Role of national reference laboratory for Avian Influenza in Poland in surveillance and outbreak investigations

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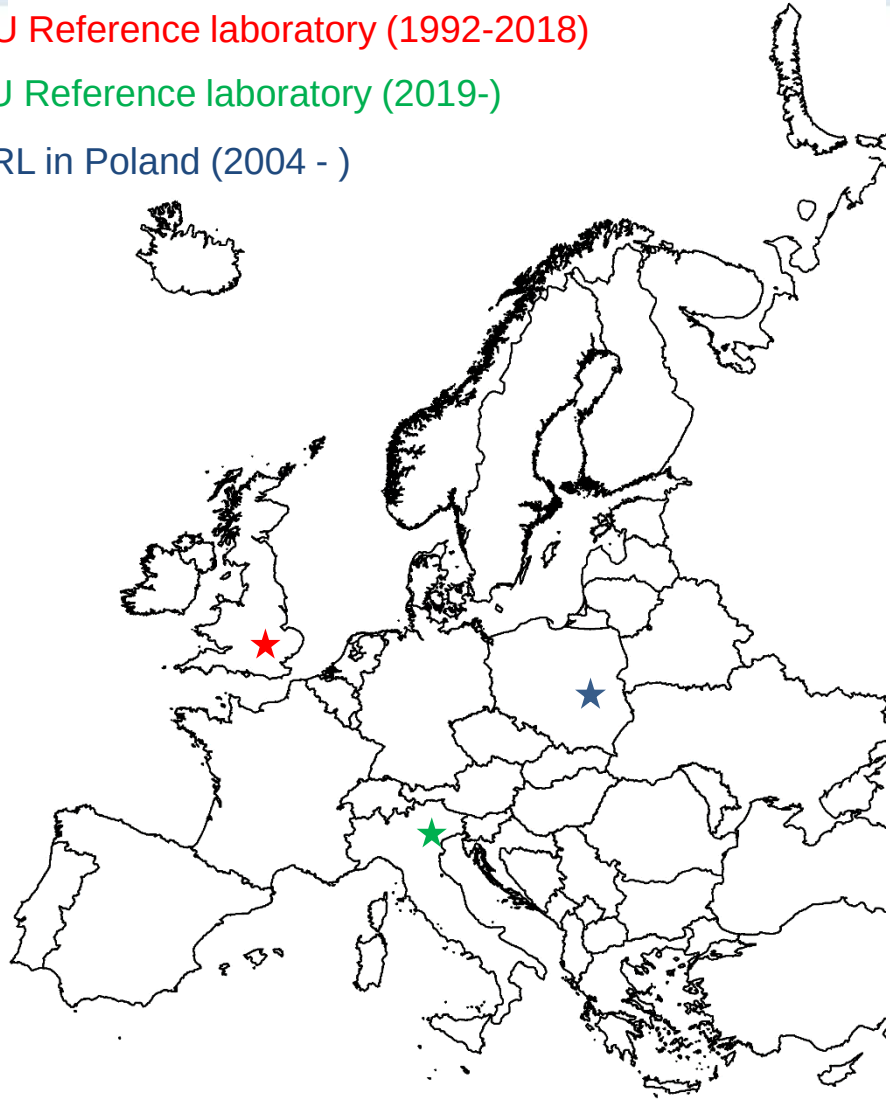
National reference laboratory (NRL) for Avian Influenza and Newcastle disease in Poland

★ EU Reference laboratory (1992-2018)

★ EU Reference laboratory (2019-)

★ NRL in Poland (2004 -)



Diagnosis and surveillance for avian influenza in the EU (including Poland)

COUNCIL DIRECTIVE 2005/94/EC

of 20 December 2005

on Community measures for the control of avian influenza and repealing Directive 92/40/EEC

COMMISSION DECISION

of 4 August 2006

approving a Diagnostic Manual for avian influenza as provided for in Council Directive 2005/94/EC

(notified under document number C(2006) 3477)

COMMISSION DECISION

of 25 June 2010

on the implementation by Member States of surveillance programmes for avian influenza in poultry and wild birds

Surveillance systems

Syndromic surveillance („diagnosis for exclusion”)

