Acute Infectious Bursal Disease in Poultry: Situation in Europe & Principles for Vaccination

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**Background**

**Immunosuppressive viral diseases**

- Highly contagious diseases, very resistant viruses
- **Target** = lymphoid cells
  - CAV: T-cells, Thymus
  - IBDV: B-cells, Bursa of Fabricius
- Destruction of lymphocytes
  - immunosuppression
  - disease
  - death
- Two forms of the diseases:
  - **clinical**: young chick
  - **subclinical**: older birds
Impact of IBD

- **Direct losses**: due to the acute forms of the diseases
  - vvIBDV: up to 25% in broilers, 60% in light breeds
  - CAV: up to 50% mortality in the first week of life

- **Indirect losses**: due to subclinical disease
  - secondary infections consecutive to acquired immunodeficiency
  - impaired growth, condemnation of carcasses…
  - estimation of the economic impact of subclinical forms
    (McIlroy et al., 1989, 1992; Mc Nulty et al., 1991):
      - IBDV: 10-15% decrease in financial return
      - CAV: 10% lower net income

- **Increased use of antibiotics and chemicals**
- **Inability to properly process low pathogenic viral strains or live vaccines**
  - circulation of these strains and change through passage in birds
  - residual pathogenicity of live vaccine strains
Viral Interactions

IBDV → CAV → MDV
Background

- Epidemiomonitoring & diagnosis vary considerably among countries → exact situation?
  - Prevalence of the acute & immunosuppressive forms
    » Are seldom accurately evaluated
    » Different forms might co-exist
  - Viruses responsible for the different forms of the disease cannot always be easily distinguished
    » Need for good assessment of antigenic, pathotypic & genetic tools for the characterization of IBD strains

- Vaccination failures in different parts of the world
In order to increase knowledge of the epidemiology of IBD
   – Member Countries should establish systems of diagnosis and epidemiometrics aimed at revealing the incidence and prevalence of the acute and immunosuppressive forms of the disease
   – Member Countries should submit infected samples to expert laboratories for the characterization of the viral strains, encouraging in particular cooperation between countries that are at different levels of development

Research on very pathogenic strains should be supported, preferably within the framework of coordinated international programs aiming at
   – the identification of possible virulence markers
   – the establishment of specific prophylactic measures

Vaccines against IBD and/or appropriate vaccination methods be developed, in order to allow more effective vaccination of young birds with maternal immunity

(N. Eterradossi, 1995)
COST 839 = 20 countries
Scientific Programme

Field

Working Group 1
Epidemiology

Working Group 3
Vaccination

Working Group 2
Diagnosis & Economic Impact

Working Group 4
Immunology & Pathogenesis

Working Group 5
Molecular Virology

Research
Infectious Bursal Disease

“Infectious Bursal Disease is a highly contagious viral infection of the young chick (3 to 6-week-old) which is characterised by the destruction of immature B lymphocytes in the Bursa of Fabricius”

Infectious Bursal Disease Virus

- Birnavirus (since 1984)
- 2 segments of dsRNA
- 50-60 nm, non enveloped, icosahedral
- Highly resistant in the environment and contaminated premises
IBDV = RNA virus

High mutation rate of the RNA polymerases

----> Antigenic variation

----> Increased virulence
Genomic Organization of IBDV

**SEGMENT A**

- VP5
- VP2
- VP4
- VP3

**small ORF**

**polyprotein ORF**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Weight</th>
<th>Function</th>
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</thead>
<tbody>
<tr>
<td>VP1</td>
<td>90 kDa</td>
<td>RNA polymerase- Replicase</td>
</tr>
<tr>
<td>VP2</td>
<td>40-45 kDa</td>
<td>Structure: external capsid</td>
</tr>
<tr>
<td>VP3</td>
<td>30-32 kDa</td>
<td>Structure: internal capsid</td>
</tr>
<tr>
<td>VP4</td>
<td>28 kDa</td>
<td>Non structural protease</td>
</tr>
<tr>
<td>VP5</td>
<td>17 kDa</td>
<td>Regulation &amp; cell lysis</td>
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**SEGMENT B**

- VP1

<table>
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<th>Protein</th>
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**Genomic Genomic Genomic**
Large ORF in Segment A

