Vaccine development, preparedness, and availability, based on HPAI strains

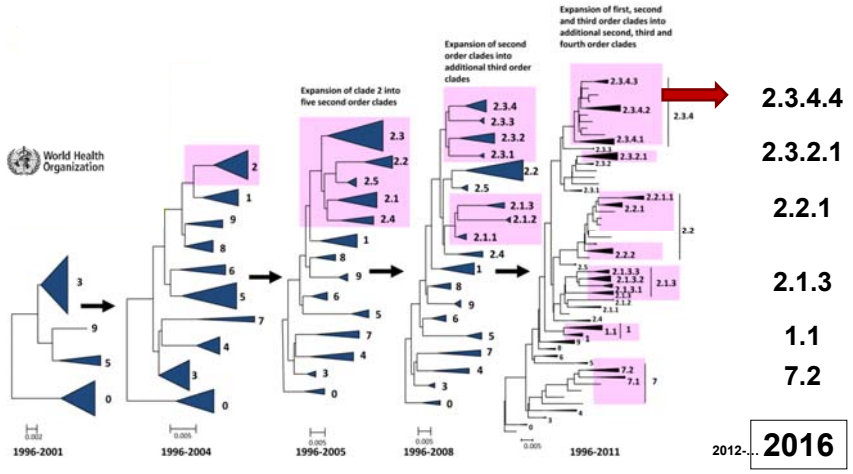
David L. Suarez, Acting Laboratory Director
Southeast Poultry Research Laboratory
U.S. National Poultry Research Center

USDA

USDA Agricultural Research Service

1

H5 Goose/Guangdong-lineage Clades



- **Since 1996 – H5N1 hemagglutinin changes – e.g. DRIFT (similar to human seasonal flu)**
- **Since 2008 – reassortment of NA genes (N5, N6, N8, N2, N3)**

2

Vaccination for Avian Influenza

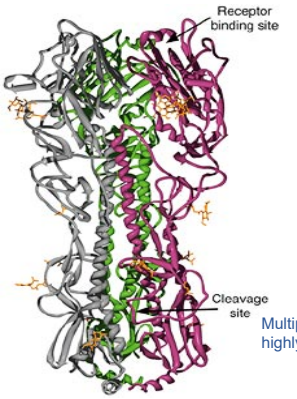
- Vaccination protects from disease but not infection (no sterilizing immunity)
- Primary protection from antibody to hemagglutinin protein
- Limited protection from antibody to neuraminidase protein (don't have to match N subtype)
- Cell mediated immunity from viral-vectored vaccines can add additional protection
- No protection from antibodies to internal proteins
- No cross protection between subtypes-i.e. H5 vaccines only protect against H5 viruses

USDA Agricultural Research Service

3

Hemagglutinin (HA) Protein

- Homotrimer
- 16 different HAs identified thus far
- Functions:
  - -binding to host cell
  - -membrane fusion
- Cleavage site = virulence determinant
- Major antigenic protein of AIV
- Target of neutralizing antibodies
- Undergoes antigenic drift

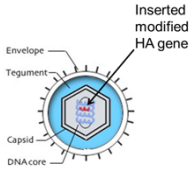


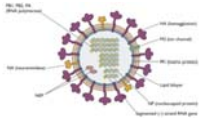
Multiple basic aa = highly pathogenic AI

www.accessexcellence.org

4

Age of Biotechnology for Avian Influenza Vaccines






Recombinant Vectored-Live	Reverse Engineered	RNA Particle	Protein Expression
HVT, FPV, NDV	Reverse genetics killed adjuvanted	Alphavirus RPH5	Baculovirus
Cell mediated and Antibody protection	Antibody protection	Cell mediated and Antibody protection	Antibody protection

- **Easier to antigenically match hemagglutinin to new outbreak virus with current biotechnology**
- **Faster to obtain conditional license for non-replicating vaccines**
- **Live viral vectored vaccines require more safety testing**

USDA Agricultural Research Service

5

Prediction of Vaccine Protection

- Antibody levels, as measured by hemagglutination inhibition (HI) tests, most common measure of immune response
  - Best to use challenge virus as antigen as measure of protection
  - Common to use vaccine antigen for HIs to show best response
- HI titers of 40 or above to challenge virus provides reliable protection and consistent reduction of virus shedding
  - HI titers below 40 are inconsistent predictor of protection
  - For live viral-vectored vaccines, HI titers are often below 40 but good protection can still be observed
- Comparison of hemagglutinin amino acid sequence can also be used
- Cell mediated immunity contributes to protection, but measuring cell mediated immune response hard
- Challenge studies measuring viral shedding best measure

USDA Agricultural Research Service

6

Amino Acid Sequence Comparison

- Comparison of amino acid sequences provides quick comparison between vaccines and field strains
- Although predictive of protection, a relatively small number of amino acid changes can potentially greatly affect antigenicity
- Homologous vaccination (99%+) provides best protection
- Recommend 95%+ sequence similarity
- 90-95% may be protective, but probably should be combined with other vaccines
- Don't recommend viruses with lower than 90% sequence similarity

