

# Identification of Emerging Swine Viruses & What to do with them

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K-State Swine Day  
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# Where There is Smoke, There is Fire

## 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.



**Courtesy of Dr. Butch Baker ISU**



Courtesy of Dr. Matt Ackerman

# "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

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

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)





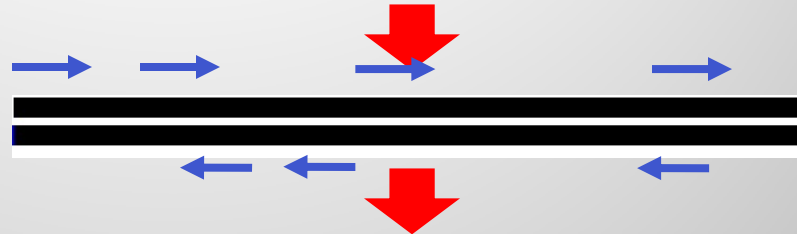
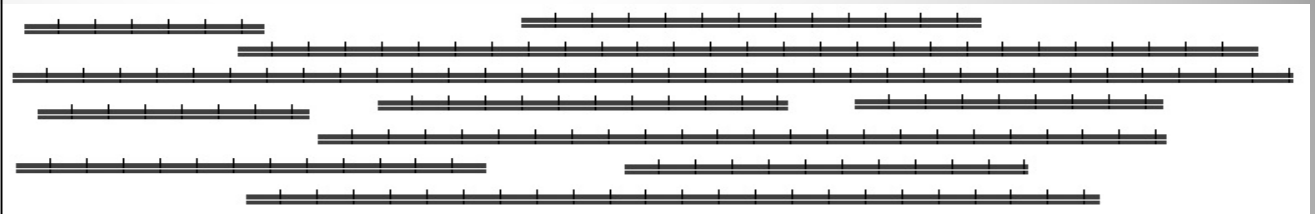
Metagenomic viral  
RNA and DNA  
(sample pretreated  
with DNase/RNase  
cocktail)

Random hexamer  
with 5'-20bp barcode

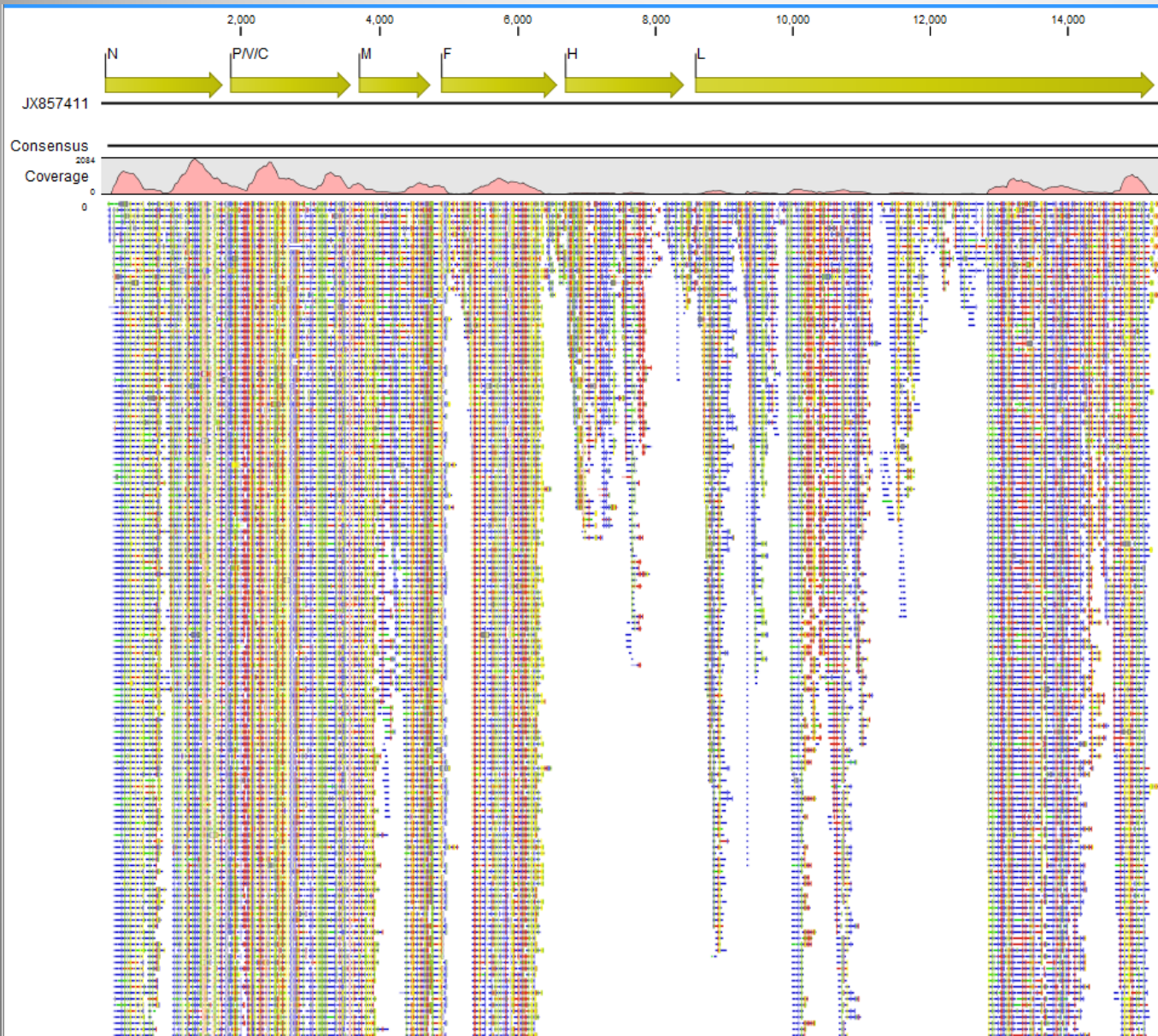
Reverse Transcription and  
Second Strand Synthesis  
(RNA → cDNA → dsDNA)

