Role of antibody detection (DIVA) in surveillance

for freedom from HPAI and diagnostic tools to assess the effectiveness of vaccination

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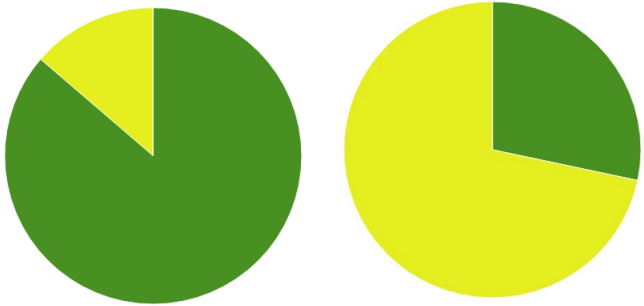
Founded as OIE

Immune responses

- Innate immunity
- Adaptive immunity
  - Cellular effectors
  - Humoral effectors
    - Antibodies
- Effects of vaccine type, formulation, species, age etc.

- ELISA (NP, HA subtype)
- HI (HA subtype)
- VNT (functional assay)
- Blotting



Herpesvirus vector vaccine

Inactivated vaccine

Defining the objectives of sero-surveillance (1)

- Vaccination coverage
  - How many birds per flock have received immunization?

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- Immunization level
  - How many birds per flock responded immunologically to immunization, and, if so, to what degree?

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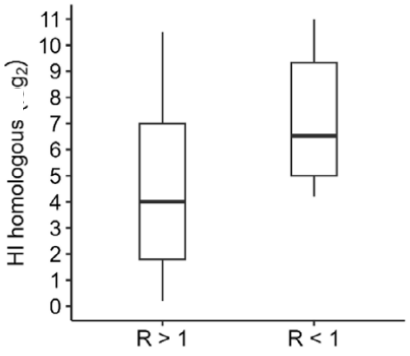
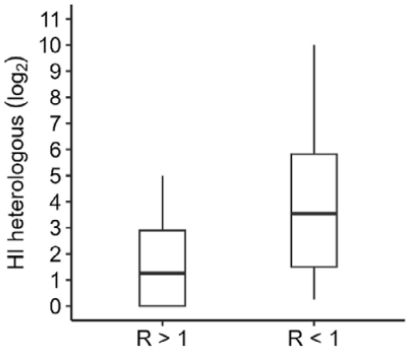
- Efficacy of protection (ideal aim always is: $R_0 < 1$)
  - How many birds per flock are protected from
    - Clinical disease
    - Infection with HPAIV
    - Excretion of HPAIV

“Doctor, is my flock protected?”

- Defining a serological threshold for protection
  - Assay? HI (quantification)
  - Antigen? Vaccine antigen (homologous), field virus (heterologous); differences in titres can be substantial (antigen distance): Ideally, vaccine and field virus antigen are identical!

Defining a serological threshold of protection?





- HI is used as a standard assay
  - HI antibodies accepted as a surrogate of virus neutralizing antibodies
  - Titres vary according to vaccine type, species, breed, age etc. and can only be used as a proxy
  - Likelihood of protection increases with higher HI titres
  - Correlation is more substantial when heterologous antigen is used

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Defining the objectives of sero-surveillance (2)

- Obtaining evidence for field virus infections in DIVA-vaccinated flocks
  - Target: NP-specific antibodies (NP is the most abundant viral protein, highly immunogenic)
  - Retrospective evidence: Allow up to 14 days post infection for NP-specific antibodies to develop
  - Sero-surveillance of vaccinated flocks only in conjunction with use of **n e g a t i v e - m a r k e r** vaccines (**DIVA**, "differentiate vaccinated from infected animals")
  - NP acting as a marker; missing in DIVA vaccines, present in field viruses: Thus, only field virus infected birds develop NP-specific antibodies

“Doctor, is there field virus in my vaccinated flock?”

- “… has there been field virus infecting the flock…” (14 days til seroconversion!)
- Has a DIVA vaccine been used? Yes!
- Run an NP ELISA on blood samples from the flock
  - How many samples do we need to consider? (At least 20 per unit; depends on *pre-defined* exclusion prevalence!)
  - Sensitivity and specificity of the assay play an important role:
    - Low sensitivity: NP positives are missed (false-negative)
    - Low specificity: Likelihood to detect false-positives increases (99.8% spec. means 2 false-positives/1000 tests!)

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DIVA-vaccinated flock has NP antibody-positive birds

- Corrupted vaccination scheme (a non-DIVA vaccine has been used [somewhen in the past])
- Unspecific NP ELISA (false-positive reactants)
- Field virus incursion at least 1-2 weeks ago (may be longer)
  - What kind of field virus?
    - HP H5 (the one against which the flock has been vaccinated)?
    - Any other LP influenza virus (NP protein is highly conserved); e.g., H9N2 or the like

- Active swab monitoring using RT-qPCR required to resolve

Current conclusions

- Sero-surveillance has limitations
  - time delay until incursions become detectable
  - restriction to antibody measurements
  - restriction of high-throughput antibody assays
  - its costs (blood sampling, material [ELISA], working time [HI])
- Sero-surveillance is useful for
  - confirming retrospectively freedom from infection (non-vaccinated or DIVA-vaccinated flocks)
  - estimating immunization levels
- Sero-surveillance could be enhanced by
  - improved ELISAs, new (surrogate) targets
  - careful integration into full surveillance concepts

Thank you, we are collecting your questions for a joined discussion round.