Emerging Pathogens of Veterinary Importance: Schmallenberg Virus

Soren Alexandersen and Zhidong Zhang

National Centres for Animal Disease

Canadian Food Inspection Agency

Acknowledgement: Martin Beer, FLI-Riems, Germany
The viruses and other agents worked on at NCAD cause severe negative impact on animal health, the economy and directly or indirectly on human health.

What may be next: Likely an RNA virus or a single stranded DNA virus originating in another species and perhaps being vector-borne.

Important to do surveillance for potential new pathogens: One World – One Health. Not only preparing for the next outbreak, but doing surveillance and disease control world-wide.
But, not all new infections jump from animals to humans, also from humans to animals or animal-animal.

Examples:
Swine vesicular disease likely from a human virus
Ebola-Reston and Nipah in pigs likely from bats

pH1N1??

From Woolhouse and Gaunt, 2008

ECOLOGICAL ORIGINS OF NOVEL HUMAN PATHOGENS

Holmes E C PNAS 2010;107:1742-1746
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Schmallenberg virus – a newly discovered virus in Europe

- Background
  - Start, Methods and Etiology
  - Epidemiology
  - Transmission
  - Clinical signs

- Set-up/development of diagnostic assays at NCFAD

Impact on trade

- E.g. Russia has banned shipments of ruminants from March 20 2012
- USA and Japan: bovine semen and bovine embryos. **Canada: testing!**
- The OIE scientific committee has endorsed recommendations for trade (Feb 2012).
Clinical signs in adult cattle (DE)

- Since August 2011 increased number of requests to BTV Lab to analyse samples; new BTV cases?
- In North Rhine-Westphalia cases of drastic milk drop and fever reported; similar signs in the Netherlands (?)
- In September first samples sent to FLI/Institute of Diagnostic Virology for further investigations (M. Holsteg, LWK NRW und R. Jungblut, VUA Arnsberg)
- All tests for „classical“ diseases were negative:
  - BTV, EHDV, FMDV, BHV-1, MCFV, BVDV, RVFV, BEFV
  - Virus isolation on bovine cells negative (no CPE)

Metagenomic analysis of 3 pooled blood (plasma) samples from diseased dairy cows from Schmallenberg
### Metagenomic analysis

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A new Orthobunyavirus - SBV

Verdachts-Proben
BH64/11
BH66/11
BH72/11

- 12 positive samples
- 6 cattle farms
- 11 adult cattle
- 1 stillborn twin calf
- All in North Rhine-Westphalia
- Close to Dutch border
- 100 samples from farms in other regions were negative
A novel Orthobunyavirus-Infection in German cattle

Since August 2011 farmers and veterinarians in North Rhine-Westphalia (Germany) and The Netherlands have reported clinical disease in cattle and suspected a new introduction of Bluetongue disease. The main clinical signs were fever and a significant drop of milk yield for several days, in some cases also diarrhea and abortions. Samples were submitted to the German national reference laboratory for bluetongue disease at the Friedrich-Loeffler-Institut, Insel Riems.

Diagnostic analyses excluded BTV, FMDV, BVDV, BHV-1, MCFV or exotic viruses like EHDV, Rift Valley fever virus or bovine ephemeral fever virus.

Therefore, 3 pooled samples from a farm with acute signs of the disease (fever greater than 40 C and milk drop of less than 50 percent) were investigated using metagenomic analysis with a Genome Sequencer FLX instrument (ROCHE). The analysis yielded 6 sequence fragments with homology to the L and the M segments of viruses from the genus Orthobunyavirus. Further metagenomic analyses led to the detection of sequences homologous to Orthobunyavirus S segment. The sequences were related to genomic sequences of Shamonda-, Aino-, and Akabane-virus, viruses which are mainly transmitted by Culicoides spp.