- early warning surveillance system

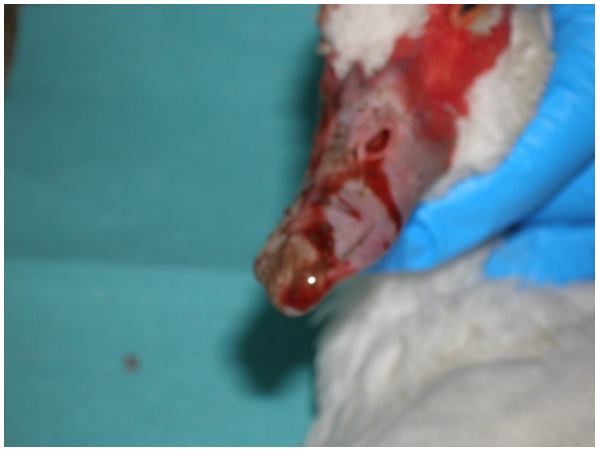
- samples from flocks with inapparent or atypical clinical signs, for which the presence of avian influenza virus cannot be immediately excluded

✓ slight increase in mortality (especially in waterfowl)
✓ drop in egg production
✓ drop in feed and water intake

Passive surveilance

- In poultry: typical clinical course - high mortality, nervous signs, cyanosis, haemorrhages etc.
- In wild birds: mostly carcasses of dead birds, occassionally samples from moribund birds

