Classical Swine Fever

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CSF and some major problems

Diagnosis

Vaccination

Gaps
Genome Organization and Virion Structure of CSFV

Family: Flaviviridae  
Genus: Pestivirus  
Species: CSFV  
BVDV-1 &-2  
BDV

Genome: +RNA (12kb)
ORF: 1
Proteins: 12
Some Facets of the Infection

Photo: Mennerich-Bunge
Slow Progression of CSFV Infection in Herds

K. Depner

28.09.2012

Stiftung Tierärztliche Hochschule Hannover
University of Veterinary Medicine Hannover, Foundation
**„High Risk Period“ (HRP)**

<table>
<thead>
<tr>
<th>Region</th>
<th>HRP (weeks)</th>
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<tbody>
<tr>
<td>Great Britain (1986)</td>
<td>4</td>
</tr>
<tr>
<td>Netherlands (1992)</td>
<td>6</td>
</tr>
<tr>
<td>Belgium (1993)</td>
<td>3</td>
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<tr>
<td>Germany (1997)</td>
<td>8</td>
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<tr>
<td>Netherlands (1997)</td>
<td>6</td>
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<tr>
<td>Spain (1997)</td>
<td>9</td>
</tr>
<tr>
<td>Great Britain (2000)</td>
<td>8</td>
</tr>
<tr>
<td>Germany (2006)</td>
<td>10</td>
</tr>
</tbody>
</table>

*Time span between introduction of the virus into a free region or country, respectively, until detection of the first outbreak. During this time the virus may spread without limitations.*
High Density of Domestic Pigs

3441 pig holdings/1422 km²

Source: Friedrich-Loeffler-Institute
Relative Risk and Distance from Primary Outbreak

Fritzemeier, Teuffert et al. 2000
Many millions of pigs are held under poor biosecurity settings (swill feeding, uncontrolled trade etc.)
Wild Boar

A steadily growing population
Laboratory diagnosis
Postnatal CSFV Infections

**Acute course**

- **Weeks p.i.**
  - 1: Incubation period
  - 2: „Atypical“ symptoms
  - 3: Convalescence
  - 4: „Typical“ symptoms
  - 5: Death
  - Virus shedding
  - Antibodies

**Chronic course**

- **Months p.i.**
  - 1: Incubation period
  - 2: „Atypical“ symptoms
  - 3: Death
  - Virus shedding
  - Antibodies
Laboratory Diagnosis of CSFV

• **Virus (antigen, nucleic acid) detection**
  • Immunohistochemistry
  • Virus isolation
  • Antigen-ELISAs
  • RT-PCR
  • Sequencing of amplicons

• **Antibody detection**
  • Virus neutralization tests (VNT, NPLA, NIFT)
  • Antibody-ELISAs
Nucleic acid detection: RT-PCR

- High sensitivity (earlier and longer detection of virus compared to virus isolation; sensitivity compared to VNT 100% vs. 70% (Depner et al. 2006))
- Allows pooling of samples
- Highly specific (differentiation of CSFV from other Pestiviruses)
- Quick results (3-5 hours)
- Commercial kits for RT-qPCR (CSFV) are available
- Most EU laboratories use (additional) in-house protocols
Real Time Multiplex PCR

• Reliable screening method for differential diagnosis of CSF
• Rapid and efficient detection of Pestiviruses (CSFV)
• Differentiation from viral pathogens that cause similar clinical signs, e.g., ASFV, SHV-1, PPV, PCV-2, PRRSV (-EU/-NA), Influenza-A virus
Multiplex RT-qPCR Screening Swine

- **FAM:** EU-PRRSV + NA-PRRSV
- **Cy5:** PCV2
- **HEX:** IC (β-Aktin)

- **Texas Red:** „early warning system“ included for FAD („red telephone“) using the same color channel
  - Classical swine fever virus (CSFV)
  - African swine fever virus (ASFV)
  - Suid herpesvirus 1 (SuHV-1)
  - Foot-and-mouth disease virus (FMDV)
Diagnostic samples (Co-infections)

Serum pig: CSFV + PRRSV + PCV2

Tonsil wild boar: CSFV + PCV2

FMDV lesion: FMDV + PCV2
Antibody Detection

• Neutralization assays are the “gold standard” and should be used in doubtful cases and for confirmatory reasons.
  - Useful for small sample numbers only: Time-consuming (~ 4 days), labour-intensive, require cell culture and live CSFV, BSL3 level!