108 kDa

Polyprotein

45-50 kDa

VPX or VP2a

55-60 kDa

VP2b

vVP2

40-45 kDa

VP4

28 kDa

VP3

32 kDa
The IBDV particle

From Bottcher et al., 1997

dsRNA

| VP1
| VP3
| VP2

VP3 trimers

VP2 trimers

From Bottcher et al., 1997
Molecular Basis of Antigenicity in IBDV

VP2

variable region

hydrophilic peaks

point mutations

inside

outside

Serotype 1

Serotype 2

Subtype
Classification of IBDV as pathotypes

**Apathogenic** (Serotype 2)  no mortality, no bursal lesions

**Pathogenic** (Serotype 1)

- mild  no mortality, increasing bursal lesions
- intermediate
- intermediate plus
- Old classical  increasing mortality
- Variant
- Classical European
- Very or hypervirulent

No Standardisation

Absence of virulence markers: immunological (MAbs) or molecular (sequences)
Epidemiology of IBD (1)

- **Before the 80s:** Satisfactory control of the disease by vaccination
  - High morbidity, low mortality (1-2%)
  - --> subclinical disease

- **Serological survey:**
  - High prevalence of antibody in breeder flocks
  - -----> passive transmission of MDA + OEV boost of breeders
  - -----> in major parts of the world, IBD was essentially subclinical
    - » indirect economical losses due to immunosuppression

**But...**
“There is nothing permanent except change”

Heraclitus
Since the mid-80s: Vaccination failures in different parts of the world

- USA (1984): slight increase in specific mortality (2-3%)
- Europe (1987): high mortality (broilers 5-15%, layers up to 60%)

--- > clinical disease

Cause for failure:

- USA: antigenic drift: VARIANT IBDVs
- Europe: increased virulence: HYPERVIRULENT or vvIBDV

Consequence

- USA: change in vaccines
- Europe: change in vaccination schedules
Worldwide geographical distribution of the very virulent forms of IBDV.
In black, countries where acute have been reported. In grey, countries where no acute forms have been reported. In white, countries with no report (updated from Eterradossi, OIE Technical Report, 1995).
Diagnosis of Acute IBD

- **Field: Clinical symptoms & lesions during the acute phase**
  - **Clinical picture**: Acute phase exacerbated (Stuart, 1989: “Acute IBD”):
    » Rapid onset of the disease followed by rapid death or recovery
    » Prostration, diarrhoea, ruffled feathers, trembling
  - **Gross lesions**: Target organ: Bursa of Fabricius: inflammation & haemorrhages
  - Lesions in non bursal lymphoid organs: spleen, thymus, caecal tonsils
  - Disseminated haemorrhages:
    » muscles (thigh & pectoral)
    » intestinal tract (proventriculus-gizzard)
  - Dehydratation: pale, swollen kidneys
Diagnosis of Acute IBD

- **Laboratory**: samples collected during the acute phase
  - Histopathology on BF or non bursal lymphoid organs
  - AGID, immunofluorescence, immunostaining on suspicious organs

- These tests are not strain specific
- Diagnosis more confused if
  - Not during the acute phase of IBDV
  - Subclinical infection
  = risk factor for next flocks → need for further characterization
Typing of isolates as vvIBDV

- **Live birds versus laboratory testing**
  - **Live birds**
    - Reproduction of the disease in SPF chicks as compared with reference strains
    - Cross-protection: inoculation of classical strains followed by challenge with suspected material
  - **Laboratory**
    - Inoculation of suspect material to
      - *cell culture*: not for vvIBDV field strains
      - *embryonated SPF-eggs*: no pathognomonic lesions, no difference in titers
    - Needs further characterization!
    - Cross-neutralization in SPF-eggs

- **Conclusion**
  - Tedious, time consuming, restricted to well-equipped laboratories
  - Need for rapid discrimination
Laboratory tools

Figure 2: The laboratory tools for IBDV characterisation.