USDA Agricultural Research Service

7

H5 amino acid Sequence Comparison

	TKIA	GsGu	DKCh	SwHu	GyWA	GWEg	TKIr	DKPo	CKHi	CKQU	TKIA
turkey/Iowa/010094/2022 Cl 2.3.4.4b		91.0	93.1	91.0	95.1	98.4	87.0	87.8	83.3	79.7	85.2
Goose/Guangdong/1/96 Cl 0	91.0		91.0	94.2	90.5	92.1	90.9	94.2	87.7	81.9	90.0
duck/China/E319-2/2003 Cl 2.3.2	93.1	91.0		96.5	92.6	94.4	89.4	91.9	87.0	81.5	88.7
Swan/Hungary/4999/2006 Cl 2.2	91.0	94.2	96.5		91.9	92.1	90.1	92.6	88.0	83.4	89.2
gyrfalcon/Washington/2014 Cl 2.3.4.4c	95.1	90.5	92.6	91.9		96.1	87.3	88.7	85.3	80.7	87.1
green-winged teal/Egypt/2016 Cl 2.3.4.4	98.4	92.1	94.4	92.1	96.1		87.1	88.4	84.3	80.8	86.2
turkey/Ireland/1378/1983	87.0	90.9	89.4	90.1	87.3	87.1		93.5	87.5	82.7	89.2
duck/Potsdam/1402-6/86	87.8	94.2	91.9	92.6	88.7	88.4	93.5		90.3	83.7	92.2
A/chicken/Hidalgo/28159-232/1994	83.3	87.7	87.0	88.0	85.3	84.3	87.5	90.3		88.1	93.6
chicken/Queretaro/CPA-04673-1/2019	79.7	81.9	81.5	83.4	80.7	80.8	82.7	83.7	88.1		86.0
turkey/Wisconsin/1968	85.2	90.0	88.7	89.2	87.1	86.2	89.2	92.2	93.6	86.0	

USDA Agricultural Research Service

8

H5 amino acid Sequence Comparison

	TKIA	GsGu	DKCh	SwHu	GyWA	GWEg	TKIr	DKPo	CKHi	CKQU	TKIA
turkey/Iowa/010094/2022 Cl 2.3.4.4b		91.0	93.1	91.0	95.1	98.4	87.0	87.8	83.3	79.7	85.2
Goose/Guangdong/1/96 Cl 0	91.0		91.0	94.2	90.5	92.1	90.9	94.2	87.7	81.9	90.0
duck/China/E319-2/2003 Cl 2.3.2	93.1	91.0		96.5	92.6	94.4	89.4	91.9	87.0	81.5	88.7
Swan/Hungary/4999/2006 Cl 2.2	91.0	94.2	96.5		91.9	92.1	90.1	92.6	88.0	83.4	89.2
gyrfalcon/Washington/2014 Cl 2.3.4.4c	95.1	90.5	92.6	91.9		96.1	87.3	88.7	85.3	80.7	87.1
green-winged teal/Egypt/2016 Cl 2.3.4.4	98.4	92.1	94.4	92.1	96.1		87.1	88.4	84.3	80.8	86.2
turkey/Ireland/1378/1983	87.0	90.9	89.4	90.1	87.3	87.1		93.5	87.5	82.7	89.2
duck/Potsdam/1402-6/86	87.8	94.2	91.9	92.6	88.7	88.4	93.5		90.3	83.7	92.2
A/chicken/Hidalgo/28159-232/1994	83.3	87.7	87.0	88.0	85.3	84.3	87.5	90.3		88.1	93.6
chicken/Queretaro/CPA-04673-1/2019	79.7	81.9	81.5	83.4	80.7	80.8	82.7	83.7	88.1		86.0
turkey/Wisconsin/1968	85.2	90.0	88.7	89.2	87.1	86.2	89.2	92.2	93.6	86.0	

USDA Agricultural Research Service

9

H5 amino acid Sequence Comparison

	TKIA	GsGu	DKCh	SwHu	GyWA	GWEg	TKIr	DKPo	CKHi	CKQU	TKWI
turkey/Iowa/010094/2022 Cl 2.3.4.4b		91.0	93.1	91.0	95.1	98.4	87.0	87.8	83.3	79.7	85.2
Goose/Guangdong/1/96 Cl 0	91.0		91.0	94.2	90.5	92.1	90.9	94.2	87.7	81.9	90.0
duck/China/E319-2/2003 Cl 2.3.2	93.1	91.0		96.5	92.6	94.4	89.4	91.9	87.0	81.5	88.7
Swan/Hungary/4999/2006 Cl 2.2	91.0	94.2	96.5		91.9	92.1	90.1	92.6	88.0	83.4	89.2
gyrfalcon/Washington/2014 Cl 2.3.4.4c	95.1	90.5	92.6	91.9		96.1	87.3	88.7	85.3	80.7	87.1
green-winged teal/Egypt/2016 Cl 2.3.4.4	98.4	92.1	94.4	92.1	96.1		87.1	88.4	84.3	80.8	86.2
turkey/Ireland/1378/1983	87.0	90.9	89.4	90.1	87.3	87.1		93.5	87.5	82.7	89.2
duck/Potsdam/1402-6/86	87.8	94.2	91.9	92.6	88.7	88.4	93.5		90.3	83.7	92.2
A/chicken/Hidalgo/28159-232/1994	83.3	87.7	87.0	88.0	85.3	84.3	87.5	90.3		88.1	93.6
chicken/Queretaro/CPA-04673-1/2019	79.7	81.9	81.5	83.4	80.7	80.8	82.7	83.7	88.1		86.0
turkey/Wisconsin/1968	85.2	90.0	88.7	89.2	87.1	86.2	89.2	92.2	93.6	86.0	