Promedmail 19.11.2011

A new Orthobunyavirus - SBV

Cell culture isolation using insect cells and BHK/VERO

Further sequencing & experimental infection
Orthobunyavirus - SBV

Family: Bunyaviridae  Genus: Orthobunyaviruses
- Enveloped, spherical - Diameter from 80 to 120nm
- Segmented Primarily Negative-stranded RNA
- Comprises over 170 named viruses

- **Genome with 3 Segments (S, M, L)**
- **Transmission by insect vectors** (Culicoides spp.)
- **Simbu serogroup**: mild clinics, but congenital disorders!
Orthobunyaviruses

- All known Orthobunyaviruses are vector-borne
- Pathogens of veterinary importance within this genus
  - Akabane virus (Simbu serogroup)
    - Congenital malformations: arthrogryposis-hydranencephaly syndrome
    - Bovine epizootic encephalomyelitis mainly in cattle < 2 years (photo on the right)
  - Cache Valley virus (Bunyamwera serogroup)
- Medically important viruses within the Bunyavirus family
  - Oropouche virus, Crimean-Congo hemorrhagic fever virus, Sandfly fever virus, Rift Valley fever virus, Sin Nombre virus and Puumalavirus

Detection of Akabane virus antigens in the bovine neuron and nerve axons (Midbrain)

Akabane virus-positive granules (dark brown color) are observed in the cytoplasm of a neuron (an arrow) and nerve axons (arrow heads). Kono et al. BMC Veterinary Research 2008 4:20
## Simbu serogroup

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Saeed et al, JGV 2001
S segment

705nt; 96% similarity to Shamonda An5550 (coverage 97%)

Arch. Virol. 16 May 2012

L and S gene ~96% identical to Shamonda

M gene ~82% identical to Sathuperi (only ~50% to Shamonda)

Reassortant?
From Africa, Asia, India, Australia, or???
Clinical Signs

• Incubation
  • In experimentally infected cattle, the virus has been shown to cause short vireamia (peaking around 4 days after infection and disappearing at day 6)

• Adult animals – from Aug/Sept 2011
  • The virus causes mild clinical signs in adult cattle. The clinical signs disappear within a few days to a week
    • A reduction in milk yield
    • Fever
    • Diarrhoea.
  • No clinical signs have been reported in small ruminants (sheep and goats)

• Foetus/newborn animals – from Dec 2011
  • The virus may infect the foetus and cause birth deformities in sheep, cattle and goats
    • Arthrogryposis, torticollis, brachygnathia, hydrocephalus, and other severe brain malformations
    • may be born dead or die soon after birth due to the deformities

http://www.defra.gov.uk/ahvla/2012/01/12/schmallenberg-virus/
Clinical picture in lambs

Arthrogryposis, torticollis, brain hypoplasia, brachygnathia inferior, skoliosis

Pictures: Courtesy of Dr. Brügmann, LVI Oldenburg
Clinical picture in calves

Deformation of the vertebral column, torticollis, brachygnathia inferior in a calf

Hydranencephaly & cerebellar hypoplasia

Pictures: Courtesy of Dr. Martin Peters, SVUA Arnsberg, Germany

Photos: Holsteg, LWK NRW
Animal trial SBV: body temperatures

Experimental infection of cattle

R07 - 4ml blood from 4 different blood samples s.c.
R08 - 4ml blood from 4 different blood samples i.v.
R09 - KC-cell supernatant s.c. and i.v.

Body temperature

RT-qPCR (L1)

VNT titre
PID 21: 1/15
>PID40: 1/160

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**Transplacental infection**

Trächtigkeit Rind: ca. 9 Monate

Höchste Empfänglichkeit im 1. Drittel

Trächtigkeit Schaf: ca. 5 Monate

Fehl-, Früh- oder Schwergeburten, missgebildete Kälber und Lämmer Monate nach der akuten Infektion der Mutterschafe!
Susceptible Species

- SBV-infections detected only in ruminants so far
  - Cattle
  - Sheep
  - Goats
  - Bison
  - Roe & Red deer (*antibody detection*)
  - Alpaca & Mouflons (*antibody detection*)

Unlikely to cause illness in people based on Europe-wide risk assessment.
Diagnostics

**Virus detection**
- real-time RT-PCR (e.g. S3-PCR, Hoffmann et al)
  > highly sensitive and specific
- virus isolation (KC cells + BHK)
  > not sensitive

**Serology**
- neutralisation test („gold standard“)
- indirect immuno-fluorescence
- ELISA (first commercial test available)
  > good correlation of the different methods (> 95%)

**Samples:**
Acute: EDTA blood and **Serum**
PCR on malformed newborns:
- **CNS/brain** (Ct 11 bis 39)
- Blood/spleen
- other tissues (e.g. Lnn.)
- alternative materials:
  - Meconium
  - Amniotic fluid (for swap)

Some animals are positive in the brain or blood or other tissues only!