From Di Fabio et al, 2000


Characterisation of the vvIBDV strains

- **Pathotyping**
  - Up to 100% mortality in SPF chickens
  - 50-60% mortality in layers, 25-30% in broilers
  - Break through higher levels of MDA
  - not possible anymore to protect passively broilers

- **Antigenicity**
  - Panel of neutralizing Mabs → no (major) antigenic drift
  - High cross-neutralization indices measured in SPF embryonnated eggs
  - Classical serotype 1 vaccines still satisfactory on SPF birds

- **Molecular characterisation**
  - Sequencing of the variable domain of the VP2 gene
  - Amino acid residues substitutions at positions 222Ala, 256Iso, 294Iso and 299Ser
    - = Genetic fingerprints of vvIBDV
  - RT-PCR of vVP2 followed by sequencing and/or RE Analysis
Mortality rates

- Challenge of 5-week-old Lohmann SPF chickens (n=25-30) with $10^5$ EID$_{50}$ of IBDV strains of varying virulence in isolated conditions by the oculonasal route.

- vvIBDV induce higher mortality rates than classical virulent IBDVs
- BUT direct comparison of results from different labs is often difficult due to differences in the challenge protocols (breed & age of chickens, virus dose, route of inoculation…)
- Confusion in nomenclature
- Need for standardized challenge model
Multiplication rates

- Titration of virus in BF of birds dying (or sacrificed) 3-4 days after challenge

  - Higher multiplication rates for more virulent IBDVs
    - 1 attenuated IBDV particle → 10 particles
    - 1 classical virulent IBDV particle → $10^2$ to $10^3$ particles
    - 1 vvIBDV particle → $10^4$ to $10^5$ particles

- In accordance with:
  - Van der Marel et al. (1990) and Eterradossi et al. (1997) using quantitative ELISA
  - Moody et al. (2000) using quantitative RT-PCR
Standardized IBDV challenge

- **Comparison with reference IBDV strains**
  - Check for absence of contaminants (Reo, Adeno, CAV)
  - Same viral dose: $10^5\text{EID}_{50}/\text{bird}$ (CAM route for egg titration)
  - Same chickens (n= 25-30)
    - Breed: AFSSA or Lohmann SPF chickens
    - Age: 4 to 5-week-old
  - Isolation units
  - During an observation period of 7 days
Pathotyping: standardized challenge

Classification based on molecular tools:
(French Mabs & vVP2 sequences)
96108 & 849VB: European vvIBDV
Henan & GX: Chinese vvIBDV
Cu1wt & F52/70: Classical European
CJ801: Attenuated Chinese

Conclusions
- Different breed susceptibility to the challenge
- Likely differences in mortality within the same antigenic & molecular viral lineage
- Clear-cut differences between vvIBDV and cvIBDV when considering clinical signs at D3
- Mortality rates is poorly relevant for classification of IBDVs as pathotypes
Laboratory tools

Figure 2: The laboratory tools for IBDV characterisation.

VIRUS ANTIGENICITY

Monoclonal antibody

Epitope (antigen)

IBD VIRUS

Nucleic acid

GENETIC RELATEDNES

PATHOGENICITY

Challenge

From Di Fabio et al, 2000
Rapid characterisation of the vvIBDV strains

- **Antigen capture tests on infected BF**
  - Capture by general antibodies, revelation by panels of specific neutralizing Mabs
  - **US panel**: no antigenic variation on vvIBDVstrain
  - **French panel (Eterradossi et al.)**: combination of 2 criteria:
    - Amount of Ag in the BF during the acute phase
    - Lack of binding of Mabs 3 & 4 (Ala 222) on all vvIBDV tested so far
  - **BUT** altered antigenicity on AA 222 alone cannot be considered as marker of enhanced virulence as variant strains and vaccine Bursine 2 also do not react with Mabs 3 & 4
  - So far, there is no Mab that specifically binds only to vvIBDV strains
Rapid characterisation of the vvIBDV strains

- **Molecular typing**
  - Minute amounts of virus
  - RT-PCR of the variable domain of the VP2 gene: best evolutionary clue
  - RE Analysis: *e.g.*
    - AA 222: *Bst*NI cuts classical IBDVs but not variant and vvIBDVs
      *Bsp*MI cuts only vvIBDVs
    - AA 245: *Sac*I cuts classical but not vvIBDVs
  - **BUT**
  - Same profiles → no conclusion
  - Different profiles → might be significant (risk of silent mutations at nucleotide level that do not affect the AA)