Source: PIWet-PIB Puławy



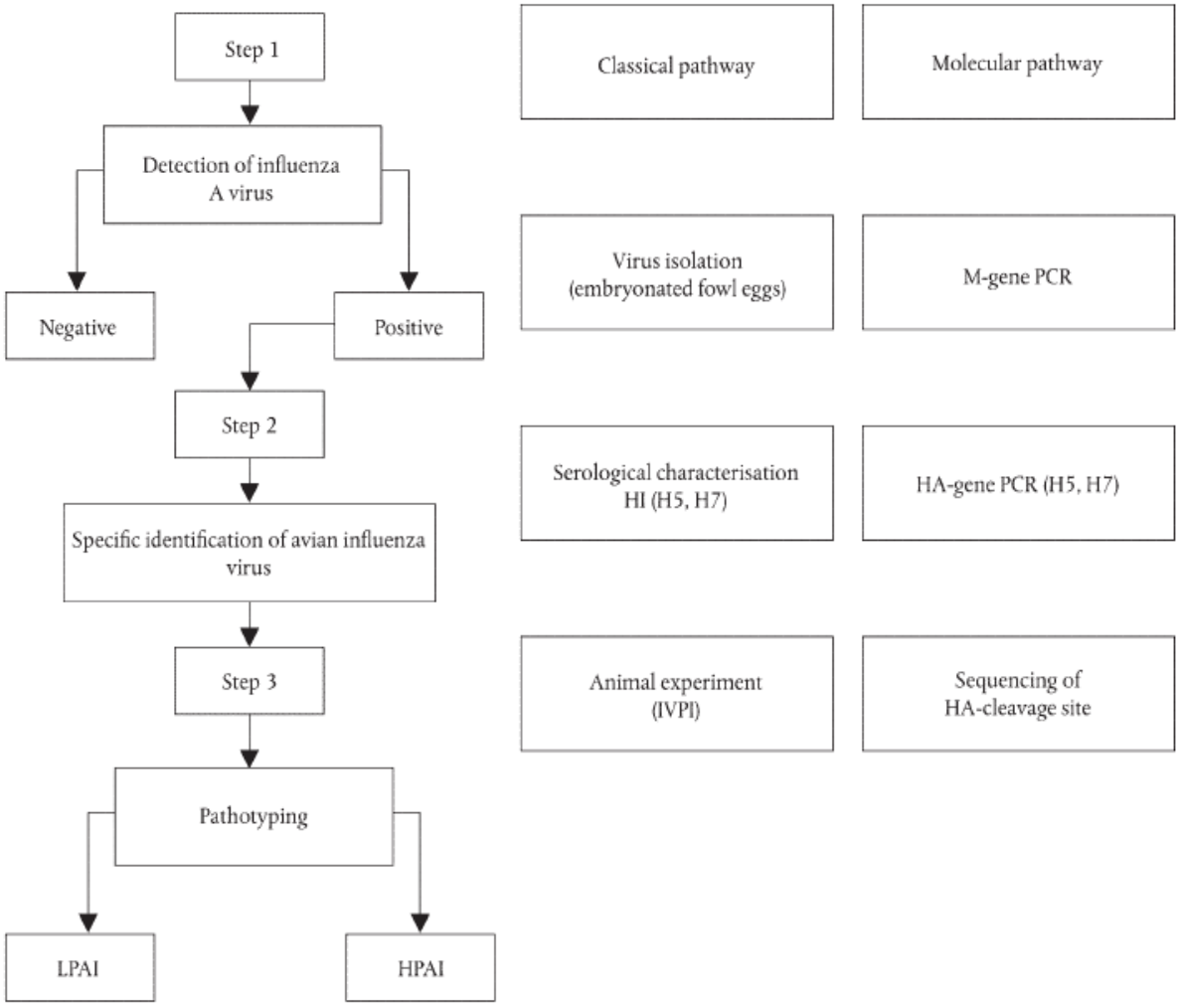
Source: A. Piróg-Komorowska

Active surveilance

- Apparently healthy birds (domestic and wild)
- Early detection of low pathogenic avian influenza (subclinical or artypical infections)
- Early detection of highly pathogenic avian influenza in species of birds in which subclinical infections are possible (ducks and geese)

Diagnostic methods

Overview of diagnostic steps for confirmation of Avian Influenza

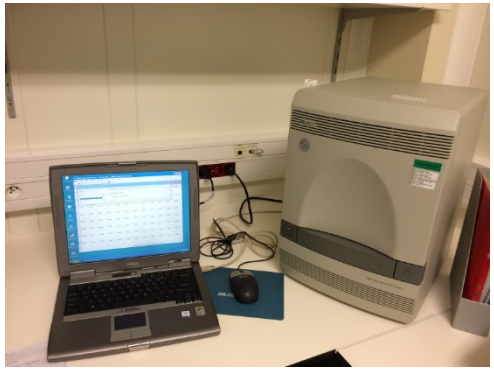


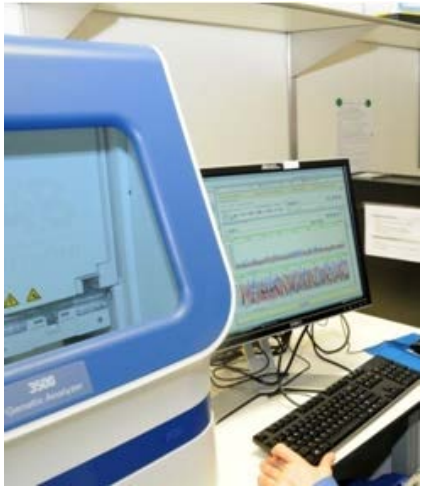
Detection of virus by molecular methods

A. Detection of avian influenza virus (M gene) by real time RT-PCR

B. Detection of H5, H7 (N1, N6, N8 etc) subtypes by real time RT-PCR

C. Assessment of virulence (conventional RT-PCR H5 or H7, sequencing)





Source: PIWet-PIB Puławy

Protocol for N1 PCR developed by Thai scientists:

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Available online at www.sciencedirect.com
SCIENCE DIRECT

Journal of Virological Methods 131 (2006) 143–147

Journal of Virological Methods
www.elsevier.com/locate/jviromet

Single step multiplex real-time RT-PCR for H5N1 influenza A virus detection

Sunchai Payungporn[a], Salin Chutinimitkul[a], Arunee Chaisingh[b], Sudarat Damrongwantanapokin[b], Chantanee Buranathai[b], Alongkorn Amonsin[c], Apiradee Theamboonlers[a], Yong Poovorawan[a,*]

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Detection of virus by conventional methods

