Control measures for Bovine Respiratory Syncytial Virus

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Introduction

BRSV characteristics important for control

Biosecurity

Vaccination

Current research

Conclusions
Introduction

Bovine respiratory disease (BRD)

One of the most costly diseases in calves

• Direct losses:
  » treatments
  » animal losses
  » reduced average daily gains
    even from subclinical disease:
    untreated cattle with lung lesions at slaughter Griffin 2014

• Indirect losses:
  » increased (x2) risk not to calve, later calving, dystocia Van der Fels Klerx 2002

Controlling BRD will increase profits and welfare
Respiratory disease

Aetiology

• Several viral and bacterial pathogens
  • BRSV, BCoV, PIV-3, BHV-1, BAV-3, BVDV etc.
  • *Mannheimia haemolytica, Pasteurella multocida, Trueperella pyogenes, Histophilus somni, Mycoplasma spp.* etc.

• Large proportion of healthy animals harbours potentially pathogenic bacteria in respiratory tract
  • will amplify at favourable conditions Angen 2009, Holman 2015

• Viral co-infections common Valarcher 2006, O’Neil 2014

• No pathogen identified in many cases, despite optimal sampling

• Non-specific techniques enable to discover new pathogens Ng 2015

Controlling BRSV is only one part of the solution

Introduction
Respiratory disease

Clinical outcome affected by host and environment:

1. Factors influencing immunity

   • Poor calving conditions Waltner Toews 1986

   • Failure of passive transfer
     » 1-7 days of age
       » serum total protein <5.7 g/dl Windeyer 2014
     » 2-3 weeks of age
       » serum total immunoglobulin <7.5g/l Pardon 2015

   • Stress Senthilkumaran 2013, 2015

Management is crucial in BRD control
Respiratory disease

Clinical outcome affected by infection pressure:

2. Factors influencing infection dose and diversity of pathogens

- Herd size Klein-Jöbstl 2015
- Calf group size Svensson 2006
- Number of susceptible calves >100-120 days of age Smith 2014
- Microclimate in calf pen
  - Bedding and ventilation Lago 2006, Nordlund 2008

By decreasing infection dose we can help the immune system to win
The role of BRSV in BRD

- Epidemic and sporadic disease outbreaks
  - 86% of outbreaks (n=21) in Norwegian dairy herds Klem 2014
  - 60% of outbreaks (n=10) in Danish dairy herds Uttenthal 1996
  - 30% of outbreaks (n=24) in Dutch veal calf operations Pardon 2011
  - 30% of outbreaks (n=20) in French suckler beef herds Valarcher 2006
  - 12% of outbreaks (n=1364) in Irish dairy and suckler beef herds O’Neil 2014

- Seroconversion or low BRSV-specific antibody titres = BRD risk
  (dairy herds, veal calf operations and fattening units)
Introduction

The role of BRSV in BRD

May cause severe disease as single pathogen
High morbidity in groups with many naïve animals (1-6 month old)
Usually low mortality: 0 to 2-3% (up to 20-30%)

Destroys muco-ciliary barrier, change innate immunity (IFN α/β↓) Valarcher 2003

Predispose for:
- co-infection with other viruses
- bacterial colonisation of lung
The aims of BRSV control

1. Reduce clinical signs and associated losses
   - Vaccines that protect against clinical signs but less against virus shedding

2. Stop virus circulation at herd or regional level
   - High biosecurity +/- vaccines that protect well against virus shedding
Virus characteristics

Classification and survival in environment

_Pneumovirus_ in _Paramyxoviridae_ family
Closely related to caprine, ovine and human (H)RSV

RSV takes lipid envelope from host membrane
Sensitive to detergents and UV-B radiation
Limited survival outside host

- Several hours on protection wear  
  Kingston 1968, Hall 1980

Virus survival will matter for biosecurity measures
Virus characteristics

Stable RNA genome -
Fusion (F) surface protein

- Important for virus fusion with the cell membrane
- Induces protective immune responses

Genetic stability enable vaccines to induce protection against several circulating strains

Amino acid conservation

BRSV strains: 98% 85%
BRSV vs. HRSV: 81% 30%

(Hall 2000)