- Sequencing and phylogenetic comparison should be preferred but are expensive
- RT-PCR + RE analysis for rapid screening of strains
Molecular Epidemiology based on vVP2

- **Serotype 1**
  - Recent vvIBDV
  - Old African vvIBDV
  - Classical virulent
    - F52/70, Cu1 wt, STC
  - Attenuated IBDVs
  - Variant US strains
  - Old European strains
    - (Hungary, Poland)
  - Australian strains
    - OH, 23/82

- **Serotype 2**
Rapid characterisation of the vvIBDV strains

- **Conclusions**
  - The usefulness of molecular tools for epidemiological monitoring is considerable to predict the relative similarities and differences among IBDV strains
  - **BUT** antigenic and molecular investigations must be related to the field situation, with a good characterisation of the circulating strains in terms of:
    - **Prevalence**: is this strain dominant in the field?
    - **Virulence**: pathogenicity in well-controlled conditions as compared with reference strains
    - **Antigenicity**: cross-protection
  - = Evolutionary (not virulence!) markers
  - Occurrence of new and diverging lineages of vvIBDVs should not be excluded in the future
  - **Continuous surveillance of the field situation is necessary**
Risk factors for vvIBDV

- High resistance → long persistence, highly effective horizontal transmission
- Northern Europe (Denmark, Sweden, Norway & Finland): sporadic vvIBDV outbreaks → risk factor analysis (M. Folden-Jensen et al., 2000):
  - Spatio-temporal factors: previous outbreak (7 km & 10 days)
  - Slaughtering practices: “thinning” = high risk factor
  - No association with previous outbreak in same farm
- Subclinical infections = risk for next flock
- Monitoring is extremely important
- Good biosecurity measures are essential
  - All in – all out
  - Cleaning, disinfection, disinsectisation…
  - Down time
  - Good distance between farms
  - Restricted accesses…
Control of Infectious Bursal Disease

- **Impact**: immunosuppression, disease & death
  - Direct losses: mortality & disease
  - Indirect losses: secondary infections, impaired growth, condemnation of carcasses
  - Public Health: increased use of antibiotics and chemicals

- **The protection**
  - Well correlated with the levels of neutralising antibodies
  - VP2: structural protein, highly conformation dependent neutralising epitopes
  - Sub-unit VP2 protein: full protection of SPF chickens

- **The control**
  - Eradication is virtually impossible in the field
  - Hygienic measures alone are most often inadequate
  - Vaccination is thus essential but regular failures of classical schedules
  - Interference of maternally derived antibody (MDA) has became the major problem in the establishment of the vaccination programme
Viral diseases = predominant pathology in industrial flocks
Vaccination is essential for control
Poultry probably the most vaccinated livestock in the world
Immunosuppressive Viral Diseases (IBDV-CAV) = major threat to the poultry industry since decades
  - No fully satisfactory vaccine
  - Interference with other vaccinations
Furthermore, immunosuppression is one of the most common causes for a failure of the general vaccination program and successful IBD vaccination is considered as a key factor in the establishment of a satisfactory control schedule.
Equation for protecting chickens from disease:
Potent & safe vaccines > < fully immunocompetent chicken
How Poultry Disease Spreads
Biosecurity

- IBDV is highly resistant to physical & chemical agents
- Persistence of virus despite disinfection
- No vaccine can solve the problem if major precautions are not taken
- All-in/all-out
- Cleaning: dry followed by hot water (60°) at high pressure
- Disinfection (2x):
  - accurate dose, temperature, contact time…
  - if field virus suspected or known: 20% formalin, 20-25°C, 70% relative humidity
- Desinsectisation of premises
- Down time before restocking
Poultry Vaccination

= Mass vaccination

- Up to 50,000 birds in a single space
- Need for effective methods
- Aim = vaccinating high enough proportion of birds in the flock
- Proportion will depend on
  - Type of infectious agent involved
  - Current epidemiological situation
- List A diseases: maximized protection
- Many other situations: minimizing the economic impact of disease
Basic Principles

- Introduction of an attenuated or inactivated disease into a live body to produce a degree of immunity through an immune response
- Concept of immunological memory: vaccinated birds will respond to a second exposure or challenge with a more rapid, strong and effective immune response
- Immune system is ready to recognize antigen, produce antibodies and develop memory cells for secondary response (« priming »)
Vaccination failure

