Endemic non-notifiable avian influenza virus infections in poultry

NRL Avian Influenza

Friedrich-Loeffler Institute, Germany

Foto: C. Illing
Avian influenza virus subtype H9N2

• H9N2 became a wider problem in poultry in China in the early-mid 1990s
• Since then endemic in poultry in many Asian, Middle East, and North African countries
• Several sublineages of H9N2 viruses emerged:
  – “G1”, most prevalent, represented by A/quail/Hong Kong/G1/97, further diversified into at least four co-circulating genetic groups A, B, C and D.
  – Y280, Korean wild bird, Eurasian wild bird, American lineage
• H9N2 “G1” viruses bear zoonotic potential:
  – Total of 38 active human H9N2 infections reported from China, Bangladesh and Egypt

Fusaro et al., 2011; Shahsavandi et al., 2012; WHO, 2018
Zoonotic transmission of AIV H9N2

https://flutrackers.com/forum/forum/flutrackers-high-pathogenic-h5n1-h1n08-h5n8-h5n6-h5n3-tracking-outbreaks-spread/745429-flutrackers-global-cumulative-h9n2-partial-case-list-1998-2017
Phylogeny of H9N2 hemagglutinin
Detection of H9N2 in Germany in poultry

Layer chickens

Lower Saxony, 1994-5
Detection of H9N2 in wild birds in Germany since 2010

Active nationwide wild bird surveillance since 2006

Cluster of H9N2 infections in breeding colony of Grey leg geese at Lake „Max-Eyth“, Stuttgart
Detection of H9N2 in Germany in poultry

Active and passive poultry monitoring
- Monitoring programmes of poultry industry
- Governmental surveillance programmes

- Lower Saxony, 1994-5
- SW Germany, 2012-3
- Lower Saxony > 2013
H9N2 - why bother?

- Substantial mortality (up to 30% in endemic regions in Asia) due to
  - Opportunistic co-infections: ORT, Eco, IBV
  - Suboptimal rearing (Ca- and water deficiency)
  - Adverse environmental conditions (T°C)
- Substantial economic losses [Germany, turkeys]
  - Increased co-morbidity (respiratory disease)
  - Suboptimal performance (weight gain, egg production, increased carcass condemnation at slaughter)
- Zoonotic propensity of G1 lineage
H9 receptor binding

Avian (2,3′) - Q234L - Mammalian (2,6′)

Imai and Kawaoka, 2012
Wan and Perez, 2007

Modelled human receptor

Globular head

190-Helix

220-Loop

130-Loop

Current Opinion in Virology
All H9N2 from Germany belong to the Eurasian wild bird lineage.
Neuraminidase N2

- N2s recruited from different Eurasian wild bird sources
Updated phylogenies

Hemagglutinin

Neuraminidase

Updated phylogenies
H9N2 HA cleavage site

H9 HA, Eurasian wild bird lineage

Turkey, Germany, >2013

PAASNR*G

Turkey, Germany, 2016-7

PAASKR*G
Deletions in the N2 stalk region

H9N2, NA, Germany

<table>
<thead>
<tr>
<th>Sequence – stalk domain</th>
<th>Δ</th>
<th>Wild bird</th>
<th>Poultry</th>
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<td>PI VERNIEIVYNNTVEKELCPKLTETY 0</td>
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<tr>
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<tr>
<td>PI_________________________IETY 23</td>
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<td>2016/17</td>
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- **NA stalk deletions**
  
  Mutations signalling full viral adaptation from anseriform to galliform hosts since 2013
Experimental infections in chickens
H9N2 A/tk/DE-NI/AR234/2013

**Occulo-oronasal infection,** 10⁶ EID₅₀

**RT-qPCR**

**OP swabs**

- Choane: S1 sheds virus at day 10, S2 remains virus negative

**CL swabs**

- Cloaca: Sentinels never shed virus

**Serology**

<table>
<thead>
<tr>
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<th>AR234/13</th>
<th>R1885/11</th>
<th>A/TK/Wis/66</th>
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<td>S442</td>
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**EA G1 US**

| H9N2-K | 10 | 6 | 4 | 10 | 0 |
| H9N2-S | 10 | 6 | 4 | 10 | 0 |

**HAH (log₂)**
Vaccination efficacy

**Vaccination:** AR234/13 H9N2 EA;
**Challenge:** 21 dpvac EA AR234 (homologous) or G1 1855/11 (heterologous)

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<tr>
<th>OP swabs</th>
<th>CL swabs</th>
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<td>250</td>
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</table>
Efficient replication of H9N2 A/tk/DE-NI/2013 in SPF chickens

Mono-infection in SPF chickens did not induce clinical symptoms

IVPI=0

Vaccination induced protective immunity against homologous challenge

H9N2 duplex RTqPCR based on H9 „Monne“-PCR and „Hoffmann“ N2 is highly sensitive and specific (not shown)
Summary: H9N2, Germany

• Constant infection pressure of H9N2 from wild bird populations but secondary spread drives outbreaks (no P2WB spill back yet)

• Currently no evidence for increased pathogenicity (cleavage site), zoonotic potential (RBE configuration) and antigenic drift (HI assays)

• Risk of reassortment with other IAV co-circulating (sporadically) in turkeys:
  - H1N1, H1N1pdm, HP H5N8, H6Nx

• Recent detection of H9N3 and H9N8
Attempts to control H9N2 in Germany

• Non-notifiable
  All actions organized and carried out by poultry industry
  - Optimized diagnosis (serology, RT-qPCR)
  - Sanitation, nucleus herds free of H9N2

• Prevention of endemicity failed
  – Vaccination programme was established (private initiative)

• Autologous vaccination
  – Rules established by regional government
  – Autologous isolate for each affected (group of) holding
  – NRL approves: H9N2, no H5/H7 contaminants
  – Formulation of vaccines by approved producer, use limited temporarily (15 months)
Delayed success to control H9N2 in Germany

- Reduced number of outbreak only since 2017
  - No clear-cut reporting of cases
  - No stringent control of vaccination efficacy

- Transboundary transmission to Poland (pullet trade)
  - Świętoń E et al. 2018, Virus Genes, PMID: 29052126;
  - Smietanka K et al. 2014, Avian Pathol., PMID: 25132323.

- Repeated new introductions of H9Nx
  - 2015: H9N2
  - 2017/18: H9N3, H9N8

Do we expect a better control of H9N2, if infections were notifiable?
Non-notifiable AIV at global perspectives

• May induce substantial economic losses

• Interferes with H5/H7 surveillance
  – Serosurveys using NP-specific ELISAs
  – NA-releated cross reactions in HI assays

• Interferes with serological DIVA
  – Negative marker vaccines

• Public health concerns (H9N2)
  – Direct zoonotic properties (G1 lineage)
  – Donor of genome segments in reassortments with zoonotic HPAIV (gs/GD H5Nx, H7N9, H10Nx)
FLI-IVD | Martin Beer

FLI-NRL | Christian Grund | Mahmoud Naguib | Rokshana Parvin
        | Diana Parlow | Aline Maksimov

FLI-AISEQ | Elke Starick | Kathrin Steffen | Günther Strebelow
          | Susanne Köthe

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Anicon® | Martin Liman | Klaus-Peter Behr

Vaxxinova® | Bernd-Andreas Schwarz

Free University of Berlin | Hafez M. Hafez

Veterinary Investigation Centres | Sample provision