7)	≥ 512
H3N2	8/109 (7,3) <sup>2</sup>	32
H6N8	1/31 (3,2) <sup>2</sup>	16
H9N2	23/109 (21,1) <sup>2</sup>	128
C-ELISA	101/112 (90,2)	

<sup>1</sup> re-tested, <sup>2</sup> all positive also with H5

## **Analysis of serological results:**

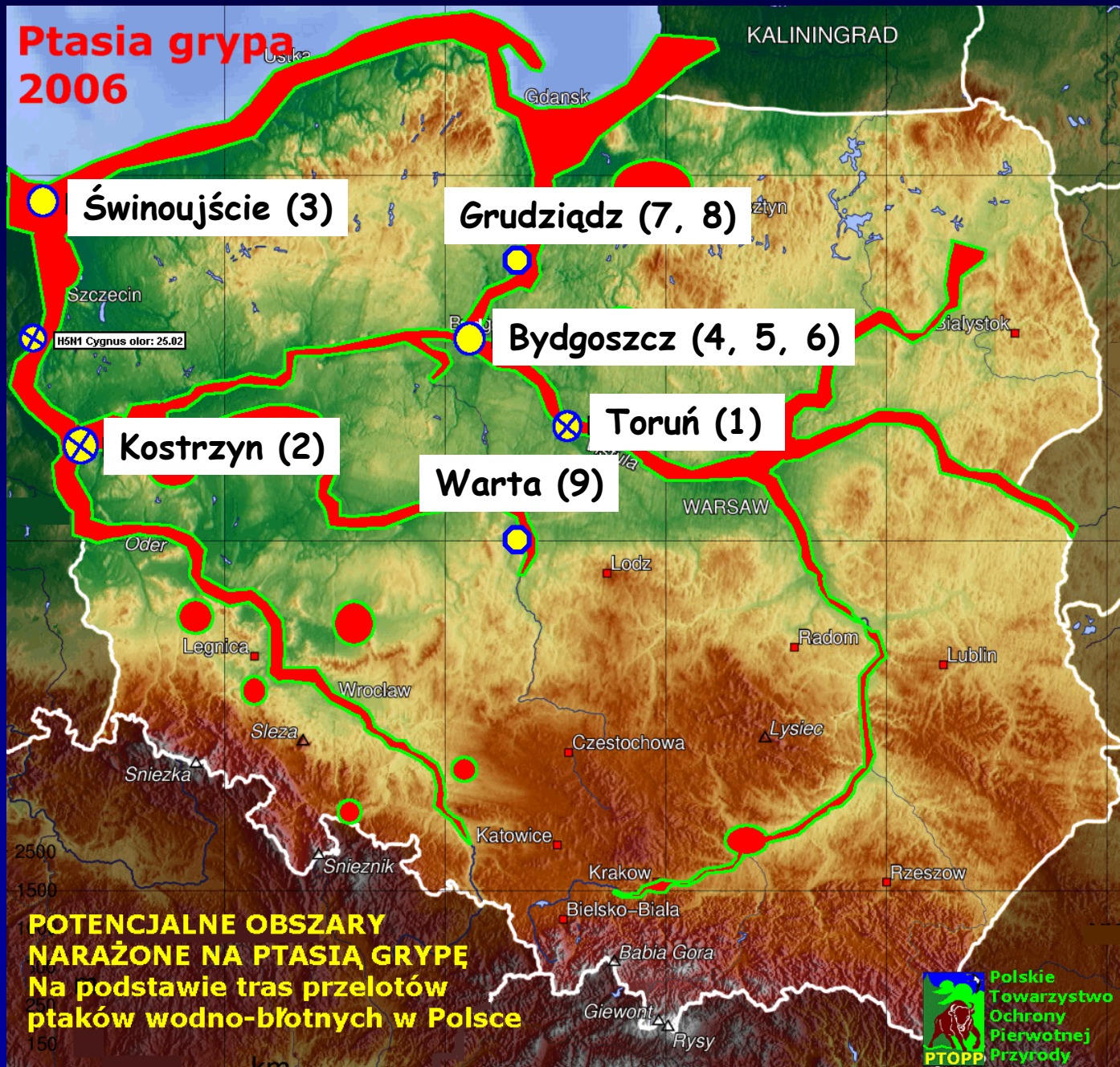
- **10 swans negative in all tests**
  - **17 swans positive only in C-ELISA**
  - **2 swans positive only in HI/H1N1 (positive in C-ELISA)**
  - **1 swan positive in all tests**
- 
- **cross-reactions?**
  - **co-infection?**

## HPAI/H5N1 outbreaks in wild birds in Poland

Place (number of outbreaks)	Data of 1st outbreak	Number and species of birds
Toruń (1)	05.03.2006	37 Mute Swans (32 live i 5 dead)
Kostrzyń nad Odrą (1)	10.03.2006	2 mute swans 1 hawk 1 grey heron
Świnoujście (1)	11.03.2006	1 goosander
Bydgoszcz (3)	11.03.2006	19 mute swans
Grudziądz (2)	29.03.2006	2 mute swans
Warta (1)	07.05.2006	1 mute swan



# Ptasia grypa 2006





I. Brown et al., Rome 2006

# Summary and conclusions

- first HPAI H5N1 cases were diagnosed in Poland on March 5th 2006 in two dead swans found in the city of Torun by RT-PCR followed by virus isolation and sequencing
- results were confirmed by VLA Weybridge (CRL for EU)
- apparently healthy swans belonging to the same flock were locked up in an aviary and subjected to additional tests
- one swan in the aviary died and found to be H5N1 positive
- 32 swans positive by RT-PCR were euthanised, remaining 80 birds were set free
- further tests (serological) confirmed that the birds were infected with AIV/H5 (most of them), but also possibly with other subtypes, during lifespan
- infected swans shed small amounts of the virus detected only by molecular methods
- preliminary study suggests that Polish AIV isolates are closely related to other HPAI H5N1 isolated from wild birds in Europe

Thank you for  
your attention !