• Poor administration of the vaccine
  - trivial causes: expiry date, inappropriate storage…
  - dosage
  - water quality (pH, chlorine)
  - water quantity
  - vaccine stability (milk products)
  - vaccination procedure
    - number of nipples
    - thirsting
    - distribution of water (dye)
    - dead space
• Improper timing (interference MDA)
• Field pressure
  - agent or serotype not in the vaccine
  - increased virulence
Inactivated vaccines

- Virus grown in cell culture or SPF embryonated eggs or SPF chickens and inactivated
- SQ or IM inoculation
- In adjuvant (mineral oil emulsion or aluminium hydroxide)
- Safe but less effective, more expensive
- Mainly used for booster vaccination of layers or breeder flocks before lay (16-20 weeks) to produce high, uniform and persistent antibody titres in hens prior to lay

→ Passive protection of broilers
Live IBDV vaccines

- No naturally low pathogenic strains
  - La Sota, Hitchner lentogenic NDV strains
  - HVT (serotype 3) against Marek’s disease

- Cell or egg adapted attenuated strains
  - IBDV: mild, intermediate or hot strains

- Mainly administered by drinking water, but nebulisation is also possible

- Efficacy: strong, long-lasting humoral and cell-mediated immune responses

- Safety: residual pathogenicity, immunosuppression, risk of reversion to virulence
Live IBDV vaccines

- Classification based on virulence & breakthrough titre (day X)

- **Mild strains**: many passages in cell cultures, used before the emergence of vvIBDV, highly sensitive to MDA, useless in countries with vvIBDV (Europe)

- **Intermediate**: lower passages in cell culture, can break through higher MDA, some residual pathogenicity

- **Intermediate plus → Hot**
  - Higher breakthrough (2-3 half-life times earlier: 6 days in broilers, 11 in layers)
  - Increasing residual pathogenicity
  - Attenuated by passages in eggs with sometimes back passages in chickens to increase efficacy (do not grow in cell culture)
  - Very expensive
  - Not registered in many European countries (do not comply to E.P.)
Breakthrough titres

From the IBD information site: http://www.gumboro.com
Safety: residual pathogenicity

- Intermediate plus or hot strains:
  - Disease in SPF chickens
  - Gross lesions: Bursal damage
  - Impaired growth
  - Clinical CAV at later age (3-4 weeks)
  - Vaccination failures (NDV, IB...)
  - Immunosuppression: secondary infections
Safety: residual pathogenicity

🔹 E.P. monograph on live IBDV vaccine:
  - **Safety**: measurement of immunosuppression in SPF chickens:
  - At the time after administration when maximal bursal damage is likely to be present:
    » Administer 1 dose of Hitchner B1 strain Newcastle disease vaccine (live)
    » The degree of immunosuppression is estimated from the comparative seroresponses 2 weeks after vaccination and protection rates after challenge.
All IBDV live vaccines (including the mild ones) protect SPF chickens against mortality.

They do not protect equally against bursal damage.

Good correlation between SN antibody & protection.

But a good protection, including against mortality, can be achieved without SN antibody.

This indicates a role of CMI in protection.

Only live vaccine provide good CMI.

Good protection: humoral + CMI responses.
The interference of Maternally Derived Antibody
Interference of MDA

- Variant & vvIBDV\textsubscript{s} can break through higher MDA levels
- No (longer) passive protection of broilers
- Need for live vaccination but interference of MDA
- **USA**: higher breakthrough linked to antigenic variation
- **Europe**: vvIBDV\textsubscript{s}
  - Role of modified epitope (Mab 3-4) not demonstrated
  - Higher multiplication rate
- Antigenic variation & modified virulence can interfere with the establishment of the vaccination schedule
- Age of vaccination?
Decay of MDA in Broilers

SN titre

OEV +

OEV -

Very virulent or variant strains

Hot vaccines

Intermediate plus

Intermediate vaccines

Mild vaccines

Age (weeks)
slaughter
Decay of MDA in Broilers

SN titre

OEV+

OEV-

Age (weeks)

slaughter
Estimation of the optimal time of vaccination

◆ **Theory:**
  – No OEV before lay → low and poorly uniform levels of passive antibody
  – OEV vaccination before lay → high and homogeneous levels of MDA