PCR Amplification  
using primer identical  
to 20bp barcode

Amplicon pools  
generated from  
randomly amplified  
virus nucleic acid



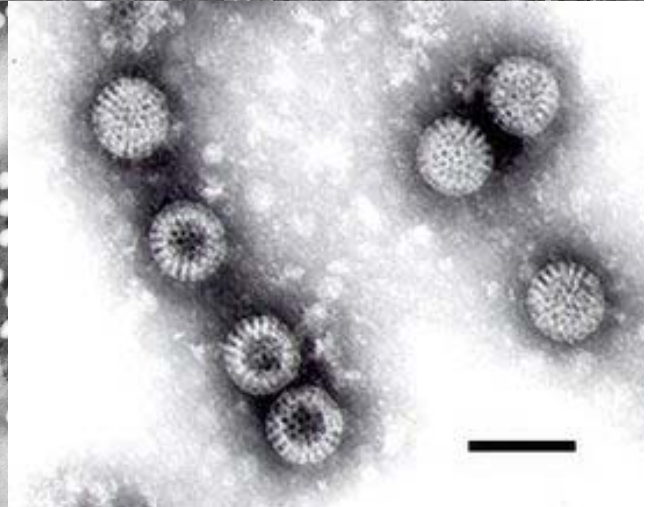
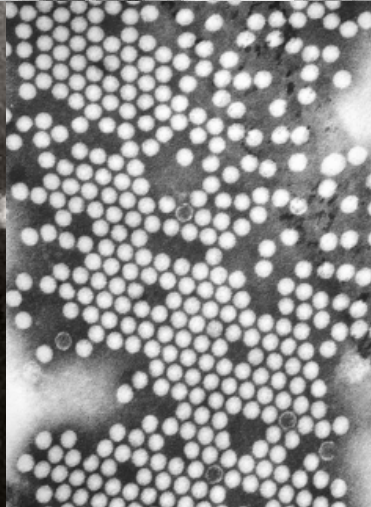
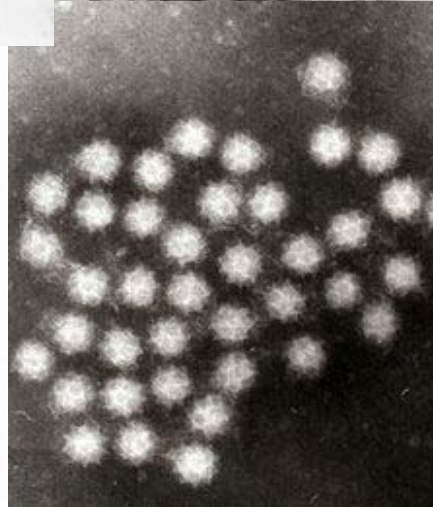
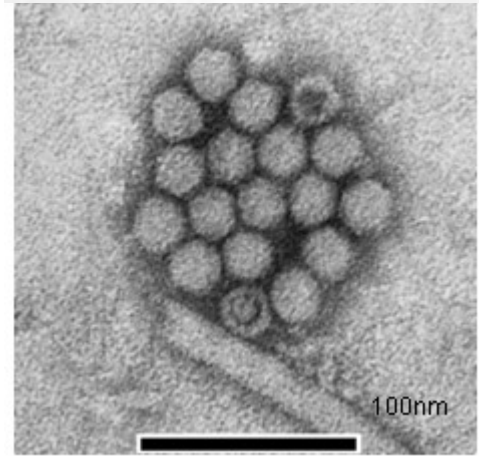
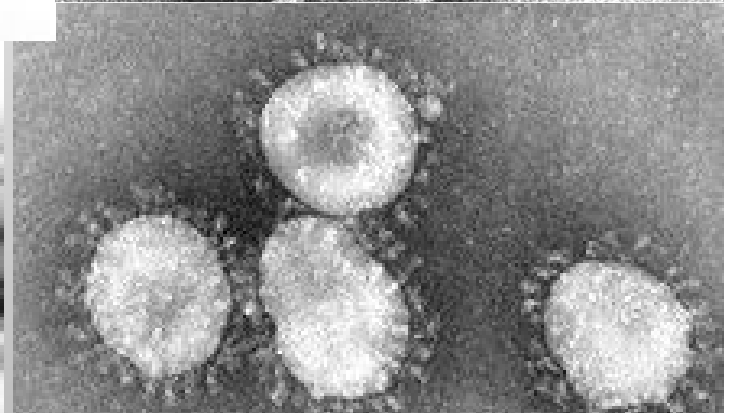
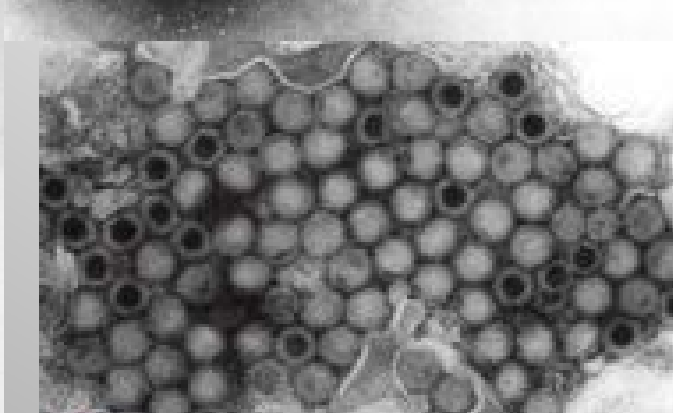
