Identification of a Hepatitis C Virus-like virus in cattle

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Third Global Conference of OIE Reference Centres,
Incheon (Seoul), Korea (Rep. of), 14 - 16 October 2014
Identification and Characterization of Novel Viruses
From Farm Animals
(TTU Emerging Infectious Diseases, German Centre for Infection Research)

Background
Detailed knowledge on the spectrum of animal viruses is important for a better preparedness with regard to future challenges.

Major Aims
- Detection and characterization of novel viruses from cattle and pigs
- To determine whether the newly identified viruses have the potential to infect humans or are already present in the human population
Project

Identification and Characterization of Novel Viruses From Farm Animals
(TTU Emerging Infectious Diseases, German Centre for Infection Research)

Results (12/2013 – 08/2014)

Set-up of protocols for screening of serum and fecal samples from cattle, pig, sheep, and goats by HTS --> identification of several novel viruses including

- Kobuviruses
- Hokuviruses
- Pegiviruses
- Parvoviruses
- Circoviruses
- Hepaciviruses
- .....
Screening of serum samples

Analysis of pooled serum samples (n=7) from dairy herds via Next-Generation-Sequencing (Illumina MiSeq and HiSeq) (collaboration with Nicole Fischer (UKE Hamburg) and Adam Grundhoff (HPI Hamburg))

1st run: 972 reads → 7 contigs (448 – 870 nt)
comparison with GenBank database: similarities found to - HCV, GB virus B, Bat hepacivirus

→ Set-up of RT-PCR
→ Identification of the virus-positive animal from the pool („B1“)

2nd run: assembly of 8,841 nucleotides, including one open reading frame (2,779 aa)
The **Flaviviridae**

Virus Taxonomy
9th Report of the ICTV
(Simmonds, Becher, et al., 2012)
Hepatitis C virus (HCV)

- 130 – 150 million people suffer from chronic hepatitis C
- Up to 500,000 patients die each year due to hepatitis C related disease

http://www.hepatitisc.uw.edu/
www.who.int
GB virus B

- Liver tropism
- Causes hepatitis in New World monkeys
- GB virus B does not infect humans or chimpanzees
- Natural host?
- Used as a surrogate model for HCV infection
Identification of novel HCV-like viruses

Since 2011...

....HCV-related sequences in dogs (Kapoor et al., 2011)
....HCV-related sequences in horses (Burbelo et al., 2012; Lyons et al., 2012)
....HCV-related sequences in bats (Quan et al., 2013)
....HCV-related sequences in rodents (Drexler et al., 2013)

Hepaciviruses seem to be more widespread and show a greater diversity than thought before!

→ Origin of HCV?
Hepacivirus – genomic organization

Hepacivirus genome

Virus Taxonomy
9th Report of the ICTV, 2012
(Simmonds, Becher et al.)

Bovine hepacivirus

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>E1</th>
<th>E2</th>
<th>p7/p13</th>
<th>NS2</th>
<th>NS3</th>
<th>NS4A</th>
<th>NS4B</th>
<th>NS5A</th>
<th>NS5B</th>
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<td>261</td>
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<td>GBV-B</td>
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<td>192</td>
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<td>119</td>
<td>208</td>
<td>620</td>
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<td>248</td>
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<td>590</td>
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<tr>
<td>New</td>
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<td>204</td>
<td>624</td>
<td>54</td>
<td>250</td>
<td>396</td>
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</table>
Phylogenetic analysis

Neighbor-Joining Complete ORF

Hepatitis C virus/1a M62321
Hepatitis C virus/4a Y11604
Hepatitis C virus/6a Y12083
Hepatitis C virus/3a D17763
Hepatitis C virus/5a Y13184
Hepatitis C virus/2a D00944
Hepatitis C virus/7a EF108306
NPHV-H3-011 JQ434008
NPHV EF369 11J JX948116
NPHV-G1-073 JQ434002
NPHV-B10-022 JQ434004
NPHV-A6-006 JQ434003
Canine hepacivirus/AAK-2011 JF744991
NPHV-NZP-1 JQ434001
Bat hepacivirus/PDB-829 KC796074
Bat hepacivirus/PDB-452 KC796090
Bat hepacivirus/PDB-491.1 KC796078
Bat hepacivirus/PDB-445 KC796091
Rodent hepacivirus/RMU10-3382/GER/2010 KC411777
Rodent hepacivirus/NLR08-365/NEL/2008 KC411796
Rodent hepacivirus/SAR-46/RSA/2008 KC411807
Rodent hepacivirus/SAR-3/RSA/2008 KC411806
Bovine hepacivirus B1
Hepatitis GB virus B AF179612
Bat hepacivirus/PDB-112 KC796077
Hepatitis GB virus A AF023424

