The Emergence of Schmallenberg virus in Europe

Wim H.M. van der Poel
Central Veterinary Institute of Wageningen University and Research Centre
EPIZONE European Research Group

USAHA meeting, Greensboro, USA, Oct 2012
The emergence of Schmallenberg virus in Europe

Presentation outline

- Schmallenberg virus (SBV) discovery
- Schmallenberg virus disease
- Diagnostics
- Epidemiology
- Characterization
- Scientific support studies European Commission
Detection of a novel orthobunyavirus
Schmallenberg, Germany, Nov 19\textsuperscript{th} 2011

- Fever and milk drop in 20 cattle farms, August 2011
- 3 pooled blood samples (1 farm)
- Genome sequencer FLX
- Sequences of orthobunyaviruses
  - Shamonda (96% homology S-segm.)
  - Aino, Akabane (70% homology, aa)
- RT-PCR assay targeting L-segment
- 100 blood samples (4 farms) \(\geq 9\) positives
First detection of Schmallenberg virus disease in cattle, The Netherlands, 8th Dec 2011

- Acute diarrhea and milk drop in dairy cattle, Sept 2011,
- 18/50 samples (8 farms) RT-PCR-positive (Ct<35)
- None of blood samples of controls positive: 0/115
- Schmallenberg virus associated with clinical diarrhea and milk drop in dairy cattle
VIRUSTRACK project CVI

Early detection of “new” and/or emerging viruses in animal reservoirs

- Implementation Micro-array screening (Incl. software)
- Complete protocol (incl. sample processing) for molecular detection and characterization of “new” and/or emerging viruses