USDA Agricultural Research Service

10

Principals of Inactivated Vaccines for AI

- Virus shedding is related to levels of antibody and antigenic relatedness of vaccine to challenge strain
- Higher HI titers correlate to lower virus shedding
- Closer antigenic relatedness correlate to lower virus shedding
  - Measured by amino acid similarity
  - Measured by HI cross neutralization
- Examples
  - HI titer of 40 with 100% match of vaccine to challenge-good protection
  - HI titer of 320 with 94% match of vaccine to challenge-good protection
  - HI titer of 40 with 94% match of vaccine to challenge-poor protection

USDA Agricultural Research Service

11

Recombinant Live Vaccines

- Several live recombinant vaccines are now available in different countries
  - Fowlpox
  - HVT
  - NDV
  - RNA replicon (alphavirus)
- Live vaccines stimulate cell mediated immunity as well as humoral immunity (and possibly mucosal immunity)
- HI titers are generally low with these vaccines and protection levels are difficult to predict
- Difficult to quantitatively determine cell mediated immunity

USDA Agricultural Research Service

12

Is a bad vaccine worse than nothing at all?

- A marginal vaccine may reduce or prevent clinical signs, but not reduce virus shedding
- Clinically normal birds shedding large amounts of virus will increase risk of virus transmission
  - Sick birds are less likely to be moved
  - Human exposure to infected birds may increase
  - Lose disease signs as an epidemiologic surveillance tool

USDA Agricultural Research Service

13

Optimal Vaccines

- **The closer the match of the vaccine to the challenge strain the better the protection (autogenous vaccine)!**
- Immunogenic vaccines that produce high levels of antibody to HA protein-adjuvants are important
- Persistence of the immune response
- Vaccine is at a cost acceptable to poultry industry?
- No good options for mass vaccination once birdsare placed on farms

USDA Agricultural Research Service

14

Practical Application

- Single dose vaccination unlikely to provide long term protection
- Multiple dose vaccination is needed for layers and turkeys
- Prime/boost vaccination in general provides wider protection (live viral vectored prime with killed boost)
- Combination vaccines (different antigens) may provide a broader immune response

USDA Agricultural Research Service

15

DIVA

- **D**ifferentiate **I**nfected from **V**accinated **A**nimals
- **DIVA** principle primary application is to assure trading partners that livestock have not been exposed to infectious virus i.e. **differentiate vaccinated only and vaccinated and then infected poultry**
- Inexpensive, reliable, and high throughput differential serologic test needed to make DIVA surveillance testing viable
- For countries that do not export poultry, DIVA vaccination probably not a major priority

USDA Agricultural Research Service

16

Approaches of DIVA

- Sentinel birds
- Flock status
- Heterologous Neuraminidase Strategy
- Non-structural protein 1 (NS1)
- Matrix protein 2 (M2)
- Positive marker vaccines
- Subunit vaccine approach
- Increased rRT-PCR of poultry and poultry products

USDA Agricultural Research Service

17

Subunit Vaccines and DIVA

- For vaccines that only express HA and/or NA proteins (viral-vectored vaccines)
- Use of currently available AGID or ELISA tests allow for DIVA differentiation
- Shown experimentally to work
- Should work with many of next generation vaccines

USDA Agricultural Research Service

18

Issues with Current DIVA Strategies

- Validation or fitness for purpose not completed for any method
  - No internationally recognized companion DIVA tests are available
  - Poultry with high HI antibody levels may not seroconvert well to challenge
    - Will this require surveillance on a larger number of samples
  - Because immune response is not uniform, will the standard be zero tolerance of antibody
  - Measurement of serologic response is delayed by at least a week-will this be sufficient to assure freedom of infection

USDA Agricultural Research Service

19

Conclusions

- Homologous vaccination is the goal
- Many types of vaccines are commercially available
- Quality vaccine is needed as well as quality administration
- Multiple doses of vaccine are needed
- Maternal antibody will affect the quality of the vaccination
- DIVA surveillance is possible, but trade partner acceptance is still required
- Vaccination should not be used in place of good biosecurity and other control measures

USDA Agricultural Research Service

20