Identification of Emerging Swine Viruses & What to do with them

Dick Hesse
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The emergence of porcine circovirus 2b genotype (PCV-2b) in swine in Canada

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Abstract — Since late 2004, the swine industry in the province of Quebec has experienced a significant increase in death rate related to postweaning multisystemic wasting syndrome (PMWS). To explain this phenomenon, 2 hypotheses were formulated: 1) the presence of a 2nd pathogen could be exacerbating the porcine circovirus 2 (PCV-2) infection, or 2) a new and more virulent PCV-2 strain could be infecting swine. In 2005, 13 PMWS cases were submitted to the Quebec provincial diagnostic laboratory and PCV-2 was the only virus that could be found consistently by PCR in all 13 samples. The PCR detection results obtained for other viruses revealed the following: 61.5% were positive for porcine reproductive and respiratory syndrome virus, 30.8% for swine influenza virus, 15.4% for porcine parvovirus, 69.2% for swine torque teno virus (swTTV), 38.5% for swine hepatitis E virus (swHEV) and 84.6% for Mycoplasma hyorhinis; transmissible gastroenteritis virus and porcine respiratory coronavirus (TGEV/PRCV) was not detected. Sequences of the entire genome revealed that these PCV-2 strains belonged to a genotype (named PCV-2b) that has never been reported in Canada. Further sequence analyses on 83 other Canadian PCV-2 positive cases submitted to the provincial diagnostic laboratory during years 2005 and 2006 showed that 79.5% of the viral sequences obtained clustered in the PCV-2b genotype. The appearance of the PCV-2b genotype in Canada may explain the death rate increase related to PMWS, but this relationship has to be confirmed.
“Globally Speaking”

Since we live in a Global Community, is there anything out there that we should be looking for???
## Swine Health Information Center
### List of Emerging Swine Threats

<table>
<thead>
<tr>
<th>Disease/Agent</th>
<th>Virus/Agent</th>
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<tbody>
<tr>
<td>Foot and mouth disease virus</td>
<td>Porcine deltacoronavirus</td>
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<tr>
<td>Classical swine fever virus</td>
<td>Porcine parainfluenza 1 virus*</td>
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<tr>
<td>African swine fever virus</td>
<td>Atypical swine pestivirus*</td>
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<tr>
<td>Pseudorabies virus*</td>
<td>Influenza C virus*</td>
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<tr>
<td>Influenza A virus*</td>
<td>Porcine respiratory coronavirus*</td>
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<tr>
<td>Nipah virus*</td>
<td>Hemagglutinating encephalomyelitis virus*</td>
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<tr>
<td>Ebola-Restin*</td>
<td>Encephalomyocarditis virus*</td>
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<tr>
<td>Porcine epidemic diarrhea virus</td>
<td>Hepatitis E virus*</td>
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<tr>
<td>PRRS virus (Chinese high path)*</td>
<td>Porcine adenovirus*</td>
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<tr>
<td>PRRS virus</td>
<td>Porcine kobuvirus*</td>
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<tr>
<td>Porcine teschovirus (Teschen/PTV1)*</td>
<td>Porcine sapovirus*</td>
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<tr>
<td>Japanese encephalitis virus*</td>
<td>Orthoreovirus*</td>
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<td>Getah virus*</td>
<td>Sendai virus*</td>
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<tr>
<td>Transmissible gastroenteritis virus</td>
<td>Porcine cytomegalovirus*</td>
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<tr>
<td>Menangle virus*</td>
<td>Vesicular stomatitis virus*</td>
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<tr>
<td>Porcine circovirus</td>
<td>Chikungunya virus*</td>
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<tr>
<td>Circovirus 3*</td>
<td>Rabies virus</td>
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<tr>
<td>Porcine rotavirus</td>
<td>Porcine bocavirus*</td>
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<tr>
<td>Swine vesicular disease virus</td>
<td>Porcine astrovirus*</td>
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<tr>
<td>Vesicular exanthema of swine virus*</td>
<td>Swine pox virus*</td>
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<tr>
<td>Porcine rubulavirus*</td>
<td>Porcine sapelovirus*</td>
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<tr>
<td>Seneca Valley virus*</td>
<td>Porcine torovirus*</td>
</tr>
<tr>
<td>Porcine parvovirus</td>
<td>Swine papillomavirus*</td>
</tr>
</tbody>
</table>

* Bat origin alpha coronavirus
“Bug Hunting”
New Tools of the Trade

• Non Biased Diagnostics--Deep Sequencing
  454 Pyrosequencing
  • MiSeq
  • Ion Torrent
• Micro Array
• Proteomics
  – Mass Spec
    • MALDI- TOF
• Genome Sequence—Molecular Signature
Next Generation Sequencing (NGS) Tools at KSVDL
MiSeq (Illumina)
Metagenomic viral RNA and DNA (sample pretreated with DNase/RNase cocktail)

Random hexamer with 5'-20bp barcode

Reverse Transcription and Second Strand Synthesis (RNA -> cDNA->dsDNA)

PCR Amplification using primer identical to 20bp barcode

Amplicon pools generated from randomly amplified virus nucleic acid
Full genome sequence of porcine parainfluenza 1 (PPIV1) virus from a nasal swab

- 11 M reads
- 52,111 mapped to PPIV1
  - 0.45% reads
- 361x average coverage
I have EMs to fill in here
bocavirus, parvovirus, sapovirus, picobirnavirus, various small circular DNA viruses, kobuvirus, astrovirus, enterovirus, rotavirus, adeno-associated virus, porcine adenovirus 5, orthoreovirus, posavirus and porcine parainfluenza virus 1
Nasal swabs are suitable specimens for viral surveys using metagenomics.

From Nasal Swabs:

bocavirus, hemagglutinating encephalomyelitis virus, parvovirus, porcine cytomegalovirus, sapelovirus, transmissible gastroenteritis virus, picobirnavirus, various small circular DNA viruses, kobuvirus, astrovirus, enterovirus, rotavirus and adeno-associated virus
From Tissues or Serum

- Porcine Circovirus 3
- Atypical Pestivirus
- Porcine Parvovirus 6
- Posavirus
New or never formally identified (no publication) by Ben Hause by NGS at KSVDL:

- Porcine parvovirus 6
- Porcine pestivirus 1 (new species)
- Posavirus (new genus)
- Porcine parainfluenza virus 1
- Porcine enterovirus G
- Porcine Circovirus 3
We found a new Virus
What Now??

• Look at the Crystal Ball and Predict Significance
• Diagnostic Epidemiology
  – Molecular
  – Seroprofile
• Koch’s Postulates
  – Single Agent
  – Multiple Agents
• Vaccine Development
• Management Changes
Critical Elements to Emerging Disease Investigations

• Curious Field Veterinarians
• Curious Diagnosticians
• Strategic Collaboration
• Freedom to Investigate
  – Understanding Boss
  – Reasonable Workload
• Tools and Budget

You have to do the Research
Global and National Communication Networks

- Read the Literature
- Sentinel Surveillance Networks
- Field Investigations
- Trip Reports/Presentations
- Skype
- “Did you hear” phone calls/e-mails
- Bar “Shop Talk”
Emerging Disease Gaps

- Vaccines and Control Strategies
- NAHLN and $$$
- “Mission Restriction”
- Attitude—it is their problem—not ours
- Proactive vs. Reactive
- Funding across species lines
- Surveillance Programs
  - Active
  - Passive
Gaps

• Those who forget the mistakes of the past are doomed to repeat them

• *Cry Wolf*—the Sky is falling—separate the wheat from the chaff

• *We can do Anything, we can’t do Everything* — Priority Setting
Questions