Avian Coronavirus
Infectious Bronchitis Virus

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• Infectious bronchitis virus is a highly contagious avian coronavirus
• Multiple serotypes and variants of the virus exist
• Infectious bronchitis virus is found worldwide
• Control is primarily through the use of modified live vaccines but...
• The disease is extremely difficult to control because different serotypes and variants do not cross protect
The Disease

- Mild upper-respiratory tract disease
  - Sneezing, tracheal rales, gasping, coughing, watery eyes, nasal mucus, and swollen sinuses
- Decreased egg quality and production in layer birds
- Some strains can cause lesions in the kidneys (Nephritis).
- Morbidity ~100%
- Mortality varies with complicating infections (E. coli)
IBV History of Serotypes

• IBV was first recognized circa 1930, and for about 15 years Mass was the only known serotype.

• In 1956 Jungherr et al. described a new serotype of IBV from Connecticut and designated it Conn.

• Now there are many recognized serotypes and countless variant viruses
The first IBV vaccines

- Drs. Beaudette and Hudson in 1937 discovered that IBV could be grown in embryonated eggs
- Dr. van Roeckel reported on a “planned exposure” to control IBV in 1942
- The first USA vaccine was developed in the 1950s using the van Roeckel M-41 strain which is a Mass type virus isolated in Massachusetts in 1941
- The M-41 strain is the parent strain for most of the Mass type vaccines used in the USA
IBV is a coronavirus

- Single stranded RNA genome 28+ kb (largest known)
- 4 structural proteins (Spike, Envelope, Membrane, Nucleocapsid)
  - Spike glycoprotein on the surface of the virus structurally it has two subunits S1 & S2
  - Antigenic diversity is due to variability in spike
IBV- Serotypes

• Spike mediates virus-cell attachment and entry into the cell
• Spikes have several regions on their surface (epitopes) that induce neutralizing antibodies.
• Epitopes on spike are unique for each IBV type
• Neutralizing antibodies bind to spike and prevent infection
IBV Diagnosis

- Virus isolation and rising antibody titers are the traditional methods for making a definitive diagnosis
- Mabs (Mass, Conn, Ark)
- Genotyping

- Collection of samples (timing is important)
  - Tracheal swabs (choanal cleft) and/or cecal tonsils
  - Placement of sentinel chickens
Sample Submission

- Live birds
- Whole tracheas
- Tracheal or choanal swabs in PBS or viral transport media (25 samples/flock, pool 5 swabs/tube)

**International**

- Swabs in a 1:1 solution of PBS and molecular biology grade buffered phenol (pH 4.5)
- Allantoic fluid in a 1:1 solution of fluid to phenol
- Swabs or tissue spotted onto FTA cards
Finders Technology Associates (FTA) cards

- Filter paper for inactivation of infectious agents
- Cotton-based cellulose membrane
- Contains lyophilized chemicals that inactivate most bacteria and viruses.
- Immobilizes and stabilizes nucleic acid (RNA and DNA) for subsequent PCR testing
- Samples can be safely transported via regular Postal Service.
  - With the proper permit samples can be submitted from outside the USA
Diagnosis- Serology

- **ELISA**
  - Not type specific
  - Detects IgG antibodies against the virus
  - Can be positive by 2 weeks PI
- **Agar gel precipitin (AGP) test**
  - Detects IgM
  - Can be positive by 7 or 8 days PI
- **Hemagglutination inhibition (HI)**
  - Type specific... *almost*
  - IBV treated with neuraminidase will agglutinate RBCs
  - HA antigen for Ark, Mass, Conn, JMK, and DE072
  - Serum reacted with each antigen and an inhibition titer is determined.
  - Cross reactivity prevents definitive type specific results
- **Virus neutralization (embryos, cell culture or organ culture)**
  - Type specific
  - Detects IgG approximately 2 weeks PI
  - Two methods
    - Alpha- constant serum diluted virus
    - Beta- diluted serum constant virus
Diagnosis- Virus Isolation

• 9-11 day old embryonating eggs

  • Inoculate CAS
  • Typical lesions in embryos are: stunting, hemorrhagic, curled embryos, with ureate deposits in the kidneys.