Valarcher 2000

Larsen 2000
Infection characteristics

Epidemiology

High seroprevalence in adults worldwide
- Areas with high cattle herd density / animal exchanges Elvander 1996, Widgren 2010
- Big herds Norström 2010


Persistence suggested
- Repeated outbreaks in the same herd
- Antibody fluctuations without virus circulation Van der Poel 1993, 1997
- Virus mRNA in lymph nodes >2 months after infection Valarcher 2001
  → active virus protein production
- Importance?
  - Does not stay in every herd after outbreaks Ohlson 2013
Infection characteristics

Transmission

Rapid spread in naive areas Inaba 1972

  - Some seem free in endemic areas Ohlson 2013

1. Direct transmission:
  - Introduction of animals/ direct contact

    Large droplet spread Hall 1981

    Airborne spread
      within a building Mars 1999
      between close but separate buildings?
      not between farms at distance Ohlson 2010
Infection characteristics

Transmission

2. Indirect transmission:
   - HRSV on hands in contact with contaminated surfaces  Hall 1980
   - Herds that do not provide boots for visitors – increased BRSV risk  Ohlson 2010
   - Anecdotes of visits by animal professionals before outbreaks
     
     Viral load on contaminated surfaces / exposure factors
     Number of contacts with sensitive individuals

- **What is the hygiene required to reduce spread below R1?**
  - Influenza A able to spread by personnel despite coveralls and hand hygiene  Allerson 2010
  - BRSV outbreaks also in herds without purchase or visitors

Applicable hygien might be enough in bigger picture
Infection characteristics

Pathogenesis and pathology

Bronchiolitis and interstitial pneumonia

Virus induced cytopathology
  Destruction/shedding of bronchiolar epithelial cells and pneumocytes
  Syncytia – virus spread by hiding, contribute to narrowing bronchioli

Immune mediated pathology
  Oedema and cellular infiltration
  Goblet cell proliferation – mucus production

→ bronchiolitis obliterans

Atelectasis and emphysema
Immune mediated pathology

Main reason for no HRSV vaccine:

- vaccine-induced exaggerated disease following natural infection

- associated with formalin inactivated vaccine

- one similar report from cattle in the field (suboptimal controls) 
  Schreiber 2000

considering large number of doses used:
not frequent problem in cattle

Vaccine safety is an important issue
Infection characteristics

Days of infection

Virus detectable
Clinical signs starting

Virus decrease

Practical window of detection

Virus clearance:
- Apoptosis of epithelial cells Viuff 2002
  enhanced by cytotoxic T cells Taylor 1995
- Epithelial cell shedding Liesman 2014
- Neutrophils phagocytose BRSV in lumen
- Macrophages phagocytose neutrophils / debris

Wanted (by vaccination):
1. T lymphocyte memory: cytotoxic T cell response
2. B lymphocyte memory:
   - airway IgA
   - neutralising antibodies
Infection characteristics

Days of infection

BRSV-specific maternally derived antibodies, MDA

Can be neutralising
Protective but only at high levels Kimman 1989

- Present in tissue rather than in upper airway lumen
- IgG₁
Immune responses

BRSV-specific maternally derived antibodies (MDA)
Inhibits the humoral response to infection and vaccination

5 calves vaccinated s.c. with commercial vaccine
   calves with highest BRSV-specific MDA at vaccination
   - lowest antibody response
   - suboptimally protected

Hägglund et al. 2004

+ vaccin-induced BRSV specific IgG1
**Immune responses**

Duration of immunity is short in calves

Calves might develop disease at first reinfection  
Blodörn 2015

- Not clear how many infections are required to get a long lasting immunity

  Adults can have long lasting IgG (years)  
  Klem 2014

Virus shedding without clinical signs, as for HRSV?
virus and infection characteristics for control: summary

Virus

- easy to spread by direct contact
  - virus shedding occurs before clinical signs

- seems to be easy to spread by indirect contact
  - may survive on material for hours (overnight ?)
    - but easy to kill by detergent

- genomic stability favours effect of vaccination

Vaccines

- should induce CTL, airway IgA and neutralising antibody
  - = efficacy and safety
Main target population for protection

