Challenges in controlling viral diseases of poultry

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Outline of the presentation

• Important poultry diseases
• Laboratory based diagnosis of diseases – different types and fit for purpose
• Vaccines - ideal vaccines, poultry vaccine technology and aim of vaccination
• Challenges in controlling RNA viruses (AIV, NDV & IBV) and DNA viruses (MDV)
The main disease challenges

• Threats to Avian Species.
  – Respiratory diseases such as NDV, IBV.
  – Immunosuppressive/oncogenic agents such as IBDV, MDV, ALV and CAV.
  – *Eimeria spp* (Avian Coccidiosis).
  – Pathogenic *E. coli & Mycoplasma spp*

• Threat to Humans and Avian Species.
  – Avian Influenza virus.
  – *Campylobacter jejuni and Salmonella enterica*. 
Diagnosis of diseases

- Presumptive diagnosis versus definite diagnosis.
- Definite diagnosis requires confirmation based on:
  - isolation of the organisms
  - serology assays
  - molecular assays (PCR & sequencing)
- The need to differentiate between infected and vaccinated animals (DIVA);
  - zoonotic diseases
  - to eradicate a disease
Serology based diagnostics

- Detecting antigen or antibody.
- Performance (specificity & sensitivity), cost and applicability.
- ELISA, a serology test for numerous diseases.
- Non ELISA such as AGID, HA/HI, IFAT, and neutralization test.
- The use of monoclonal antibody based assay able to detect specific strains/subtypes and for DIVA strategy.
Diagnostic Test

Field (on site) testing
- Dipstick – antigen detection
  - Turn around time
  - Cost per test
  - Multiplexing
  - Specificity
  - Sensitivity

- ELISA, PCR, NASBA, LAMP, Microarray – detection of antigen, antibody, DNA/RNA

Laboratory testing
**PCR based diagnostics**

- **Different formats**;
  - Nested PCR - very sensitive, prone to contamination
  - Multiplex PCR - detect more than 1 targets
  - In situ PCR – on tissue sections (latent infection)

- **Different detection methods**;
  - Conventional agarose gel
  - Real-time PCR;
    - more rapid and sensitive
    - able to quantify virus titer (virus shedding)
Sequencing of PCR products

- Identification of specific-strains or subtypes.
- Identification of virulence markers.
- Identification of specific mutations.
- Origin, geographical distribution and evolution of the virus.
- Next-generation sequencing (NGS) technology allows:
  - rapid & cost effective genome-wide sequencing
  - metagenomic based detection of viruses
Vaccines and Vaccination

• An ideal vaccine:
  • produce the same immune protection which usually follows natural infection but without causing disease
  • generate long-lasting immunity
  • interrupt spread of infection

• Aim of vaccination (prophylactic):
  • sterilizing immunity (prevent infection)
  • disease immunity (prevent disease)
  • blocking immunity (prevent spread of disease)
Poultry vaccine technology

- Live attenuated and killed vaccines in different combinations: mono-, bi-, tri, & polyvalent.
- Recombinant vaccines available commercially – limited choice and only based on:
  - Viral vector vaccines - Fowlpox virus (FP 9), Herpesvirus (HVT), Adenovirus.
  - Reverse genetics vaccines – primarily for RNA viruses, AIV and NDV.
  - Bacterial vector vaccines – Salmonella.
Immunosuppression

**Noninfectious**
- Space
- Air quality
- Toxins
- Temperature

**Infectious**
- Lymphotrophic viruses that destroy lymphocytes and/or macrophages
- Indirectly by releasing immunosuppressive mediators/cytokines

**Increased susceptibility to infection**
- Transient or permanent
- Generalized or specific

**Poor vaccine induced immunity**

**Chemicals**
- Toxin
- Steroid

**Proinflammatory cytokines**
- Ammonia

**Toxin**
- Space
- Air quality
- Toxins
- Temperature

**Bacterial**
- MDV
- CAV
- AIV
- IBDV
- ALV

**Indirectly by releasing immunosuppressive mediators/cytokines**
- TGF-β
- IL-10

**Proinflammatory cytokines**

**Maternal antibody**
• Avian influenza virus
• Newcastle disease virus
• Infectious bronchitis virus
• Marek’s disease virus
HA and NA are immunogenic surface glycoproteins