Species:
Human
Horse, dog
Bat
Rodent
Cattle
Primate
Bat
**Acute vs. chronic infection?**

**Sample acquisition**

<table>
<thead>
<tr>
<th>Date</th>
<th>August 2013</th>
<th>February 14</th>
<th>May 14</th>
<th>August 2014</th>
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<tbody>
<tr>
<td>Initial sampling</td>
<td>40 animals</td>
<td>35 animals</td>
<td>34 animals</td>
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</table>

**Intra-herd prevalence: 35 %**

Some animals were able to clear the infection

Other animals are chronically infected for over six months

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<th>Date of sampling</th>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
<td>neg</td>
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<tr>
<td>4</td>
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<td>31</td>
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<tr>
<td>39</td>
<td>pos</td>
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<tr>
<td>40</td>
<td>neg</td>
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</tbody>
</table>
Prevalence of BoHCV in Germany

- Establishment of RT-PCR: broadly reactive PCR-primers (highly conserved region, NS3)

- Number of serum samples investigated for BoHCV: n=320
  - 120 serum samples: South of Germany (12 herds)
  - 200 serum samples: Animals submitted to the Clinic for Cattle (TiHo) in 2014

→ Identification of five positive animals
→ Prevalence: 1.6%
Neighbor-Joining
NS3 (270 nt)

Human
Horse, Dog
Bat
New World Monkey
Cattle
BoHCV: Clinical and pathological signs

Liver enzymes

BoHV positive: n=12
BoHV negative: n=38

AST  GGT  GLDH

BoHV pos.    neg.
BoHV pos.    neg.
BoHV pos.    neg.
BoHCV: Clinical and pathological signs

Dairy cow 463/14

- 4 years old
- Holstein Friesian
- Teat injury → Clinic for Cattle, TiHo
- Necropsy
- Macroscopic and histological analyses

→ No signs of liver inflammation or liver damage attributable to a viral infection
## qRT-PCR

<table>
<thead>
<tr>
<th>Tissue [RNA 1:5]</th>
<th>Ct value</th>
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<tbody>
<tr>
<td>Cerebrum</td>
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<tr>
<td>Udder</td>
<td>35,2</td>
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<tr>
<td>M. psoas major</td>
<td>No Ct</td>
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<tr>
<td>Heart</td>
<td>38,2</td>
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<tr>
<td>Lung</td>
<td>36,8</td>
</tr>
<tr>
<td>Kidney</td>
<td>31,6</td>
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<td>Spleen</td>
<td>33,1</td>
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<tr>
<td>Liver</td>
<td>24,8</td>
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<tr>
<td>Lymphnode (Lung)</td>
<td>33,9</td>
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<tr>
<td>Lymphnode (Rumen)</td>
<td>37,7</td>
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<tr>
<td>Lymphnode (Intestinum tenue)</td>
<td>31,1</td>
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<tr>
<td>Lymphnode (Intestinum crassum)</td>
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<td>Lymphnode (Caecum)</td>
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<tr>
<td>Lymphnode (Liver)</td>
<td>30,1</td>
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<tr>
<td>Serum</td>
<td>27,5</td>
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Summary

- Identification of a so far unknown Hepacivirus in cattle
- Genomic organization typical for the genus *Hepacivirus*
- Comparative sequence analysis: closest relationship to GB virus B
- Prevalence of BoHCV infection 1.6 %
- Persistent and non-persistent infections
- Evidence for liver tropism
- So far, no obvious clinical or pathological signs in infected animals
Outlook

- Establishment of serological assay
  -> sero-prevalence in cattle and other species including human?

- Clinical consequences of infection within infected herds?

- Virus propagation in cell culture?

- Establishment of an *in-vivo* infection model

- Can BoHCV serve as a surrogate infection model for HCV?
Acknowledgements

Institute of Virology, TiHo
Christine Bächlein
Alex Postel
Ester Barthel
Franziska Peest
Hossein Naghilouy Hidaji

Institute for Pathology, TiHo
Andreas Beineke
Kathrin Becker

Clinic for Cattle, TiHo
Jürgen Rehage
Claudia Raschka

UKE/HPI, Hamburg
Nicole Fischer
Adam Grundhoff
Daniela Indenbirken
Malik Alawi

Veterinary Practice Legau
Jenny Offinger

Bundesministerium für Bildung und Forschung
DZIF