Viruses EPIZONE microarray

1200 viruses/ 15,000 oligo’s
# Microarray detection van Schmallenbergvirus

<table>
<thead>
<tr>
<th>name</th>
<th>ProbeName</th>
<th>Serum (-)</th>
<th>Brain Sheep SL (no spike)</th>
<th>Serum 4</th>
<th>brain Sheep SL4 (no spike)</th>
<th>Serum (-)+1.5 ssc</th>
<th>brain sheep SL+1.5 ssc (no spike)</th>
<th>Serum 4 +1.5 ssc</th>
<th>brain Sheep SL4+1.5 ssc (no spike)</th>
<th>spike/control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lelystad virus</td>
<td>11138120_v5_8681</td>
<td>34609</td>
<td>0</td>
<td>24028</td>
<td>26</td>
<td>36171</td>
<td>0</td>
<td>20577</td>
<td>0</td>
<td>s</td>
</tr>
<tr>
<td>Lelystad virus</td>
<td>11138120_v5_8682</td>
<td>38220</td>
<td>158</td>
<td>25313</td>
<td>64</td>
<td>42074</td>
<td>35</td>
<td>21143</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Lelystad virus</td>
<td>11138120_v5_8683</td>
<td>60744</td>
<td>121</td>
<td>32517</td>
<td>55</td>
<td>47636</td>
<td>0</td>
<td>25005</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lelystad virus</td>
<td>11138120_v5_8684</td>
<td>53247</td>
<td>142</td>
<td>29103</td>
<td>213</td>
<td>41150</td>
<td>17</td>
<td>21910</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Lelystad virus</td>
<td>11138120_v5_8685</td>
<td>45940</td>
<td>34</td>
<td>29027</td>
<td>21</td>
<td>45512</td>
<td>34</td>
<td>25518</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Lelystad virus</td>
<td>11138120_v5_8686</td>
<td>21604</td>
<td>97</td>
<td>15554</td>
<td>163</td>
<td>24005</td>
<td>78</td>
<td>13264</td>
<td>205</td>
<td></td>
</tr>
<tr>
<td>Porcine circo virus</td>
<td>12280941_v5_10826</td>
<td>302386</td>
<td>37</td>
<td>309763</td>
<td>0</td>
<td>257726</td>
<td>0</td>
<td>238390</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Porcine circo virus</td>
<td>12280941_v5_10827</td>
<td>207046</td>
<td>8</td>
<td>190863</td>
<td>0</td>
<td>206793</td>
<td>0</td>
<td>166479</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Golden hamster intracisternal A-particle H18</td>
<td>161591_v5_4529</td>
<td>11307</td>
<td>0</td>
<td>13733</td>
<td>8</td>
<td>10887</td>
<td>0</td>
<td>12398</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Golden hamster intracisternal A-particle H18</td>
<td>161591_v5_4530</td>
<td>10943</td>
<td>0</td>
<td>14518</td>
<td>0</td>
<td>10363</td>
<td>0</td>
<td>12352</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Golden hamster intracisternal A-particle H18</td>
<td>161591_v5_4531</td>
<td>18838</td>
<td>0</td>
<td>23023</td>
<td>0</td>
<td>16087</td>
<td>0</td>
<td>18855</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Golden hamster intracisternal A-particle H18</td>
<td>161591_v5_4532</td>
<td>12435</td>
<td>7</td>
<td>18981</td>
<td>0</td>
<td>12567</td>
<td>0</td>
<td>16450</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bovine circo virus</td>
<td>9631282_v5_1860</td>
<td>239190</td>
<td>95</td>
<td>232280</td>
<td>33</td>
<td>236829</td>
<td>183</td>
<td>203142</td>
<td>202</td>
<td></td>
</tr>
<tr>
<td>Bovine circo virus</td>
<td>9631282_v5_1861</td>
<td>229055</td>
<td>0</td>
<td>234431</td>
<td>0</td>
<td>224214</td>
<td>0</td>
<td>186732</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bovine circo virus</td>
<td>9631282_v5_1862</td>
<td>186126</td>
<td>0</td>
<td>178731</td>
<td>0</td>
<td>174622</td>
<td>97</td>
<td>153240</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Bovine circo virus</td>
<td>9631282_v5_1863</td>
<td>92753</td>
<td>3</td>
<td>75475</td>
<td>6</td>
<td>104144</td>
<td>0</td>
<td>76872</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bovine circo virus</td>
<td>9631282_v5_1864</td>
<td>259560</td>
<td>0</td>
<td>241831</td>
<td>12</td>
<td>265359</td>
<td>0</td>
<td>199342</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bovine circo virus</td>
<td>9631282_v5_1865</td>
<td>134871</td>
<td>0</td>
<td>123025</td>
<td>0</td>
<td>136289</td>
<td>7</td>
<td>102647</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Bovine circo virus</td>
<td>9631282_v5_1866</td>
<td>221624</td>
<td>83</td>
<td>174566</td>
<td>105</td>
<td>185085</td>
<td>1</td>
<td>149108</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bovine circo virus</td>
<td>9631282_v5_1867</td>
<td>130764</td>
<td>0</td>
<td>107570</td>
<td>0</td>
<td>102845</td>
<td>93</td>
<td>87230</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Yaba-like disease virus</td>
<td>12056159_v5_14453</td>
<td>829</td>
<td>215</td>
<td>6252</td>
<td>368</td>
<td>406</td>
<td>35</td>
<td>327</td>
<td>205</td>
<td>p</td>
</tr>
<tr>
<td>Sabo virus</td>
<td>18677729_v5_11888</td>
<td>608</td>
<td>481</td>
<td>694</td>
<td>4385</td>
<td>563</td>
<td>3994</td>
<td>258</td>
<td>5472</td>
<td></td>
</tr>
<tr>
<td>Sabo virus</td>
<td>18677729_v5_11889</td>
<td>2979</td>
<td>21015</td>
<td>2397</td>
<td>20419</td>
<td>3019</td>
<td>18552</td>
<td>1457</td>
<td>23965</td>
<td>p</td>
</tr>
<tr>
<td>Shamonda virus</td>
<td>94039095_v5_12231</td>
<td>899</td>
<td>9460</td>
<td>0</td>
<td>10398</td>
<td>876</td>
<td>8764</td>
<td>0</td>
<td>13652</td>
<td>p</td>
</tr>
<tr>
<td>Shamonda virus</td>
<td>94039095_v5_12233</td>
<td>855</td>
<td>10395</td>
<td>0</td>
<td>11671</td>
<td>1025</td>
<td>11644</td>
<td>0</td>
<td>18133</td>
<td>p</td>
</tr>
<tr>
<td>Aino virus</td>
<td>30409711_v5_214</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>sel</td>
<td></td>
</tr>
<tr>
<td>Akabane virus</td>
<td>30409709_v5_216</td>
<td>14</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>sel</td>
<td></td>
</tr>
<tr>
<td>Akabane virus</td>
<td>30409709_v5_215</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>46</td>
<td>sel</td>
</tr>
<tr>
<td>Bunyamwera virus</td>
<td>221048_v5_2324</td>
<td>20</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>17</td>
<td>9</td>
<td>1</td>
<td>1 sel</td>
<td></td>
</tr>
</tbody>
</table>

**Controls**

**Orthobunyaviridae oligos**

![>500 = positive](image)