Hemagglutinin (HA)

Neuraminidase (NA)

Lipid Bilayer

Real 2-D E.M. images

PNAS 2006, 103: 19123
Avian influenza dynamics

Comparing with H5N1, H7N9 infection in poultry seems more widespread and persistent, constituting a greater threat for human

1997 >> 2004 >> 2008 >> ??

H5N1

Migratory birds
Reservoir and distanced transmission of virus

Poultry
Occasional and transient infection

Pathogenic infection

Human

H7N9

Migratory birds
Reservoir and distanced transmission of virus

Poultry
Widespread and persistent infection?

Low pathogenic infection

Human
1997 H5N1 outbreak in chickens in Hong Kong.

1999 H9N2 virus in domestic poultry in Hong Kong and Southern China.

2003 H7N7 outbreak in domestic poultry in the Netherlands.

2013 H7N9 outbreak in domestic poultry in China.

Influenza A viruses in poultry that cause diseases in humans (H9N2 undergo extensive antigenic drift and circulating in various poultry species in few countries)

Host range & pathogenicity determinants of 44 H9N2 isolates isolated from different poultry species in Bangladesh during 2008–2011.

Red indicates the residues that are critical for influenza pathogenesis, enhanced replication in mammalian hosts.

Green indicates unique substitutions in the viruses.

Source: CDC
Avian Influenza Diagnostic Tests (LPAI): Range of Detection in a Flock (Unvaccinated)

- AGID (IgM, may start to decrease after 30 days)
- ELISA (IgG)
- HI (IgG)
- Antigen Capture
- rRT-PCR
- Virus Isolation

Days Post-Infection

Dennis Senne, USDA
H5N1 Control Measures: Vaccination

Animals $\rightarrow$ H5N1 $\rightarrow$ Water $\rightarrow$ Poultry $\rightarrow$ Man $\rightarrow$ infection

**Vaccine?**

- Types of vaccines
- When to vaccinate
- Vaccination regime
- Differentiating infected from vaccinated animal (DIVA) strategy

Countries that practice vaccination
- China
- Vietnam
- Indonesia
- Egypt
Virion Structure of NDV

- M protein
- HN protein
- F protein
- P protein
- M protein
- NP protein
- L protein

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<tr>
<th>Genotypes</th>
<th>Newcastle disease virus</th>
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<tr>
<td>I</td>
<td>Low virulence, vaccine strains Australia (QV4), Ireland (Ulster)</td>
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<tr>
<td>II</td>
<td>Low virulence, vaccine strains worldwide (LaSota, B1, VG/GA), neurotropic virulent from US, TXGB</td>
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<tr>
<td>III</td>
<td>Isolated in Japan before 1960, sporadic isolation in Taiwan and Zimbabwe</td>
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<tr>
<td>IV</td>
<td>Predominant isolated in Europe before 1970</td>
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<tr>
<td>V</td>
<td>Emerged in South and Central America in 1970 then caused outbreaks in Europe, North America, Mexico</td>
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<tr>
<td>VI</td>
<td>Emerged in Asia in 1960, continued to circulate as the predominant genotypes until 1985 when genotype VII became more common. Genotype VIa commonly isolated in pigeons</td>
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<tr>
<td>VII</td>
<td>Emerged in Far East in 1990, spread to Europe and Asia, VIIc, d, e isolated from China, Kazakhstan, South Africa, including Malaysia, VIIIf, g, h represent African isolates</td>
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<tr>
<td>VIII</td>
<td>Circulating in South Africa since 1960 and continue to circulate in SEA</td>
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<tr>
<td>IX</td>
<td>First isolated in China in 1948 and continue to circulate in China</td>
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<tr>
<td>X</td>
<td>Isolated exclusively from Taiwan</td>
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*Infection, Genetics & Evolution 2010, 10, 26-35*
Newcastle Disease Virus

