Molecular Diagnostics in Veterinary Medicine Helps to Eradicate a Cattle Disease With a World-Wide Distribution

Ernst Peterhans, Institute of Veterinary Virology, University of Bern

**Biological background:**
- the BVD virus and the disease
- the BVD virus and its host → its host population

**Epidemiological background:**
- BVD is arguably the most widespread cattle disease world-wide
- the situation in Switzerland and Europe

**Is it possible to eradicate BVD?**
- diagnostics: the approach
- diagnostics: the techniques
- the Swiss BVD eradication campaign
The bovine viral diarrhea virus (BVDV)

Genus **Pestivirus**, Family **Flaviviridae**

- the closest relatives of BVDV are Border Disease virus of sheep and and Classical Swine Fever virus
- a more distant relative is Hepatitis C virus of humans
- two biotypes: cytopathogenic / non-cytopathogenic

...you can park more than 100 million of these viruses on a parking lot of 1mm² area
BVDV and its host

2 types of infection: **transient (acute)** / **persistent**

**Fetal Infection:**

- `ncp` BVDV only!

**Insemination**

- AB-neg → pos

- **Month 1**

- **gestation**
  - Months 2-4
  - Months 5-9

- **Early death**
- **Persistently infected**
- **Spreads virus lifelong**
- **immunotolerant**

- **Ncp or cp BVDV**
- AB-neg → pos

- Early death

- Persistently infected
- Spreads virus lifelong
- Immunotolerant

- Malformations
- Abortion

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BVDV and host: transient infection

**Frequently observed**

Individual animal: moderate diarrhea, coughing

Beef production: intermittent diarrhea with coughing → increases use of antibiotics

**Rarely observed**

Bleeding in internal organs:

USA/Canada 1989-92: 40'000 animals perished of 120'000 infected
Acute hemorrhagic BVD
BVDV and its host

What is so special about the persistent infection?

Fetal Infection: ncp BVDV only!!

Ncp or cp BVDV

Insemination

AB-neg → pos

Month 1

gestation

Months 2-4

months 5-9

Early death

Persistently infected

Spreads virus lifelong

immunotolerant

Ab+

months 5-9

malformations

Abortion

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BVD virus: how to outwit the host's immune system
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No antibodies against BVD-Virus - but virus carrier for life!

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Mucosal Disease: a virological accident – with a lethal outcome

Cells not destroyed

cytopathogenic

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Mucosal Disease:  
a virological accident – with a lethal outcome
Persistent infection: the consequences

ca. 12,000 animals
Why are there two types of infection?

**Transient infection**
- Insemination
- Month 1
- Gestation
- Months 2-4
- Months 5-9
- Early death
- Persistently infected
- Spreads virus lifelong
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**Persistent infection**
- Ncp or cp BVDV
- Fetal Infection: ncp BVDV only!!
- AB-neg → pos
- Early death
- Persistently infected
- Spreads virus lifelong
- Immunotolerant
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- Abortion

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Why should the virus bother to have two types of infection (transient and persistent)?
Persistent infection is a “life insurance” for the virus!
Persistently infected animals perpetuate and spread the virus in the cattle population.
Persistently infected animals perpetuate and spread the virus in the cattle population

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Persistently infected animals perpetuate and spread the virus in the cattle population
Epidemiology of BVD in Switzerland

- No ABs to BVDV (susceptible) 40%
- ABs to BVDV (protected) 60%
- Persistently infected 0.7%
  (at least on PI animal on every eighth farm)
BVD ERADICATION in Switzerland: criteria fulfilled?

- Biology of infection known in sufficient detail ✓
- Epidemiology of BVD in Switzerland known ✓
- Unequivocal identification of animals ✓
- Concept of eradication clear ✓
- Diagnostic methods established ??

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BVD-Diagnostics: *what is important?*

1. Persistently infected animals are crucial for persistence of BVD virus in population

2. Prevalence of persistently BVD virus-infected animals is low

→ persistently infected animals must be detected with the highest possible sensitivity and specificity
BVD-Diagnostics: *two major methods*

<table>
<thead>
<tr>
<th>Antigen-ELISA</th>
<th>Real-time RT-PCR</th>
</tr>
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<tbody>
<tr>
<td>detects protein</td>
<td>detects nucleic acid</td>
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</tbody>
</table>

- **sensitivity**: ++ | +++
- **specificity**: ++ | +++
- **material:** ear notch | ear notch, blood, milk
- **automatized:** yes | yes
- **pools**: no | yes
Sensitivity of different methods of virus detection

Intake of colostrum

AG-ELISA I

NS23

AG-ELISA II

E_{RNS}

Immunohistology

Real-time-RT-PCR
Development of a real-time RT PCR method for BVDV detection
Real-time RT-PCR method for BVDV detection

test originally developed by Swiss BVD reference laboratory, based on sequence information of >250 BVD field virus strains:
Collaboration with Qiagen led to significant improvement of all test characteristics:

Exceptionally high sensitivity: detection limit close to theoretical limit: 1.5 to 3 genome copies
Real-time RT-PCR method for BVDV detection

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**Suitable for automatized virus detection** of virus in milk, blood, ear notches: test suitable for mass screening
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No interference by antibodies: testing of pooled samples:
  negative pools ➔ animals uninfected
  positive pools ➔ identify!
Swiss BVD eradication programme

Diagnostic method adapted to stage of eradication:

I  Detect and eliminate persistently infected animals
   test all animals for VIRUS

II Detect and eliminate persistently infected animals
   born during years 1 and 2 after stage I
   test all animals for VIRUS

III monitor herds
   test selected animals for ANTIBODY
The logistics of the Swiss BVD eradication campaign

In the first stage (3 months), appr. 1.5 Million animals will be tested for virus

*This is possible only when all steps of the process are streamlined!*

**Farms:**

All samples labelled with barcode (milk, ear notches, blood), linked with animal data base
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**Farms:**

All samples labelled with barcode (milk, ear notches, blood), linked with animal data base

**Laboratory:**

recording of sample, pooling, RNA extraction, real-time RT-PCR, reporting of negative pools, identification of virus-positive animals in positive pools reporting of virus-positive animals: 

"robotics & electronics"
Situation in the US:

BVD 2006 CONFERENCE ORAL PRESENTATIONS
“BVD Control; The Future is Now”

Grand Ballroom II, Adam’s Mark Hotel, Denver, January 29 - 31
Control of BVD: Situation in Europe

- **S, SF, N, DK**
  - Close to BVD eradication

- **(D)** National campaign in planning stage

- **(NL)** Eradication by breeding assoc.

- **(F)** Regional programmes

- **(A)** National campaign started 2004

- **(I)** Regional programmes
National campaign to start autumn 2007

www.bvd-info.ch