---

**Microarray detection van Schmallenbergvirus**

**Schmallenbergvirus**

**Orthobunyaviridae oligos**

**Controls**

![>500 = positive](image)
Orthobunyaviruses / Schmallenberg virus

classification, genome

- "Shamonda-like" virus
- Family Bunyaviridae
- Genus Orthobunyavirus
- ssRNA virus
- 3 RNA segments

**Diagram:**
- Nucleocapsid
- Polyprotein
- Polymerase

**Illustration:**
- Glycoprotein (Gn and Gc)
- Polymerase (L)
- Genomic RNA
- Ribonucleocapsid (RNA)
- Nucleoprotein (NE)
- S segment (1kb)
- M segment (4.5kb)
- L segment (6.9kb)
- Cleavage by host protease
- Alternative initiation
- HO-3' to 5'
Simbu serogroup viruses
Main clinical and epidemiological features

- Vector transmitted: Midges and mosquitoes
- No evidence for direct transmission
- Short viremia, 3-7 days, no carriers
- Distribution: East Asia, Australia, Africa, Middle-East
- 1972-1975: >42000 deaths in calves in Japan due to Akabane virus.
- Malformations in newborn ruminants, asymptomatic infections in several other species (incl. pigs, horses, dogs). No zoonotic potential
First detection of Schmallenberg virus disease in sheep, The Netherlands, Dec 15th 2011

- Malformations in new-born lambs in at least 10 farms, Dec 2011 (GD Deventer)
- Samples of 8 lambs (4 blood samples, 8 brain tissue samples) from two farms tested by RT-PCR (CVI-WUR)
- 2 brain samples RT-PCR positive (Ct20 and Ct34)
Schmallenberg virus
Disease notification the Netherlands

- 20 December 2011
- Abnormalities in new-borne lambs, calves and goats to be reported to the Dutch Food Safety Authority (nVWA)
- Pathological examinations and laboratory testing by Animal Health Service (GD), and CVI respectively
- Results of testing and examinations reported to nVWA

Materials (virus culture and ref. samples) free available from CVI and FLI
Schmallenberg virus
Risk assessment and Biosafety

- ECDC, 22 dec 2011
- Sources: RIVM, CVI Netherlands, FLI Germany
- Schmallenberg virus unlikely to cause disease in humans (based on genetically similar viruses)
- Health of people in contact with infected animals should be carefully monitored
- FLI, RIVM, CVI: Biosafety levels
  - Laboratory: BSL2
  - Animal experiments: BSL3
Examinations in notified malformed new-born ruminants

- Max 5 lambs per farm
- Blood samples from mother animals
- Questionnaire
- Diagnostics:
  - necropsy, GD
  - RT-PCR (neonates), CVI
  - VNT (mothers), CVI
Congenital malformations lamb
The Netherlands, Jan 2012
Schmallenberg virus congenital defects
Schmallenberg virus, CVI-Lelystad, Virus isolation/ Characterization

- **Virus isolation**
  - Bovine and Ovine
  - BHK cells
  - Vero cells

- **Virus characterization/ Genome Sequencing**
  - Whole Genome approach (Illumina Hiseq 2000)
  - Micro-array
  - RT-PCR approach
Schmallenberg virus genome organisation
RT-PCR assays CVI/GD

1. SBV specific RT-PCR targeting the virus L-segment
2. SBV specific RT-PCR targeting the virus S-segment
3. Pan-orthobunyavirus RT-PCR targeting the S-segment
Schmallenberg virus reassortment
Origin?

Yanase et al., 2012

Simbu serogroup viruses

1. Akabane
2. Aino
3. Buttonwillow
4. Douglas
5. Facey’s paddock
6. Ingwavuma
7. Inini
8. Jatobal
9. Kaikamur
10. Manzanilla
11. Mermet
12. Oropouche
13. Oya
14. Para
15. Peaton
16. Sabo
17. Sango
18. Sathuperi
19. Simbu
20. Shamonda
21. Shuni
22. Thimiri
23. Tinaro
24. Utinga
25. Yaba
Simbu serogroup virus infection
Virus induced abnormalities in calves and lambs
(Parsonson et al. 1977, 1981; Kirkland et. al 2005 )

- Sheep (av. gestation period av. 147 days)
  - Sensitive period tissue damage 20-50 days gestation
  - Peak period tissue damage 28-36 days gestation

- Cattle (av. gestation period 285 days)
  - Sensitive period tissue damage 76-249 days gestation
  - Peak period tissue damage 80-120 days gestation
Virus Neutralisation Test (VNT) CVI-Lelystad (Loeffen et al., 2012)