◆ **Practice:**
  – Not always uniform
    » If bad priming of hens with live vaccine → uneven boost by OEV
    » Mixed populations of chicks from different origins
    » If subclinical infection (field challenge) during life of hens → rise in MDA

◆ **Reality:**
  – Race between vaccine and field virus
  – Depending on the infection pressure

→ **Need to evaluate the immune status of the flock in order to define the right moment for vaccination**
The Deventer formula

- First established by Dr Ben Kouwenhoven in de ’80s (mean titres)
- Replaced by the « Deventer formula » (J.J. De Wit et al.) since 1990 (distribution of titres)
- **Principle:** regular decline (half life, log₂ scale) of MDA in chicken → age of vaccination can be predicted

- **Advantages:**
  - Flexible bleeding dates for testing (1-10 days post-hatch)
  - Suitable for:
    - all types of birds (broilers, breeders, layers)
    - uniform and non-uniform titre distribution
    - all kinds of IBDV vaccines

- **Conditions:**
  - Number of samples (min 18) and quality of chicks
  - Based on VNT → correlation with each ELISA needs to be established
  - Tool, no guarantee that can help if all other conditions are fulfilled
Broilers vaccination in Europe

Interference of MDA

- Passive protection during the whole growing period

- Active protection in the presence of MDA
Broilers vaccination in Europe

- **Question**: Is it still possible to protect the broilers passively during their whole growing period??
- **Answer**: Not anymore since the emergence of very virulent IBDV (extended age susceptibility)

- **Question**: Is it possible to extent the duration of passive protection?
- **Answer**: NO!
  - inactivated OEV with bursa-derived antigen (Box et al., 1989)
  - subunit vaccines: baculovirus or yeast or prokaryotic derived VP2 antigens?

- **Question**: Is OEV boost before lay still indicated?
- **Answer**: Belgium: NO! Rest of the world: YES!
**Rationale for stopping the OEV of breeders**

- Not possible anymore to protect broilers passively during the whole growing period
- MDA not always uniform even when OEV boost (mixed populations, challenge during period of lay…)
- OEV = more expensive
- Lower age susceptibility during 2 first week of life
- « Lower » economic impact when challenge earlier in life
- Two live vaccinations, calculated by ELISA during first days of life
- Taking benefit of the heterogeneity of MDA levels: low antibody chickens will act as bioreactors and spread the vaccine when the other birds become susceptible

**Results:**

- No OEV vaccine anymore in Belgium since the 90’s
- Sporadic outbreaks but not more than in many other EU countries
- In 2002: larger outbreak in Belgium & Netherlands: same vvIBDV strains as 10 years before, poor hygiene, bad timing of vaccination
Broilers vaccination in Europe

Interference of MDA

- Passive protection during the whole growing period
- Active protection in the presence of MDA
Active protection in presence of MDA

- **Attenuated vaccine**: competition with field virus (mild-intermediate)
- **Hot strains**: residual pathogenicity, risk of reversion to virulence
- **Inactivated vaccine in young chicks**: very expensive, can be considered for high value flocks and/or in countries with high and cheap manpower
- **Recombinant vaccines**: HVT, FPV, FAV: efficacy in the young chick?
- **NAVAC**: insensitive to MDA, priming of response at young age but expensive and poorly efficient so far
- **In ovo vaccination**: with immune complexes or with HVT-VP2
Maternally Derived Antibody

- Protection against pathogens in the neonatal stage, when immunocompetence is not yet completely developed
- Maternal antibodies of birds are stored in the yolk sac (IgY ≈ IgG) and transferred to the embryo while it matures
- Interference with vaccination by specifically eliminating vaccine, so that the optimal vaccination dose is dependent on the titre of maternal antibodies

Analysis of the kinetics of the transfer of maternal antibodies from the yolk sac to the serum
NDV+ IBDV+

NDV-specific IgG
IBDV-specific Ig

ED16
ED18
ED19
ED20
ED21
Day 1
Day 2
Day 3
Transfer of maternal antibodies from the yolk sac to the chicken

NDV

IBDV
In ovo vaccination:
- low titres of maternal antibodies
- virus replication remains possible
- low vaccination doses evoke strong, long-lasting immune responses

“A window of opportunity’ exists for avoiding interference of maternal antibodies with vaccination”
New developments in the field of IBDV vaccines

- **HVT Recombinant Vaccine**
  - Insertion of genes of immunising epitopes of a disease agent (IBDV - VP2) into non-essential gene loci of a vector virus (HVT – herpesvirus of turkeys).
  - Vaccination with the recombinant virus results in immunization against both the vector virus (MDV) as well as the expressed epitopes of the disease agent (IBDV).