Calves and young stock – most disease
   Either kept in original herd or commingled in production units

1. Protection from encountering virus
   a) Calf-buying herds – direct spread most important
      i. Quarantines /welcome units - difficult to make functioning mix of animals from many origins large animal groups in same airspace sensitive individuals – high pathogen shedders no strict isolation (ventilation, staff hygiene)
      ii. Isolation of sick individuals - lower infection dose for others very difficult with many sick animals, not practiced
      iii. Make new-coming calves non-infectious at arrival stop virus introduction into their original herd

Tackle the infection at source
Biosecurity in breeders/ calf-supplying herds

...to decrease infections also in calf-buying herds

Requires
1. That routes that are difficult to control not common:
   - airborne transmission between herds at distance
   - small wild animals
   - milk truck/ milk collection
   - reexcretion of persistent virus

2. Strict hygiene of visitors, until we know level needed

3. Functioning quarantines (e.g. on pasture)

4. Or purchase of non-infectious animals
Advantages of biosecurity without vaccination

1. reducing introduction of several pathogens Van Shaik 2002
2. omit:
   - vaccine costs
   - risk of pathogen spread with contaminated vaccine
   - risk of reversion of vaccine to virulent virus
   - risk of vaccine-induced immunopathology
3. enable serologic monitoring of virus spread
Biosecurity

Possible drawbacks - biosecurity without vaccination

- No BRSV-specific MDA in calves sold to infected units
  - would respond well to vaccination
- Higher morbidity at virus introduction (low herd immunity)
- Faster and more severe disease
  - adult losses
  - more virus, better transmission
- Social isolation?
  - auctions and exhibitions
  - spontaneous visits between neighbours
Biosecurity

Proposed means of controlling BRSV by biosecurity

- Rely on buying and selling animals between free herds

- Status classification based on repeated antibody screening
  - pooled milk samples from a few first calvers
  - bulk milk

- Ensure high biosecurity
  - protection wear

- Alert system between farmers and professionals
Biosecurity

Remains to be investigated at longer term/larger scale

- BRSV circulation in herds, which:
  - do not purchase animals
  - provide means for hand hygiene, and protection wear
  - are situated in BRSV endemic area with high density of cattle herds

• If hygiene measures are not sufficient – investigate other means
Biosecurity

Will likely decrease many but not all pathogens

Will depend on education of farmers
  supported by veterinarians and farmer organisations

Not possible to implement optimally everywhere
  (neither in Scandinavia)

→ vaccination is a good tool
Vaccination

Added to calves and young stock that need to be immune before virus encounter (before shipping)

1. Reduce clinical signs (continuous basis)

2. Stop virus circulation (temporarily until virus decrease)
   requires:
   strong virological protection also in calves with MDA
   early vaccination/ immunity with rapid onset and long duration
   identification of virus shedders
   including subclinically infected yearlings and adults?
Vaccination

The easy ones - BRSV seronegatives

1. Young and no BRSV-specific MDA, but healthy

2. MDA or active immunity has waned

Parentally administrated vaccines
   good clinical protection
   at least partial virological protection (duration?)

West 1999, Hägglund 2004, Grauman & Larsen 2010, Vahl 2014 (MSD), Mackoschey 2008 (Intervet)

Examples of such vaccines on the market:
2 w + 6 w: immune at 8-10 w of age
1w + 4-5 w: immune at 6-9 w of age
Vaccination

- Immunise all animals asap.
  infection dose important factor Hall 1981

- Respect storage conditions

- Respect vaccination interval
  if too short:

  little time for competition for antigen/
  selection/expansion of cells fitting best to antigen

  “best-fit” memory cells are not formed

  ➢ low/ no memory response at boost

  ➢ immunological memory of poor quality
Vaccination

The hard ones - young calves with BRSV-specific MDA

- Immunesystem immature compared to 5 month old calf
- Corticosteroids might affect active responses after ~3 weeks
- ”MDA-vaccine-hiding” to immune system

parentally administrated vaccines not optimal Ellis 2014
Vaccination

The hard ones - young calves with BRSV-specific MDA

Maternal immunisation

Increased levels of BRSV-specific MDA in the calf

Ellis 1996, Dudek 2014

+ potentially protects even the very young calf
+ protects mother

- failure of passive transfer
- lack of optimal local immunity
- lack of memory responses
Vaccination