• Antibody ELISAs are ideal for surveillance and mass screening.
  - Commercially available kits are reliable, (relatively) easy to handle and give fast results (~ 4 h).
  - Cross-reactions with BVDV and BDV antibodies usually occur, sensitivity is inferior to the neutralization assay

• - DIVA ELISAs are currently being improved or new ones are under development

• Penside tests are under development, but not yet available

Proficiency Test - Participants

- 26 NRLs from 27 EU Member States
- 2 additional RLs from EU Member States (E)
- 5 NRLs from EU candidate countries (BiH, HR, MK, MNE, SRB)
- 2 NRLs from non MS European countries (CH, N)
- 10 NRLs from third countries (AR, CDN, CL, CN, CO, C, DOM, MEX, RUS, US)
- 4 commercial companies

- Total: 49 labs from 41 countries!
Proficiency Test - Spectrum of Tests

Number of participants:
• Total: 49
• PCR: 43
• Sequencing: 7
• VI: 33
• Ag-ELISA: 23
• Ab-ELISA: 45
• VNT: 36

Some variability in tests performed:
one laboratory performing 1 Ab-ELISA
to
another lab performing PCR, VI, 3x Ab-ELISA, 8x VNT
Viral RNA is extracted from samples of CSFV infected pigs
Transfection of cells with RNA by electroporation

1x10^7 SK-6 cells + RNA (~1-30 µg)
1 pulse, 950 µF, 180 V

Incubation at 37 C, 3 days
¼ of transfected cells

Fluorescence antibody test
MAb C16 (anti-NS3) anti-mouse-Cy3

Extracted RNA is not infectious for pigs!
Why CSFV sequencing?

1. Identification of isolates in outbreak scenarios
   - genotype?
   - nearest related isolate?

2. Tracing of virus
   - source of introduction?
   - index case?
   - epidemiological investigations during outbreak
     (evidence for indirect/direct transmission)

3. Research purposes
   - identification/characterisation CSFV isolates
   - adaptation/development of improved diagnostic tools
   - understanding of viral biology on molecular level
     (e.g. virulence, pathogenesis)
Genetic Diversity of CSFV

Group 2.2
- Western Europe 80's, 90's
- Eastern Europe 90's
- Thailand 90's
- Singapore 80's
- Asia 1980-2000's

Group 2.1
- Western Europe 80's, 90's, 2000
- Malaysia 80's
- South Africa 2005
- Asia 1980-2000's

Group 2.3
- Western Europe 80's, 90's, 2005
- Eastern Europe 90's, 2000's
- Russia 2000's
- Japan 70's
- Asia 1980-2000's

Congenital Tremor (UK, 1984)

Group 3.1

Group 3.2
- Korea 80's, 90's

Group 3.3
- Thailand, 90's

Group 3.4
- Japan, 70's
- Taiwan 90's

Central America 90's
- Western Europe 40's - 70's
- Ukraine 90's
- USA 60's
- Cuba 90's
- Malaysia 60's
- Australia?
- Vaccine strains

Group 1.2

Group 1.1
- Japan 60's
- Western Europe 40's-60's Korea 80's
- (UK, Germany)
- Thailand 90's
- Eastern Europe 90's?
- China?
- Russia

USA 40's, 50's
- Brazil 80's
- Mexico 90's

Colombia 04-07

E2-gp gene fragment (190 nt)

(Paton et al., 2000, updated by Greiser-Wilke 2008)
The EURL CSFV Database

- Collect CSFV isolates and epidemiologic data
  - Make the isolates available to other laboratories for further studies
- Allow fast determination of the group / cluster (identity) of isolates in case of new outbreaks (to support the epidemiologists)
  - Originally sequences of relatively short genomic fragments:
    - 150 nt of the 5’-NTR
    - 190 nt of the E2 glycoprotein gene
    - (409 nt of the NS5B gene)
  - Quick results (typing module):
    - Check the input sequence (orientation, errors…)
    - Output: phylogenetic tree
Welcome to the Classical Swine Fever Database@EURL

Please log in

Username:
user

Password:

Log in

For a short introduction and more information on the database, please have a look at the help menu.
Practical Application of Genetic Typing

CSF Virus Subgroups:

- **Eradicated since 1990**
  - 2.2*Hessen (dp, wb)

