Today’s perspectives for IB Control
Recent findings

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Introduction
More IB problems recently

QX
793/B
IT 02
D274
etc.
More IB problems recently

Western Europe : 2002 - 2006

Table 7. Proportion (%) of IBV per country after removing all of the 100% vaccine detections.

<table>
<thead>
<tr>
<th></th>
<th>United Kingdom</th>
<th>France</th>
<th>Germany</th>
<th>Holland</th>
<th>Belgium</th>
<th>Spain</th>
<th>Western Europe</th>
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<tbody>
<tr>
<td>793B</td>
<td>27.7</td>
<td>42.1</td>
<td>28.4</td>
<td>25.1</td>
<td>17.4</td>
<td>14.9</td>
<td>28.5</td>
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<td>Massachusetts</td>
<td>25.9</td>
<td>12.0</td>
<td>17.0</td>
<td>26.8</td>
<td>14.1</td>
<td>12.2</td>
<td>21.2</td>
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<td>Italy02</td>
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<td>9.5</td>
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<td>55.4</td>
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<tr>
<td>QX-like</td>
<td>0</td>
<td>25.5</td>
<td>37.6</td>
<td>30.3</td>
<td>39.1</td>
<td>0</td>
<td>17.3</td>
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<tr>
<td>D274</td>
<td>7.0</td>
<td>0</td>
<td>10.0</td>
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<td>22.8</td>
<td>17.6</td>
<td>6.6</td>
</tr>
<tr>
<td>B1648</td>
<td>0</td>
<td>3.2</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
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<tr>
<td>Other a</td>
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<td>2.8</td>
<td>4.1</td>
<td>1.3</td>
<td>3.3</td>
<td>0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

(After K.J. Worthington et al. Avian Pathology, 2008)
More IB problems recently

Western Europe : 2002 - 2006

(4103 samples – 59% positive for IBV)

(After K.J. Worthington et al. Avian Pathology, 2008)
More IB problems recently

Western Europe : 2002 - 2006

(After K.J. Worthington et al. Avian Pathology, 2008)
More IB problems recently

QX
793/B
IT 02
D274
etc.

QX

GA 08

Q1 (J2)

J2
Var2
More IB problems recently

TOP 10 DISEASES POULTRY
2006-2009

‘000 LSUs lost to disease p.a.

- Duck virus: 1,599
- Fowl typhoid: 2,204
- Fowl cholera: 3,801
- Pullorum disease: 4,029
- Mycoplasmosis: 13,235
- Inf. bursal disease: 27,086
- Newcastle disease: 60,371
- LPAI: 70,683
- Av. inf. bronchitis: 84,268
- HPAI: 96,721

LSU losses from all other poultry diseases: 2,760

More IB problems recently

Why?
IB : everywhere the same questions

Is it IB ?

Which vaccine(s) / which program ?

New (technology) vaccines ?

Vaccination / administration ?

Let’s go back first to some key points related to IB and IBV…
Some key points related to IBV & IB

IBV is:
- poorly resistant in the environment
- but can persist several weeks / months in birds
- and is very contagious

So that:
- Biosecurity is important
- All-in/All-out & Down period are important
- Vaccination is necessary
IBV: an ever changing virus
Some key points related to IBV & IB

IBV is known as an “ever changing” virus:
- genotype
- antigenicity
- tropism
- escape vaccine protection

But there is also stability in the change:
- good for poultry production
- good for vaccine producers & users
- hope for new protection concepts
Is it IB?
Is it IB?

Clinical monitoring

Serological monitoring

Characterisation:
Is it IB?

Clinical monitoring

Serological monitoring

Characterisation:
Is it IB?

The 4 basic tropisms of IBV

- Trachea
- Gut
- Kidney
- Oviduct
Is it IB?
Is it IB?
Is it IB?

Papers and Articles

Characterisation of an infectious bronchitis virus isolated from vaccinated broiler breeder flocks

D. Parsons, M. M. Ellis, D. Cavanagh, J. K. A. Cook

Veterinary Record (1992) 131, 408-411

Four apparently serologically closely related isolates of infectious bronchitis virus were obtained from two flocks of vaccinated broiler breeders, one mile apart, which were experiencing increased mortality and decreases in egg production. The isolates were serologically distinct from isolates previously described and capable of causing characteristic infectious bronchitis-like respiratory infection in young chicks. In one experiment, the H120 vaccine strain of the virus did not protect the trachea against challenge with the new isolates 21 days later.