The hard ones - young calves with BRSV-specific MDA

- Active immunisation might be better *if* MDA can be overcome

- Intranasal administration better than parental
  
  (MDA inhibit less in upper airway lumen)
  
  Live attenuated vaccines
  
  *e.g.* from 9 days: *immune at 2 weeks of age*

Live vaccine + simultaneous infection = more disease (?) Kimman 1989

Duration of protective immunity <4 months (booster needed) Ellis 2013

Vaccine virus shedding
  
  if transmission between animals (*in vivo* passage)
  
  risk for reversion to virulent virus
  
  circulation of new strain in the field
Experimental attenuated live vaccine:
Small hydrophobic protein gene deletion
ΔSHrBRSV

- Calves with BRSV MDA
  Vaccine virus shed almost abolished
  No spread to seronegative sentinels

- Compared to wildtype virus
  ΔSHrBRSV induced more apoptosis in vitro
  -better virus clearance

ΔSHrBRSV (i.n. i.t.) marked reduction of pulmonary inflammation in gnotobiotic calves, after vaccination

Equal, strong, virological protection against mild challenge after 6 months Taylor 2014
The hard ones - young calves with BRSV-specific MDA

- Single intranasal administration (ΔSHrBRShV)

- Two parenteral injections of subunit vaccine
  - internal human RSV proteins (N, P, M2-1)
  - Abisco 300 adjuvant (SUAbis) s.c.
  - Montanide ISA71 adjuvant (SUMont) i.m
# Lung pathology on PID 7

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*Slide K Blodörn*
Current Research

- IgA in airways (anamnestic only)
- IFNg producing T cells (respiratory lymph nodes)
- Systemic VN antibodies (anamnestic only)

- IFNg producing T cells (systemic)
- Local and systemic antibodies, non-neutralising
- Improve by including F protein
Current Research

- How to increase duration of protection with live i.n. vaccine?
  - Live attenuated vaccines induce better responses if they replicate i.n.
  1. Homologous boost
     - Inhibition of boost-vaccine-virus-replication by local responses induced by priming
     » Parenteral boost might be advantageous
  2. Heterologous boost
     Subunit vaccine (SUMont) i.m.
     - better local and systemic antibody responses after boost
     - better virological protection against challenge 7 weeks after boost

compared to homologous boost with ΔSHrBR SV i.n.  Makabi-Panzu 2014
Current Research

- DIVA compatibility (differentiation of infected from vaccinated animals)

- Goal:
  Combine with biosecurity in control programs
  Exit from vaccination at long term
BRSV or classic vaccine

Gene-deleted recombinant BRSV

Protein subunits + adjuvant

Infection with virus replication
Future perspectives

DIVA (differentiation of infected from vaccinated animals)

1. Find infected animals among vaccinated
detecting antibodies against a virus protein omitted from the vaccine

2. Protect naive animals without interfering with
    serologic monitoring of virus spread
    BRSV classification status

3. Monitor vaccine efficacy against circulating strains
   - Discover when vaccinated animals get infected and contagious
     - Contagious animals develop high levels of DIVA antibodies
     - Protected animals develop lower levels of DIVA antibodies
     - The duration of protection becomes clearer
Conclusion

• BRSV should be studied further in large, closed herds with strict visitor hygiene in endemic areas, to understand
  - the role of modes of transmission that are difficult to control

• We should aim to stop virus introduction in calf-supplying herds
  - to reduce virus circulation, additionally after commingling in production units

• We should immunise calves early, before shipping
  - maternal immunisation
  - active immunisation that overcome MDA
    • Intranasal priming and parenteral, heterologous boost?

• By combining biosecurity with DIVA vaccines that induce strong virological protection we may advance towards better control of BRSV and other diseases

• DIVA vaccines might provide possibilities for serologic monitoring of their own efficacy, can potentially be used to protect young calves and stop virus circulation, without interfering with serological studies on virus spread or herd classification status.
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