- Constant threat in many countries in the world.
- Endemic in Asia, Middle East, Africa, Central and South America.
- Vaccine use makes assessment of true geographical distribution difficult.
- Why NDV (genotype VII) is endemic in Malaysia any many countries in this region despite vaccination?
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<th>No</th>
<th>Findings</th>
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<tr>
<td>1</td>
<td>Outbreaks and spreading of virulent genotype VII is due to poor flock immunity associated with inadequate vaccination practices.</td>
<td>Vet Micro 2012, 160:17.</td>
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<tr>
<td>4</td>
<td>Current vaccine (genotype II) prevent disease but cannot stop viral shedding. When genotype-matched vaccines were used, viral shedding was significantly reduced compared to genotype-mismatched vaccines. However, no study on impact of virus shedding on disease transmission.</td>
<td>Vaccine 2009, 27, 904. Vaccine 2007, 25, 7238. Avian Pathol 2008, 37, 1. Avian Dis 2011, 55: 391.</td>
</tr>
<tr>
<td>5</td>
<td>Genotype VII NDV infected chickens showed different cytokine expression patterns, survive longer and shed longer and higher amount of viruses than genotype VIII infected chickens. The importance of this is currently been studied.</td>
<td>Comp Immunol Microbiol Infect Dis. 2013, in press</td>
</tr>
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Relationships between HI titer and challenge protection

What is the HI titer to reduce virus shedding hence, halt transmission of disease?

HI antibody titer (log2)

Death  Egg drop  Virus shedding/Transmission of disease

FAO, 1978
Virus shedding in vaccinated birds via oral and cloacal routes at different days post challenged

Unpublished data
Infectious Bronchitis Virus (IBV)

- IBV group under group 3 coronavirus with more than 50 serotypes.
- In Malaysia and SEA countries, the presence of different IBV strains is not well studied.
- Besides Mass strains, limited studies indicated the presence of Qx-like and variant IBV strains.

*Avian Dis 2012, 56: 634–641*
Mutation of IBV genome at hot spots influence the biological and immunological properties of the virus

IBV Quasispecies: Virus Persistence and Evolution

- Virus undergo mutation & recombinant
- Selection pressure by immune system, cells, host species

Variants IBV differ in:
- virulence
- escape immune response
IBV Vaccination

- Primarily comprised of live attenuated and killed vaccines.
- Immunity is short term require multiple re-vaccination.
- Vaccines contribute to emergence of variant strains and may undergo reversion to virulent strain.
- Identification of prevalent strains including new variant is important for effective control of IBV.
- New variant IBV isolates needs to be characterized and current vaccines or combinations of commercial vaccines ought to be tested for efficacy.
New infectious laryngotracheitis virus strains

- Viruses undergo mutation, reassortment and/or recombinant.
- These events are not restricted to RNA viruses.
- DNA virus such as ILTV strains can undergo intra-specific recombination producing new strain of ILTV.

*PLoS ONE 2013, 8(2): e55121.*
Marek’s disease virus: vaccines vs evolution

- Breaks in MD vaccine-induced immunity
- Breaks in MD genetic resistance
- Current vaccines failed to halt MDV evolution

Fig. 6. Step-wise evolution of virulence of MDV. Relationship between the virulence increase and the introduction of different vaccines is shown. HVT, herpes virus of turkeys; bivalent, HVT and serotype 2 (SB-1) vaccines; Rispens, CVI988 strain.

The Vet. J. 2005, 170: 175-183
Conclusion

- Pathogens are moving targets - new strains, more virulent strains, vaccine escape strains, strains that are able to resistant therapy.

- Lab based diagnosis – fit for purpose, availability, results interpretation and facility to run it (biocontainment).

- Vaccine efficiency - protection levels against infection, clinical disease and/or disease transmission.
Acknowledgements

• I would like to thank all the researchers, staff and graduate students of the animal vaccines and therapeutics research group.

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Thank You

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