- **Introduced 1993 & eradicated 1995**
  - 2.3*Spreda (dp)

- **Introduced & eradicated 1997**
  - 2.1*Paderborn (dp)
  - 2.2*Ringelsdorf (dp)
  - 2.3*Kärnten (dp)

- **Introduced 1992 & eradicated 2001**
  - 2.3*Güstrow (wb)

- **Introduced 1992 & eradicated 2005**
  - 2.3*Uelzen (dp, wb)

- **Reemerged & eradicated 2006**
  - 2.3*Guestrow (dp)
  - 2.3*Rösrath (wb)

- **Introducted 1992 & eradicated 2007**
  - 2.3*Rostock (wb)

- **Reemerged & eradicated 2009**
  - 2.3*Rösrath (wb)

*dp-domestic pig  
*wb-wild boar
Lithuanian CSF Outbreak 2011 – Phylogeny Based on Full-Length E2 Sequences (1119 nt)

No differences between isolates from 2009 and 2011 in 5´NTR and E2 fragment sequences!
=> Are isolates from 2009 and 2011 identical?

Isolates from 2009 and 2011 not identical!
(6/7 nucleotide exchanges to index case 2011)

Reliability of full-length E2 based phylogeny was confirmed in a phylogenetic study (Postel et al., Vet. Res., 2012)
Restructuring of the CSFV Database

- full-length E2 (5’NTR-E2, complete genome) sequences will be included.
- options for sequence search, sequence comparison, phylogenetic analysis and geo-referencing will be updated.
- Neighbour joining algorithm and rooting at CSFV isolate Congenital Tremor are recommended by the EURL
- Additional benefits of full length sequencing will be explored.
Vaccination
Some CSF Vaccines

Modified live Vaccines
- Several variants of the lapinised China (C-) strain
- GPE
- PAV 250
- Thiverval

Subunit Vaccines (DIVA)
- E2 expressed by baculoviruses

Live DIVA Vaccines
- CP7_E2alf (chimeric)
- plus other candidates

Reviewed by EFSA

Why Emergency Vaccination?

Breakdown of pig losses (NL 1997)*:

- Direct involvement in outbreak: 0.7 mio
- Preventive slaughter: 1.1 mio
- Welfare and other reasons: 9.2 mio

*S. Horst, personal communication

Endemic situations in wild boar
Endemic situations in backyard pigs
A Model for Emergency Vaccination against FMD in The Netherlands*

<table>
<thead>
<tr>
<th></th>
<th>Culling only</th>
<th>Culling+Vaccination</th>
</tr>
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<tbody>
<tr>
<td>Duration of epidemic (days)</td>
<td>200</td>
<td>84</td>
</tr>
<tr>
<td>Culled herds (infected and prophylactic)</td>
<td>2.425</td>
<td>178</td>
</tr>
<tr>
<td>Vaccinated herds</td>
<td>--</td>
<td>1.210</td>
</tr>
<tr>
<td>Number of culled animals</td>
<td>463.061</td>
<td>43.707</td>
</tr>
<tr>
<td>Total costs (Mio Euro)</td>
<td>1,132</td>
<td>883</td>
</tr>
</tbody>
</table>

*Meuvissen et al. 2006
SCIENTIFIC OPINION

Animal health safety of fresh meat derived from pigs vaccinated against Classic Swine Fever¹

Scientific opinion of the Panel on Animal Health and Welfare

(Question No EFSA-Q-2008-427)

 Adopted on 12 December 2008
Risk “Cull only” vs. Emergency Vaccination

Lifting restrictions after 1000 outbreak simulations

Outbreaks (%)

- Remaining risk (V+ or Ab not detected)
- Safe (provided no vaccination failure)

H.H. Thulke, Helmholtz-Centre for Environmental Research 2008
Oral Vaccination of Wild Boar

first cases 1992

1994-2008
3056 cases

2009 – 52 cases

Last case
July 2009

Source: TSN, Friedrich-Loeffler-Institut
Retrospective analysis of the oral immunisation of wild boar populations against classical swine fever virus (CSFV) in region Eifel of Rhineland-Palatinate

Stefan von Rüden a,* , Christoph Staubach b, Volker Kaden c, R.G. Hess d,e, Julia Blicke e, Sabine Kühne a, Jana Sonnenburg b, Andreas Fröhlich b, Jürgen Teuffert b, Volker Moennig a