• Chicken embryo tracheal organ culture

• Primary chicken kidney cells

• Susceptible chicks
Molecular Diagnosis

Molecular detection - real time RT-PCR

Molecular identification - Genetic typing
Real Time RT-PCR detection of IBV

*Used to rapidly screen field samples for IBV*

- Extremely rapid (20 minute test)
- Does **not** identify IBV type
- Determines the amount of virus in the sample
- Real time RT-PCR is a standard RT-PCR reaction that includes a fluorescent dye or labeled probe used to detect amplified DNA as it is being made
Cycle threshold (CT) value is the point where fluorescence in the reaction crosses a minimum value considered to be positive.
Genetic basis for molecular typing of IBV

A change in the spike gene can translate into a change in the amino acid sequence of spike
Sequencing to identify IBV type

RT-PCR
Amplify Spike

HVR 450 bp

1720 bp

Run Sequencing reaction

BLAST analysis
Basic Local Alignment Search Tool
Sequencing Analysis to Identify IBV type

### Basic Local Alignment Search Tool (BLAST)

<table>
<thead>
<tr>
<th>Sequence ID</th>
<th>Accession</th>
<th>Description</th>
<th>Score</th>
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### Phylogenetic tree

The phylogenetic tree illustrates the evolutionary relationships among various IBV strains, showing the branching patterns and distances between different sequences.
General IBV Epidemiology

• The virus is evolving-
  – New serotypes (variants) continue to arise
  – New IBV like viruses emerge in other species (turkeys, pheasants, pea fowl).

• Some IBV types remain geographically restricted whereas others do not.
• Phylogenetic analysis of IBV around the world
  • Phylogenetic analysis provides a measure of relatedness between isolates.
  • The closer the relatedness the more likely they will cross-protect
IBV types- North America

- **GA/124/11** (similar to CA/1735/04) most recent variant in the SE USA
- Georgia 07 & Georgia 08 -variants in the SE USA related to CAV viruses
- Georgia 98 (GA98)- similar to DE072
  - (Vaccine available, Lee et al. Avian Dis 2001)
- Arkansas- the most frequently isolated virus
  - Genetic drift is occurring in the Ark viruses (Jackwood et al. Avian Dis. 2005)
- California variant viruses- 3 groups recognized
  - **CAV/1737/04** (Jackwood, et al. Avian Dis. 2007)
  - Recent variants identified in 2004/2005
IBV Types - South America

- **Brazil** - BR1, BR2, BR3 genetic types 2007-2010
  - 71% of isolates are variants and 98% of those are related to 4/91
- **Venezuela** - one variant virus identified by RFLP, others probably exist
- **Chile** - Q1 IBV
- **Colombia** - Q1 IBV
  - Collaboration: Instituto Colombiano Agropecuario (ICA), Federacion Nacional de Avicultores de Colombia (FENAVI) and the University of Georgia, PDRC
  - 4 genetic groups not related to Mass, Conn or Ark vaccines. Mass and Conn molecular types also identified.
- **Peru** - several variant viruses identified not related to the Massachusetts serotype.
Q1 and QX Phylogenetic Tree

Ark

Mass

Mass/H52/ S1

Mass/H120/ S1

Mass/Mass/ S41/41 S1

Mass/Beaudette

Conn/Conn46/51 S1 vaccine

FL/FL18288/71

DE/DE072/92 S1 vaccine

GA98/0470/98 S1

Dutch/D1468/81

4/91

CH/TC07-2/07

71.2

CH/TC07-1/07

793B/4.91/91

CA/1737/04 S1

DMV/5642/06 S1

GA07/GA07/07 S1

71.2

CA/1737/04 S1

Mass/H52/ S1

Mass/H120/ S1

Mass/Mass/ S41/41 S1

Mass/Beaudette

Conn/Conn46/51 S1 vaccine

FL/FL18288/71

DE/DE072/92 S1 vaccine

GA98/0470/98 S1

Dutch/D1468/81

Chile/12139/09

China/LDL97/97 aaz09202

China/Q/98

China/Jz/86

China/T3/96

Israel/Variant2/98

B/D207/84

B/D274/84

UK/6/52/82

B/Uk/167/84

B/Uk/142/86

E/D3896/84

CA/1737/04 S1

DMV/5642/06 S1

GA07/GA07/07 S1

6/91

PP14/PP14/??