INFECTIOUS bronchitis is an acute, highly contagious respiratory infection of chickens of all ages, which affects not only the respiratory tract but also the reproductive tract (King and Cavanagh 1990). Effective vaccines have been available for many years, and vaccination schedules commonly use both live attenuated and inactivated oil adjuvanted vaccines. In the United Kingdom, the live vaccines are only of the Massachusetts serotype but the inactivated vaccines may contain a UK "variant" strain. There have, however, been reports of outbreaks of infectious bronchitis in vaccinated flocks of all ages (Cook 1983, 1984, Cook and Huggins 1996), although these occurring in laying flocks have not resulted in mortality and have usually been associated with only small decreases in egg production. The present report describes the isolation of infectious bronchitis virus from two vaccinated broiler breeder flocks in which mortality and substantial decreases in egg production occurred.

Materials and methods

Flock history

Flock A - The first signs of disease were seen in the middle house of three houses of 28-week-old broiler breeders on February 1, 1991. The flock was unusually quiet, the birds had failed to eat and 17 (0.65 per cent) of the pullets had died overnight. Mortality on the previous six days had been 0, 0, 0, 0, 0 and 0. At this time no cockerels had died but by the end of the outbreak mortality was similar in both cockerels and pullets.

Clinical examination showed that the worst affected pullets were depressed and lethargic. Other observations included: closed eyes, swollling around the eyes and under the jaw, birds standing in corners with wings held away from their bodies, head shaking, panting and a respiratory sniff. After the initial mortality the litter became wet. All three houses on the farm were affected within about four days of the first clinical signs. Between 28 and 30 weeks of age 374 pullets (1.3 per cent) died.

Flock B - This was a flock of 55-week-old broiler breeders situated about one mile from flock A. A rise in mortality was noticed in one pen in one of two houses of 14,800 birds on February 2 with mortality over four days being 0, 20, 20 and 0. Mortality rose in the neighbouring pen two days later. Egg production fell from 51.9 per cent to 29.5 per cent over a period of four weeks, before showing some recovery (Fig 1). Post mortem findings were similar on both farms. The birds had died in good condition, the carcasses were pale and there was a mild tracheitis and excess mucus in the mouth. There was food in the crop but none in the intestines, the kidneys were pale and swollen and the liver showed varying degrees of congestion. No bacteria were isolated from the livers. All the birds were...

FIG 1: Percentage egg production in two broiler breeder flocks during the four weeks before and after clinical respiratory signs were observed and infectious bronchitis virus was isolated which failed to recover fully (Fig 1). There was some loss of egg colour but no abnormally shaped shells, the thickness of which remained good.
Is it IB?

Pale 'wet' pectoral myopathy in broiler affected with 4/91 infection.

(Picture: P. Cargill, UK, 1993)
Is it IB?

(Picture: Brazil, 2004)
Is it IB?

(Picture: Brazil, 2004)
Is it IB?
Is it IB ?

(Picture : Brazil , 2004)
Is it IB?
Is it IB?
Is it IB?
Is it IB?

Clinical monitoring

Serological monitoring

Characterisation:
Serological monitoring of IB (broilers)

- SPF vaccinated at D 1
- Broilers vaccinated at D 1
- + field infection

Serological monitoring of IB (broilers) (After J. Gelb, 1998)
Serological monitoring of IB (broilers)

Mean ELISA titre at slaughter can indicate IBV infection

Panel showing the number of flocks with different mean ELISA titre (log 2) levels. The graph indicates a comparison between control flocks and IFT-IB positive flocks. The data is reported in the paper "Serological monitoring of IB (broilers)", by J.J. De Wit, 1992.
Is it IB?

Clinical monitoring

Serological monitoring

Characterisation:
- isolation
- serotyping
- genotyping
- pathogenicity in vaccinated birds
Characterisation of IBV: serotyping

Cross-seroneutralisation on a given medium (EE, CC, Trach.Ring)

Relationship calculated from Archetti & Horsfall formula (1950)

\[ R = 100 \times \sqrt{\frac{\text{Product of heterologous titres}}{\text{Product of homologous titres}}} \]

Arbitrarily:
- \( % > 0.8 \) : same serotype
- \( 0.6 < % < 0.8 \) : relationship
- \( % < 0.6 \) : different serotypes
Characterisation of IBV: genotyping

- PCR & sequencing of (part of) S1 gene

- Fast & easy:
  "the more people do PCR, the more variant IBVs they report"

- Useful for diagnosis & epidemiology

- Little predictive value regarding pathogenicity and vaccine protection
## Characterisation of IBV: genotyping