Antibody detection in calves
SBV: Epidemiology Germany and EU

Investigation of malformed newborns:
- High rate PCR+ malformed lambs
- Low rate PCR+ malformed calves

Serology (pre-colostral) as additional tool (new case definition)

16 May 2012, more than 4000 cases, around 2/3 in sheep

Serology: 12-93% positive in DE & NL, highest in cattle

Germany, the Netherlands, Belgium, France, England, Italy, Luxembourg, and Spain
Assumptions:
Duration of pregnancy in sheep 150 d [145-155] Tagen
Risk period for SBV infection day 32 [25-38] of pregnancy.

=> Shift: 17 weeks

SBV transplacental infections peaked at the same time of the year as BTV8
Transmission

Detection of SBV-genome in midges (*Culicoides*) from autumn 2011

- **Belgium**, collected from Sept to Oct 2011
  - C. obsoletus s.s
  - C. dewulfi
- **Denmark**, collected in October 2011
  only 6 km from the German border
  - C. obsoletus group
    (obsoletus, chiopterus, dewulfi and scoticus)
- **Italy**, collected from **Sept** to Nov 2011
  - C. obsoletus group

Transmissibility of infected animals (incl. aborted foetus/lamb/calves)?
Direct contact?
The possibility of transmission by insemination or embryo transfer?
Set-up/Development of assays for diagnosis of SBV infection at NCFAD – Collaboration with FLI-DE

- Conventional one step RT-PCR
  - Viral RNA provided by FLI, Germany
  - Primers for S (217 bp) or L gene (389bp)
  - Conventional one step RT-PCR for S gene
    - RT at 60ºC and 60ºC annealing worked best
  - Conventional one step RT-PCR for L gene
    - RT at 50ºC and 56ºC annealing best

Advantages:
- Very quick set-up (days)
- Provides sequence information
Set-up of molecular diagnostic assays

- **Real-time one step RT-PCR**
  
  (NB! getting probes take some time)
  
  - Viral RNA and **protocol** provided by FLI, Germany
  - Primer and probes targeting L gene (144bp) or S gene (87bp)

  ![Graphs showing fluorescence vs. cycles for S and L genes](image)

  - **S gene**
    - Most sensitive (42 cycles)
  - **L gene**
    - Less sensitive (42 cycles)

  ![ABI graphs](image)
NCFAD-2012 samples tested by RT-qPCR

- **Samples**
  - Lamb A: Lung, Liver, Spleen, Kidney, Thymus, Adrenal gland, and Placenta
  - Lamb B: Lung, Liver, Spleen, Thymus

- **RNA extraction**
  - **Tissue homogenization:** 10% (W/V) tissue suspension prepared by grinding the tissue sample in a pestle and mortar.
  - **RNA extraction** using QIAzol and Qiagen RNeasy spin column.

- **RT-qPCR**
  - RT-qPCR for SBV was performed on an ABI SDS7900HT
  - RT-qPCR for β-actin was carried out separately on Smartcycler

**Results:** All negative for SBV and positive for β Actin
Virus isolation

- Titration of the virus on BHK cells
  - Virus: BH80/11-4/KC1/BHK6+1 (Lot no: 12V1008), TICD50=10^{5.5}
  - BHK cells: prepared by RDU

CPE on BHK cells infected with Schmallenberg virus
Serological assays
Immuno-Peroxidase Assay (IPA)

$\text{TCID}_{50} = 10^{2.5}/\text{ml}, 3 \text{ dpi}$
Serology - Virus Neutralisation Test (VNT)

- BHK cells
- Control sera
  - Positive serum from FLI, negative serum from NCFAD
  - Two-fold serial dilutions of sera, 2 wells per dilution
  - The test is read after 3dpi

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All tested serum samples from Canadian sheep are **negative** for antibodies to SBV

However, NML/Mike Drebot found antibodies to Cache Valley virus

Future Work

- “Validation” of assays
- Production of positive controls in sheep
- Immuno-Peroxidase Assay for ovine
- Generation of RNA positive control for RT-PCR
- Compare commercial ELISA with in-house assays

Unknown Factors

- Geographic distribution; virus origin?
- Climate raising disease risk?
- Host range, wild ruminants?
- Vector competency etc?
- Transmission modes?
- Viraemia length in natural infection?
- Source of virus in affected animals?
- Strong and long-lasting immunity?
- New reassortants/virulence?
- Role of production systems
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