- SBV isolate lamb brain tissue
- VERO cells CPE
- 5 days culture
- Amido black staining
- Applied for cattle and sheep sera
- Specificity, 99.4%, tested with archived serum samples
- Sensitivity >92%, based on notified farm field samples
Schmallenberg virus
Immune HistoChemistry, GD/CVI-Lelystad

Lamb brain tissue (Cerebellum)
SBV Immunostaining
(using a simbu serogroup Mab)
Schmallenberg virus
Laboratory diagnosis in affected calves

- Few calves have infectious virus in tissues / hard to find antigens
- Calves and lambs may have residual RNA in tissues
- Around 75% of all AHS calves will have antibody (in most body fluids: pericard, CSF, pleural fluid)
- Infection can be confirmed through detection of antibodies in serum of mothers
RT-PCR results per species June 2012

<table>
<thead>
<tr>
<th>Species</th>
<th>Total tested</th>
<th>Virus detected</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat-kids</td>
<td>65</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Sheep lambs</td>
<td>800</td>
<td>232</td>
<td>29</td>
</tr>
<tr>
<td>Calves</td>
<td>1,301</td>
<td>185</td>
<td>14</td>
</tr>
</tbody>
</table>

- Low number of goats-kids tested
- Highest number of positives in lambs
# VNT results per species April 2012

<table>
<thead>
<tr>
<th></th>
<th>Total tested</th>
<th>Antibodies detected</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>37</td>
<td>16</td>
<td>43</td>
</tr>
<tr>
<td>Sheep</td>
<td>458</td>
<td>422</td>
<td>92</td>
</tr>
<tr>
<td>Cow</td>
<td>899</td>
<td>860</td>
<td>96</td>
</tr>
</tbody>
</table>

### Total tested

- Goat
- Sheep
- Cow
Schmallenberg virus detections in farms
The Netherlands Dec 2011 - June 2012

Farms notified malformation in new born lambs and calves
Schmallenberg virus
Public health risk

- First risk assessment RIVM supported by FLI and CVI (ECDC publication 22 Dec 2011): “Schmallenberg virus unlikely to cause disease in humans, but infection cannot be excluded”

- Health of people in contact with infected animals should be carefully monitored

- Indicated biosafety levels: Lab BSL2, animal exp.: BSL3

- No SBV antibody in exposed humans (n=301) (Reusken et al., 2012 EID): public health risk extremely low
Schmallenberg virus seroprevalence study (Elbers et al., EID 2012)

- 1100 randomly selected dairy cows
- Stratified sampling design by province
- 2 cows per herd.
- Mean age of sampled dairy cattle: 2 years
- Virus neutralisation test (VNT) (Titers ≥ 8: positive)
Schmallenberg virus within herd seroprevalence (RT-PCR positive herds)

Dairy herd 1 (58 dairy cows):
- One malformed calf
- 34 dairy cows were tested.

Dairy herd 2 (40 dairy cows):
- Two malformed calves
- 34 dairy cows were tested.

Sheep flock 1 (800 ewes):
- 15/41 (37%) malformed lambs.
- 60 mother-ewes tested.

Sheep flock 2 (81 ewes):
- 2/30 (13%) malformed lambs
- 35 mother-ewes tested

Courtesy A. Elbers
Results seroprevalence study
Schmallenberg virus antibodies

- Estimated seroprevalence in dairy cattle in the Netherlands: **73%**
- High agreement in serological status of animals within herds (either seropositive or seronegative)
- 50% of positive samples showed titer ≥ **512**

Within-herd seroprevalence
- Sheep flock 1: **93%**  Sheep flock 2: **71%**
- Dairy herd 1: **74%**  Dairy herd 2: **100%**

Courtesy A. Elbers
Schmallenberg virus seroprevalence in dairy cattle, The Netherlands
Schmallenberg virus
RT-PCR Ct-values in culicoides
The Netherlands

<table>
<thead>
<tr>
<th>Culicoides spp</th>
<th>Ct-values heads</th>
<th>Ct-values abdomen</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Obsoletus complex</td>
<td>24.56 (19.6-36.02)</td>
<td>30.52 (24.59-36.45)</td>
</tr>
<tr>
<td>C. Chiopterus</td>
<td>31.62 (27.88-35.36)</td>
<td>22.50 (18.16-36.45)</td>
</tr>
</tbody>
</table>