A. Detection of avian influenza virus (M gene) in Specific Pathogen Free (SPF) embryonated eggs

B. Identification of HA subtype in haemagglutination inhibition test (HI)

C. Assessment of pathogenicity (evaluation of intravenous pathogenicity index – IVPI – in SPF chickens)

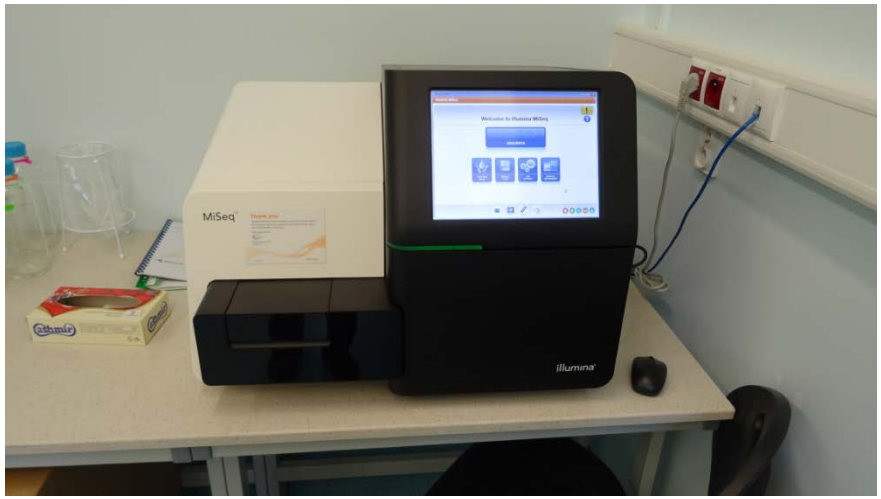
Source: PIWet-PIB Puławy

Detection of antibodies by HI methods

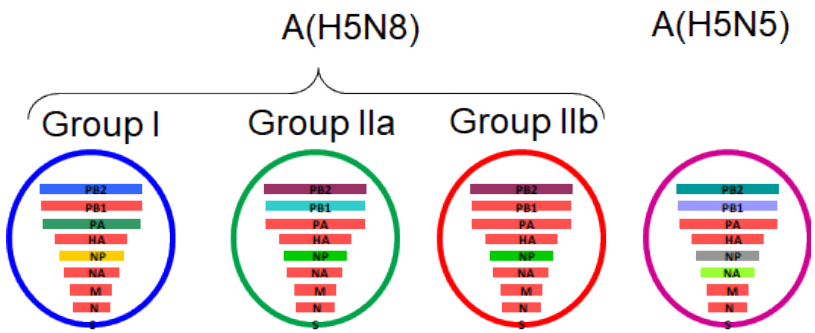
- ✓ Surveillance for AI in poultry (complement early detection systems) – evidence for exposure to H5 and H5 AI
- ✓ Compulsory for all Member States
- ✓ In case of positive results: re-sampling (swabs for molecular/virological diagnosis)
- ✓ Continuous update of antigen conformity by EURL

High throughput sequencing: genetic characterization of circulating viruses

- Use of whole genome sequencing enabled rapid identification and characterization of avian influenza virus genotypes introduced into Poland
- Collaboration with Department of Omics Analysis

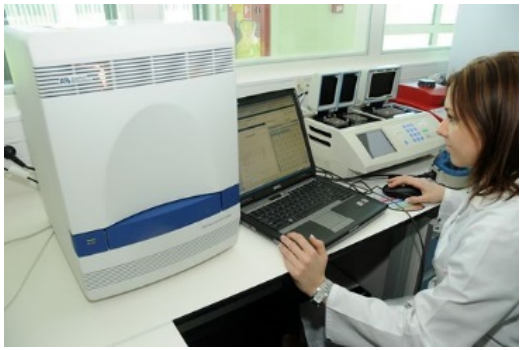


Source: PIWet-PIB Puławy



Laboratory capacity

- ✓ BSL 2, BSL 3 and BSL 3+ laboratory facility
- ✓ BSL 3+ animal facility
- ✓ Time to final results (molecular methods) < 24 hrs (usually 6-8 hours)