Ark/Ark99/73

Ark/ArkDPI/61 S1

CAL99/CA1/55/99 S1

CAL99/NE15172/95 S1

Holte/Holte/54

Gray/Gray/60

Iwak/JMK/64

SE17/SE17/77

iowa/iowa/609s/56

iowa/iowa/609/56

CAV/CAV188/95

CAV/CAV9437/95

CAV/CAV565/91

CA/CA/12495/96 S1

CA/557/03 S1

HN99 S1

JAAS/04/01 vaccine

N1/62 S1

GA08/HSp15/08 S1

GA08/Pass 4 challenge strain

GA08/GA08/08 Pass 16 S1

WA08 S1/GU301925

Q1

QX

Colombia/92075-64/12

Colombia/Ark/92072-52/12

Colombia/92073-53/12

Colombia/92072-50/12

Colombia/92072-51/12

Colombia/92074-59/12
QX IBV History

- QX IBV was first reported in China circa 1995 (LX4-type virus)
  - Currently it is the most frequently isolated IBV type in China
- In 2001-2002 reported in Eastern Europe and Russia
- In 2004-2006 reported in the Netherlands, Germany, France and Belgium
- Spread to England in 2007
  - Found in broilers, layers, breeders and backyard birds
- Causes Nephritis
- Vaccines are available

Courtesy of Dr. Guillermo Zavala
Q1 IBV History

• 1996 to 1998 Q1(also T3 and J2) IBV first isolated in China
  – 25 to 70 day-old layer chickens
  – Respiratory disease/ Proventriculitis
  – Q1, T3 and J2 are 98.9% similar

• Taiwan 2005
• Chile 2009
• Italy 2011
• Colombia 2012
• No vaccine is available at this time
Control using Vaccination

- IBV vaccines are given at 1-day of age in the hatchery and at 14 to 18 days of age in the field (broilers).
  - The vaccine can be given by course spray in the hatchery and the field or by water in the field
  - Multivalent vaccines are usually given in combination with NDV
Vaccines

- World wide
  - Mass (H52 and H120)
- USA
  - Mass (Mass 41), Conn, Ark-DPI, Holland (Mass) DE, GA98, (JMK, Fla)
- Europe
  - Mass, 4/91 (793B)
- Australia
  - Vac C, VicS, VacB2, VacB3
- Netherlands
  - Mass, D1466, D274, D1201
IVB Vaccine Efficacy

• The vaccine must have the same serotype as the challenge (field) virus (homologous protection)

• Little or no cross protection occurs between serotypes (heterologous protection)

So how do we protect chickens against variant IBV strains?
Modified live vaccines and cross-protection

- Vaccinating with more than one serotype induces cross-reacting antibodies that can provide protection.

- Some strains of IBV are particularly good at inducing these cross-reacting antibodies (Ma5 & 4/91 or DE072).
Protection: Signs/Ciliostasis/Virus Detection

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Challenge virus</th>
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<tbody>
<tr>
<td>Day of hatch</td>
<td>GA 11</td>
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<tr>
<td>Day 14</td>
<td></td>
</tr>
<tr>
<td>MA5</td>
<td></td>
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<tr>
<td>DE072</td>
<td></td>
</tr>
<tr>
<td>MA5</td>
<td></td>
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A Protection is based on:
- ≥90% protection from signs
- >50% protection against ciliostasis
- ≥90% protection against virus detection
Key Steps in the Control of IBV

1. Surveillance and identification of new variant viruses by sequence analysis is extremely important
2. Determine if new variants are pathogenic
3. Clinical case data and targeted surveys are used to determine how widespread the variant has become
4. Determine if current commercially available vaccines are efficacious
5. Development of a new vaccine for the variant if none of the commercial vaccines alone or in combination are efficacious
Thank you for your attention!