Elbers et al., EID 2012
Simbu serogroup viruses
Contamination features

- Arthropod borne viruses: labile outside vector and host
- Short viraemic period: 3-7 days No evidence persistent infection (Kirkland, 2002, Hoffmann et al., 2012)
- Experimental infection bulls with Akabane virus did not result in contaminated semen (Parsonson et al., 1981)
- In vitro exposure to akabane virus did not result in contaminated embryos (Singh et al., 1982)
Schmallenberg virus
Geographical spread and trade restrictions
Oct 2012

Schmallenberg virus geographical spread

Schmallenberg virus trade restrictions
Schmallenberg virus
European Commission, DGSanco
Scientific support studies

- Consortium
  - NL (CVI),
  - D (FLI),
  - B (CODA),
  - UK (AHVLA),
  - Fr (ANSES/INRA)

- Selected areas
  - Pathogenesis
  - Epidemiology
  - Diagnostics

- Budget:
  - 50% National funding
  - Total ~3.4M€
European commission SBV studies
5-country consortium

Area Pathogenesis

- Cattle (FLI)
- Immunity (AHVLA)
- Sheep (CVI and CODA)
- Goat (ANSES)

Pregnant and non-pregnant, seropos. and seroneg. animals
European commission SBV studies
5-country consortium

Area Epidemiology

- Horizontal transmission
  - Contact animals in infection experiments
- Transmission competent vectors
  - Retrospective study
  - Prospective study
  - Vector competence study
European commission SBV studies
5-country consortium

Area Epidemiology (continued)

• Role of semen and embryos
  • PCR testing of semen from infected bulls
  • In vitro inoculation of embryos
• Host range
  • Experimental infection of pigs
  • Testing of samples from horses and pigs
• Identify reservoirs
  • Testing of samples from deer
  • Testing of samples from zoos and wildlife centres
DGSanco SBV studies
5-country consortium

Area Diagnostics
• Harmonisation of RT-PCR assays
• Harmonisation of serological assays
• Circulation of samples of cattle, sheep and goat
• Establishment of reference panels
Simbu serogroep viruses
Prevention and Control

- Protection against vectors (midges and mosquitoes)
- Pasturing / housing of young animals together with adult animals before sexual maturity
- Posponed service/ insemination
- Vaccination, Vaccine candidate but no vaccine available yet!
**ERG Mission:** “To improve research on preparedness, prevention, detection, and control of epizootic animal diseases through cooperation, with extra attention for new and emerging epizootic animal diseases including those which may have zoonotic potential”
EPIZONE European Research Group
partner institutes
EPIZONE-European Research Group Activities/outputs

- Communication and networking
  - Website, Newsletter
- Annual meetings
  - 7th AM Brussels, 1-4 Oct 2013
- “Young EPIZONE” young scientists programme
- Scientific missions and courses
  - Classical + molecular virology 2011
- Scientific publications
- Standardized protocols and reference panels
EPIZONE-European Research Group
Activities/ outputs

- Dedicated symposia
  - BTV Brescia 2009, Arnhem 2011
  - Schmallenberg, Brighton 2012
- Databases maintenance
  - Central access via EPIZONE website
- Shared resources
  - Cells, viruses, antibodies
- EU-China Building bridges meetings
- Workshops
  - Databases workshops
  - Epidemiology workshops
Acknowledgements

Central Veterinary Institute: Willie Loeffen, Johan Bongers, Armin Elbers, Renate Hakze, Frank Harders, Norbert Stockhofe, Jeroen Kortekaas, Rob Moormann, Riks Maas, Klaas Weerdmeester, Yolanda de Visser, Els de Boer, Jan Cornelissen, Eric de Kluyver, Stephanie Vastenhouw, Ruth Bouwstra, José Harders, Bregje Smid, Wim van der Poel, Sjaak Quak, Betty Verstraaten, Bart Kooi

ASG Livestock Research: Marcel Hulst

Animal health Service Deventer: Jet Mars, Petra Kock, Kees van Maanen, Harold van der Heijden, Gerard Wellenberg, Piet Vellema, Klaas Peperkamp, Jan Vos, Gerdien van Schaik

Friederich Loeffler Institute: Martin Beer, Thomas Mettenleiter, Bernd Hoffmann, Dirk Höper, Franz Conrath,

RIVM, Bilthoven: Marion Koopmans, Chantal Reusken

EPIZONE partner-institutes: Stephan Zientara, Falko Steinbach, Ann Brigitte Caj, Anette Bøtner, Trevor Drew,
Thank you

wim.vanderpoel@wur.nl
www.epizone-eu.net