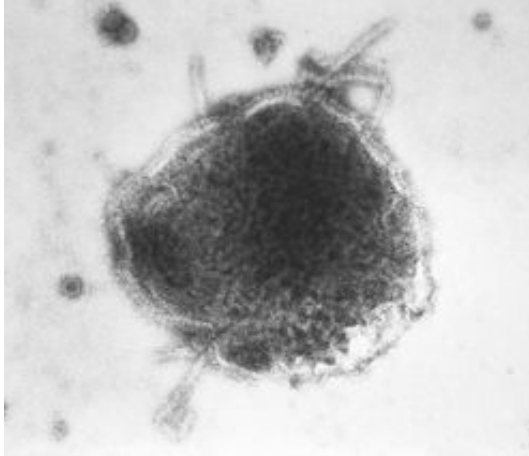
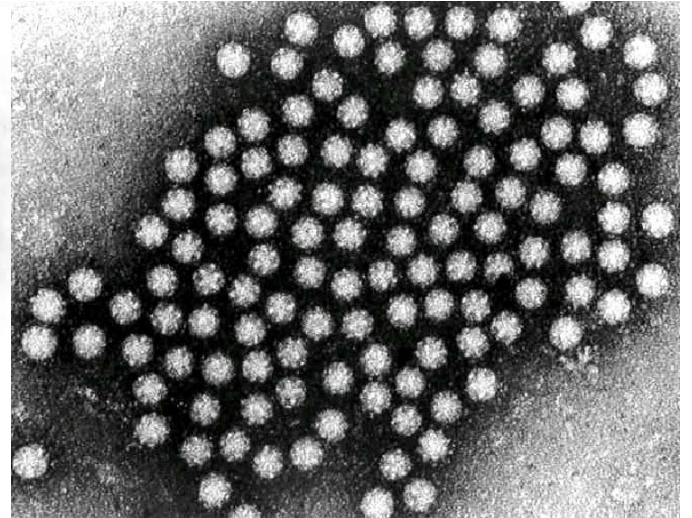
# Full genome sequence of porcine parainfluenza 1 (PPIV1) virus from a nasal swab



- 11 M reads
- 52,111 mapped to PPIV1
  - 0.45% reads
- 361x average coverage



Is to fill in here



# From Oral Fluids

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