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d State Veterinary Laboratory of Rhineland-Palatinate, 56073 Koblenz, Germany
e Ministry of Environment, Forestry and Consumer Protection, 55116 Mainz, Germany

Received 25 December 2007; received in revised form 7 April 2008; accepted 18 April 2008
Development of Virological Prevalence in Rhineland-Palatinate

Start of vaccination: Feb. 2002
Development of Seroprevalence in Rhineland-Palatinate

Start of vaccination: Feb. 2002

Serological prevalence (95% confidence interval per month (1999 – 1st quarter of 2005)
To Capitalise on the Relationship

CSFV-based chimaeras (de Smit et al., 2001):
- $E^{RN}$ replaced by BVDV-2-sequences
- E2 replaced by BVDV-2-sequences

BVDV-based chimaeras (Reimann et al., 2004, 2006):
- E2 replaced by CSFV-E2
- E1-E2 replaced by CSFV-E1-E2

DIVA ELISA:
$E^{RN}$-blocking-ELISA, E2-peptide-ELISA, E2-blocking ELISA (epitope-specific)
CSFV DIVA Vaccine CP7_E2alf

CP7

CP7ΔE2

CP7_E2-Alf

CSFV-E2 (CSFV-Alfort 187)

(Reimann et al., 2004, 2006)
Modified live marker vaccine candidate CP7_E2alf provides early onset of protection against lethal challenge infection with classical swine fever virus after both intramuscular and oral immunization

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\textsuperscript{c} Fort Dodge Veterinaria SA, Ctra Camprodon s/n Finca “La Riba”, 17813 Vall de Bèrnia, Spain

Protection 7 days after i.m. vaccination
Protection 14 days after oral vaccination
Vaccination with CP7_E2alf

Differentiation between infected and vaccinated animals possible!

Natural infection with CSFV

No differentiation between infected and vaccinated animals possible: NS3 specific antibodies are cross reactive

E\text{rms} specific ELISA: Detection of a natural CSFV infection
DIVA ELISA

Preincubation: serum + CSFV E\textsuperscript{rms} (30 min. 37°C)

Incubation: preincubation mix + conjugate (1 h 37°C)

Detection (after 30 min. RT)

- color: negative
- no color: positive

CSFV E\textsuperscript{rms} antigen

Anti-CSFV E\textsuperscript{rms} antibodies present in the sample

Anti-CSFV E\textsuperscript{rms} mAb

Anti-CSFV E\textsuperscript{rms} HRPO conjugate

Modified in accordance with the protocol of the PrioCHECK® CSFV E\textsuperscript{rms} ELISA (Prionics)
Gaps have been defined by

http://www.discontools.eu/
Gaps - Diagnosis

• Multiplex RT-qPCR should be developed further and commercialised.
• There are no reliable and easy-to perform penside tests. Further development and assessment of affordable penside tests for virus and antibodies is necessary. A consensus on the use of penside tests for the detection of CSFV is needed.
• The potential of full length in-depth sequencing for a higher resolution in molecular epidemiology should be explored in more detail.
• The methods for serological differentiation of CSFV infection from other pestivirus infections need to be refined.
• Sensitivity and specificity of serological DIVA ELISAs should be improved.
• The market potential for CSFV diagnostics is small, therefore availability of certain test kits is sometimes critical. Likewise there are no commercial incentives to develop new tests or improve existing ones.
Gaps - Vaccination

- Little is known about efficacy and safety of many locally available MLVs vaccines. Further studies on the quality of vaccines which are in use worldwide is necessary.
- The understanding of mechanism of early protection after vaccination with MLV (e.g. C-strain) and live DIVA vaccines should be improved.
- Development and production of “new” type of baits for young wild boar is not yet completely solved.
- Improvement of strategies to eradicate CSF in backyard holdings should be devised including oral vaccination.
- Efficacious and innocuous live DIVA vaccines suitable for vaccination of domestic pigs and oral administration for wild boar and backyard pigs should be developed and licensed. The license should only be valid in conjunction with the availability of a discriminating DIVA ELISA.
- Since future live DIVA vaccines are most likely genetically modified organisms there are uncertainties concerning their registration. The use of genetically modified vaccines might be problematic in some countries.
Vaccines & Diagnostics are only Part of Disease Control

LAVES (Schmedt auf der Günne)
Thank you for your attention!

EURL
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