<table>
<thead>
<tr>
<th>Flock ID</th>
<th>Commercial Laboratory</th>
<th>University of Delaware Laboratory</th>
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<tbody>
<tr>
<td></td>
<td>% Partial S1 Sequence Homology (from 479 to 788 of S1)</td>
<td>% S1 Sequence Homology</td>
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<tr>
<td></td>
<td>GA 08 Index</td>
<td>GA 08 Vaccine</td>
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<tr>
<td>GA 08 Vaccine Virus</td>
<td>99,7</td>
<td>99,7</td>
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<tr>
<td>GA 08-8225 Index case</td>
<td>100,0</td>
<td>100,0</td>
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<tr>
<td>Dbc</td>
<td>80,6</td>
<td>80,6</td>
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<tr>
<td>Shg</td>
<td>99,4</td>
<td>99,4</td>
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<tr>
<td>Cvt</td>
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<td>TWTS</td>
<td>80,7</td>
<td>80,7</td>
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<tr>
<td>Plmt</td>
<td>99,4</td>
<td>99,4</td>
</tr>
<tr>
<td>Brk-T</td>
<td>80,9</td>
<td>80,9</td>
</tr>
<tr>
<td>Brk-K</td>
<td>99,0</td>
<td>99,0</td>
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</table>
Characterisation of IBV: protection testing

- Vaccination (SPF and broilers) using various vaccines or combinations of vaccines
- Obviously the best tool
- but time consuming and expensive
Which vaccine(s) / programs?
Vaccine protection against IB

- Passive protection
- Active protection / live vaccines
- Active protection / killed vaccines
## Vaccine protection against IB

### Passive protection (IBV + E.Coli challenge)

<table>
<thead>
<tr>
<th>DAYS AT CHALLENGE</th>
<th>MDA STATUS</th>
<th>CUMULATIVE % MORTALITY (DPC)</th>
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<tr>
<td></td>
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<td>4</td>
</tr>
<tr>
<td>1</td>
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<td></td>
<td>+</td>
<td>3</td>
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<td>7</td>
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<td>14</td>
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<tr>
<td></td>
<td>+</td>
<td>0</td>
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</table>

(After A. Mockett et al. 1987)
Vaccine protection against IB

The QX story in Europe
Vaccine protection against IB

Active protection / live vaccines

- even “new” IB vaccines are still “old”:
  - attenuation / production in eggs
Vaccine protection against IB

Active protection / live vaccines

- even “new” IB vaccines are still “old”:
  - attenuation / production in eggs
  - homologous / narrow protection
  - residual pathogenicity
Vaccine protection against IB

Active protection / live vaccines

- even “new” IB vaccines are still “old”:
  - attenuation / production in eggs
  - homologous / narrow protection
  - residual pathogenicity

Strong need for new approaches / new vaccines
Vaccine protection against IB

Active protection / live vaccines

What to do in case of a new variant IBV?

Today’s vaccination strategies:

- development of an homologous vaccine
- combination of 2 different IB vaccines
Protection with a combination of IB vaccines

Comparison of the protection against a M41, 793B or Q1 challenge in commercial broilers that have been vaccinated at day of hatch with Cevac Bron 120L, Cevac IBird or the combination of both vaccines

SJAAK DE WIT¹, M. ESMANN¹, H. VAN DE SANDE¹, VILMOS PALYA², YANNICK GARDIN³

¹GD DEVENTER, ²SSIU CEVA PHYLAXIA, ³SD CEVA LIBOURNE
Material and Methods

- Day-old commercial broiler chicks, Ross breed
  - IB vaccines: Cevac Bron 120 L (H-120 strain) and Cevac IBird (1/96 strain)
  - Eye drop (0.05 ml-drop in each eye).
  - IBV challenge strains: M41, 793B-like, Q1 (“Q1a”) and Q1 (“Q1b”) from Chile,
  - Challenge dose: $10^{4.0}$ EID$_{50}$ IBV by eye drop method (0.05 ml-drop in each eye).

- 5 days post-challenge, ciliostasis test. 5 rings/bird.
  - Score 4: <25% of beating cilia;  Score 3: 25 to 50% beating;  Score 2: 50 to 75% beating;
  - Score 1: 75 to 99% beating;  Score 0: all beating.

- Protection standard: score < 10/20.
## Material and Methods

### Vaccination (d0) | Challenge strain | Challenge age (days)
--- | --- | ---
Cevac Bron 120 L | | |
Cevac Bron 120 L + Cevac IBird | M41 | 21 & 35
- | | |
Cevac IBird | | |
Cevac Bron 120 L + Cevac IBird | 793B-like | 21 & 35
- | | |
Cevac Bron 120 L + Cevac IBird | Q1a | 28
- | | |
Cevac Bron 120 L + Cevac IBird | Q1b | 28
- | | |
- | mock | 21, 28, 35
Ciliostasis protection rates

Protection rates after M41 challenge (21 days)

% protection

Ciliostasis protection rate

- Unvax Unch
- Unvax Ch
- Bron 120
- Bron 120 + IBird
Ciliostasis protection rates

Protection rates after M41 challenge (35 days)

Ciliostasis protection rate

- Unvax Unch
- Unvax Ch
- Bron 120
- Bron 120 + IBird
Ciliostasis protection rates

Protection rates after 793B-like challenge (21 days)