- **Virus-Antibody Complex Vaccines**
  - Antibody (immunoglobulin) specific for the virus is mixed in an appropriate ratio with the vaccine virus = virus-antibody complex (immune complex) vaccine.
  - The amount of antibody in the complex is so small that it does not provide passive immunity or neutralise the vaccine virus.
  - But the amount of antibody added to the vaccine is enough to delay by several days the normal course of vaccine virus replication.
  - Allows for the safe *in ovo* administration of “moderately attenuated vaccine viruses”.

In ovo Vaccination

ED0  Incubator  regularly turning
      ↓
ED18  Hatching tray  no turning
      ↓
      ↓
Transport
      ↓
Opportunity to:
immunise each individual chick
without additional stress of manipulation

“In ovo vaccination”
In ovo Vaccination: History

- *In ovo* vaccination replaces hand inoculation
  - 1982: Sharma and Burmester demonstrate efficacy of MDV vaccination in ovo
  - 1992: First automated egg injection system, the inovoject system, patented by EMBREX Inc.
  - 1995: In North America, 55% of broiler embryos were vaccinated for Marek’s disease on ED18
  - 1997: *In ovo* vaccination against infectious bursal disease (IBD) with bursaplex vaccine
  - 1999: More than 80% of U.S. broiler industry vaccinates in ovo against MD

- Reason: better controlled and less expensive

Underlying fundamental mechanisms?
Chickens are not entirely immunocompetent before the end of the first week of life.

Early immunised birds responded to ‘dead’ antigens, but later, suggesting that the antigen was stored until the immune system had matured enough to react.

Viruses may replicate in the embryo and persist, if not lethal, until an immune response can be raised.
Safety of *In ovo* Vaccination

Current poultry vaccines, except for Marek’s Disease, cannot be used as they kill the 18 day-old embryos.

Clear need for new vaccines that can be used in ovo

Development of an *in ovo* vaccine against IBDV

- Known attenuated or low pathogenic IBDV strains: unsuitable for *in ovo* vaccination
- Immune complex vaccines
Conclusion: *In ovo* Vaccination

Although the immune system is not entirely developed before the end of the first week, *in ovo* vaccination against some live viruses is very effective if:

- Viruses survive in the chick until the immune system has matured enough to react
- The viral strains and their dose are adapted to the host (18-day-old chicken embryo) to avoid toxicity
- A small window exists to avoid interference with maternal antibodies
- Defence mechanisms, other than Ig responses, could be activated by *in ovo* vaccination (non-specific)
Conclusions

- Risky period
- Estimation of age for vaccination
- Multiple vaccinations
- More invasive vaccines

- Biosecurity
- Cleaning
- Desinfection
- Desinsectisation

- MDA SN titre
- vVIBDV breakthrough titre
- Vaccine breakthrough titre

Age (weeks)
Vaccine of the future: safe priming in presence of MDA

Prime

Risk period

MDA SN titre

vvIBDV breakthrough titre

Vaccine breakthrough titre

Boost

Age (weeks)

Alternatives: priming in presence of MDA
- Recombinant vaccines (HVT)
- OEV vaccination of young birds
- In ovo vaccination
- DNA vaccination
Conclusions & Perspectives

- Clinical and subclinical IBD cause direct and indirect economical losses:
  ----> it is therefore essential to control both clinical & subclinical diseases

- More sensitive and rapid diagnosis methods:
  ----> better definition of the epidemiological situation
  ----> control of the seropositivity of breeders

- Molecular basis for virulence and pathogenesis:
  ----> attenuation and control

- More efficient, safe and economical vaccines:
  ----> “priming” vaccines might have advantages over conventional vaccines