HPAI A(H5N8/N5) in Poland 2016-2017: summary

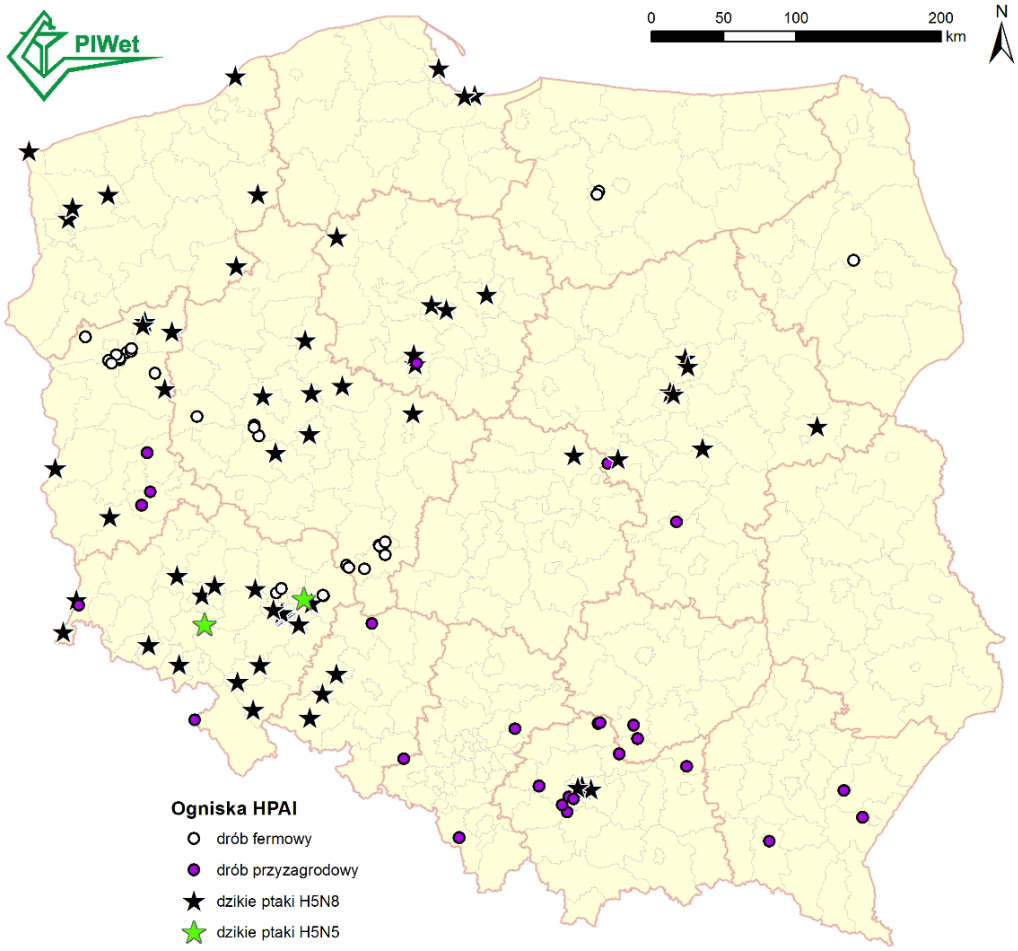
- 65 outbreaks in poultry (65xH5N8
  - 38 in commercial farms
  - 27 in backyard holdings

- 68 events in wild birds
  - 66 x H5N8
  - 2 x H5N5

- Last outbreak: 16 March 2017

- Simultaneous introduction of different genotypes of H5 clade 2.3.4.4B

- Manuscript: Swieton &Smietanka, Phylogenetic and molecular analysis of highly pathogenic avian influenza H5N8 and H5N5 viruses detected in Poland in 2016–2017, accepted for publication in Transboundary&Emerging Diseases



The role of NRL during HPAI H5 clade 2.3.4.4 epidemic

- ✓ Suspicions of Highly pathogenic avian influenza (HPAI)
- ✓ Exclusion of HPAI
- ✓ Investigations of contact holdings
- ✓ Examination of healthy meat poultry from surveillance/restriction zones prior to transport to designated slaughterhouse
- ✓ Testing of holdings after re-population
- ✓ Assessment of virulence of detected H5 viruses (*in vivo* and *in vitro*), phylogenetic analysis, molecular markers for zoonotic potentials etc.
- ✓ *Ad hoc* scientific opinions to inform decision-makers and stakeholders about risk
- ✓ Seminars and trainings for Veterinary Inspection

Thank you!