% protection

Ciliostasis protection rate

- Unvax Unch
- Unvax Ch
- IBird
- Bron 120 + IBird
Ciliostasis protection rates

Protection rates after 793B-like challenge (35 days)

- Unvax Unch
- Unvax Ch
- IBird
- Bron 120 + IBird
Ciliostasis protection rates

Protection rates after Q1α challenge (28 days)

<table>
<thead>
<tr>
<th>Protection rate</th>
<th>Unvax Unch</th>
<th>Unvax Ch</th>
<th>Bron 120 + IBird</th>
</tr>
</thead>
<tbody>
<tr>
<td>% protection</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ciliostasis protection rates

Protection rates after Q1b challenge (28 days)

% protection

Ciliostasis protection rate

- Unvax Unch
- Unvax Ch
- Bron 120 + IBird
Conclusions

- A combination of Mass type vaccine (Cevac Bron 120L) and a 793B type vaccine (Cevac Ibird) was able to induce high levels of protection in commercial broilers against homologous (M41 & 793B) challenges at 21 and 35 days of age.

- As well as high levels of protection against heterologous (Chilean Q1a and Q1b) challenge at 28 days of age.
Protection with a combination of IB vaccines

Recent results from trials conducted at GD Deventer with chickens vaccinated in the hatchery with a combination of:
a Mass type IB vaccine (Cevac Bron 120 L or Cevac Mass L) and a 793 B type IB vaccine (Cevac IBird)
Protection with a combination of IB vaccines

<table>
<thead>
<tr>
<th>IBV</th>
<th>VACCINATION</th>
<th>% PROTECTION / CILIOSTASIS AT (days):</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>793 B</td>
<td>-</td>
<td>0</td>
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<tr>
<td>793 B</td>
<td>H120 + IBird</td>
<td>100</td>
</tr>
<tr>
<td>793 B</td>
<td>H120 + IBird + Transmune</td>
<td>100</td>
</tr>
<tr>
<td>Brazil D2147</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Brazil D2147</td>
<td>Mass L + IBird</td>
<td>-</td>
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</table>
Vaccine protection against IB

Can we expect “really new” IB vaccines in the future?
New IB vaccines?

Desperately needed because of:
- lesions induced by live vaccines
- need for “universal” protection

Spike Pr.
S1
S2
New IB vaccines?

Reverse Genetics IBV:
- rIBV BeauR-M41-S1 or rIBV BeauR-4/91-S1

Recombinant vector vaccine:
- rFPV-S1
- rAdeno-S1
- rMDV-S1 (rHVT-S1)

Recombinant vector vaccine + “immune response modifiers”:
- rFPV-S1 / IL18 or rFPV-S1 / IFNγ or rFPV-S1 + IFNγ

Subunits:
- rS1

DNA:
- Plasmid DNA

Combined approaches:
Vaccination / Administration
Administration of IB vaccines

2 options?

At hatch

At farm
Administration of IB vaccines

Only 1 option

At hatch

At farm

YG - 140416
Administration of IB vaccines

CEVA-DESVAC IN LINE SPRAYER

Key Features
- Flat spray design = perfect coverage (2 or 4 flat shaped nozzles)
- Free-standing system adaptable to any convey or belt system
- Crates are not stopped for vaccination
- Cleaning In Place system (CIP) for better hygiene
- Data can be downloaded on a USB device for traceability
Administration of IB vaccines

CEVA-DESVAC IN LINE SPRAYER

- Accurate dosing (5 ml to 16 ml)
- Adjustable nozzle holders and spray arms
- Reliable air pressure regulator
- Integrated vaccine shaker system
- Alarms for blocked crates and empty vaccine tank
- Safety sensor for vaccine consumption
- Saves vaccine
Administration of IB vaccines

SPRAYER TESTING – Materials & Methods

Vaccination process

Chickens after vaccination
Administration of IB vaccines

SPRAYERS TESTING – Results

Ratio of chicks with vaccine-take
Administration of IB vaccines

SPRAYERS TESTING – Results

Evolution of the results

![Graph showing the evolution of results over time for different spray methods and sampling types. The graph compares Conjunctiva samples and Trachea samples across dates D2, D4, and D7, with various spray methods indicated by different lines and markers. The graph demonstrates the ratio of positives over time for each sampling type and method.]
Administration of IB vaccines

SPRAYERS TESTING – Results

Evolution of the results

- Eye-drop application = 100% uniform vaccine-take both in the conjunctiva and the trachea from D2;
- Complete vaccine-take at D7, indicating the efficient spreading of vaccine virus within the flocks.

Monitor of quality of vaccination requires individual samples to be taken at or earlier than D4 (more sensitive to detect differences).
Administration of IB vaccines
Administration of IB vaccines

IB protection is also passive protection

i.e. vaccination of breeders with killed vaccines
THANK YOU
FOR YOUR ATTENTION