This little piggy went to market: applications of genomics for sustainable pig health

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Why is animal health so important?

- Disease causes loss of up to 15% of potential profit
  - Mortality
  - Product condemnation
  - Treatment costs (prevention and treatment)
  - Reduced production efficiency
  - Lower genetic gain
  - Reduced consumer acceptance

Comprehensive disease control program

- Biosecurity
- Sanitation
- Eradication
- Vaccination
- Antibiotics
- Genetic resistance

Why take a genetics approach to improve animal health?

- Improves animal welfare
- Environmentally sound approach
- Sustainable improvements
- Addresses consumer concerns about antibiotics
- Animal health affects human health
  - ✓Zoonotic diseases
  - ✓Food poisoning

Evidence for Host Resistance: Porcine Respiratory and Reproductive Syndrome (PRRSV)

- PRRS is a 700 million dollar issue
- Variation in severity among infected pigs (exp. challenges)
  - Antibody response and lung lesions:
  - Meishan < Duroc (Halbur et al., 1998)
- Evidence of genetic resistance relative to reproductive failure (Lewis et al., 2009)
  - matings per conception (PRRSV neg.) $h^2 = 0.04$
  - matings per conception (PRRSV pos.) $h^2 = 0.46$

The PRRS Host Genetics Consortium (PHGC)

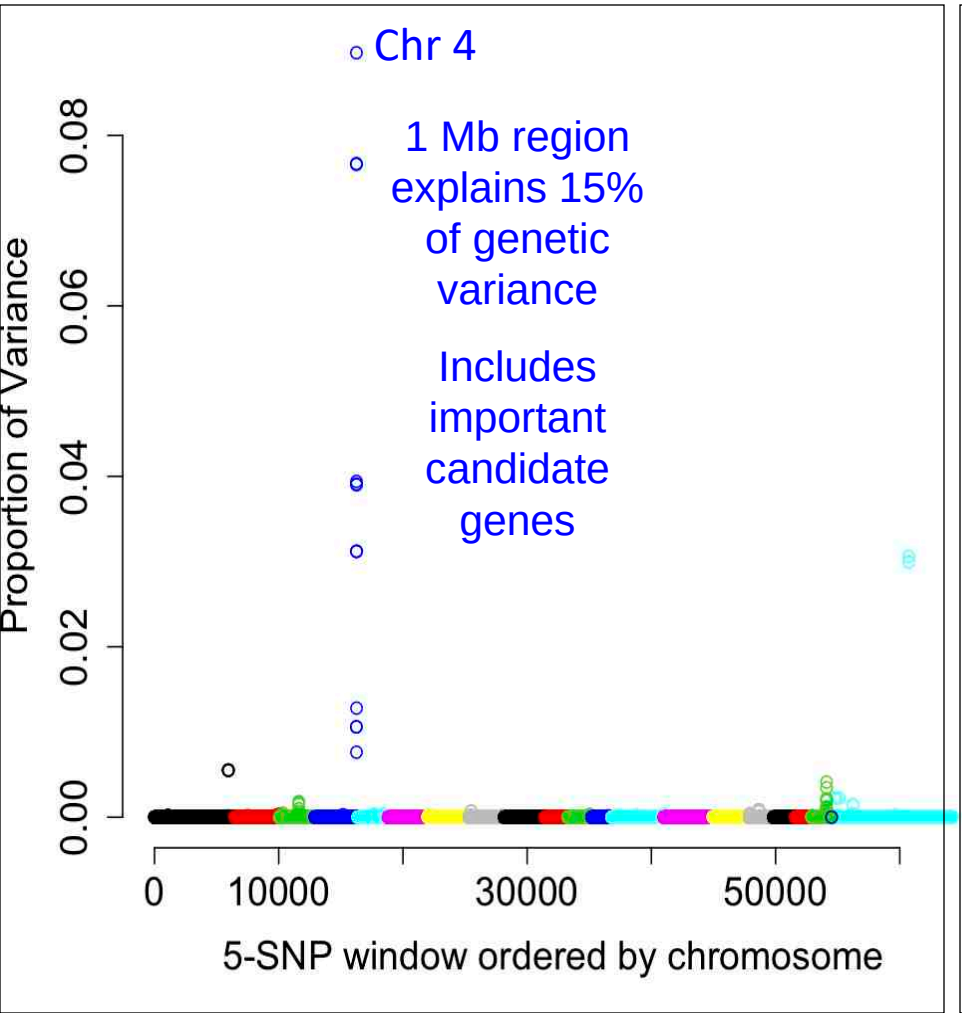
Joan Lunney. USDA BARC Bob Rowland, KSU

- Understanding the role of host genetics in resistance to PRRSV infection, and the effects of PRRS on pig health and related growth.
- Uses a nursery pig model to assess pig resistance/ susceptibility to primary PRRSV infection.
- After acclimation, pigs infected with PRRSV and followed for 42 days post infection (dpi).
- Blood samples collected at 0, 4, 7, 10, 14, 21, 28, 35 and 42 dpi, and weekly weights recorded.

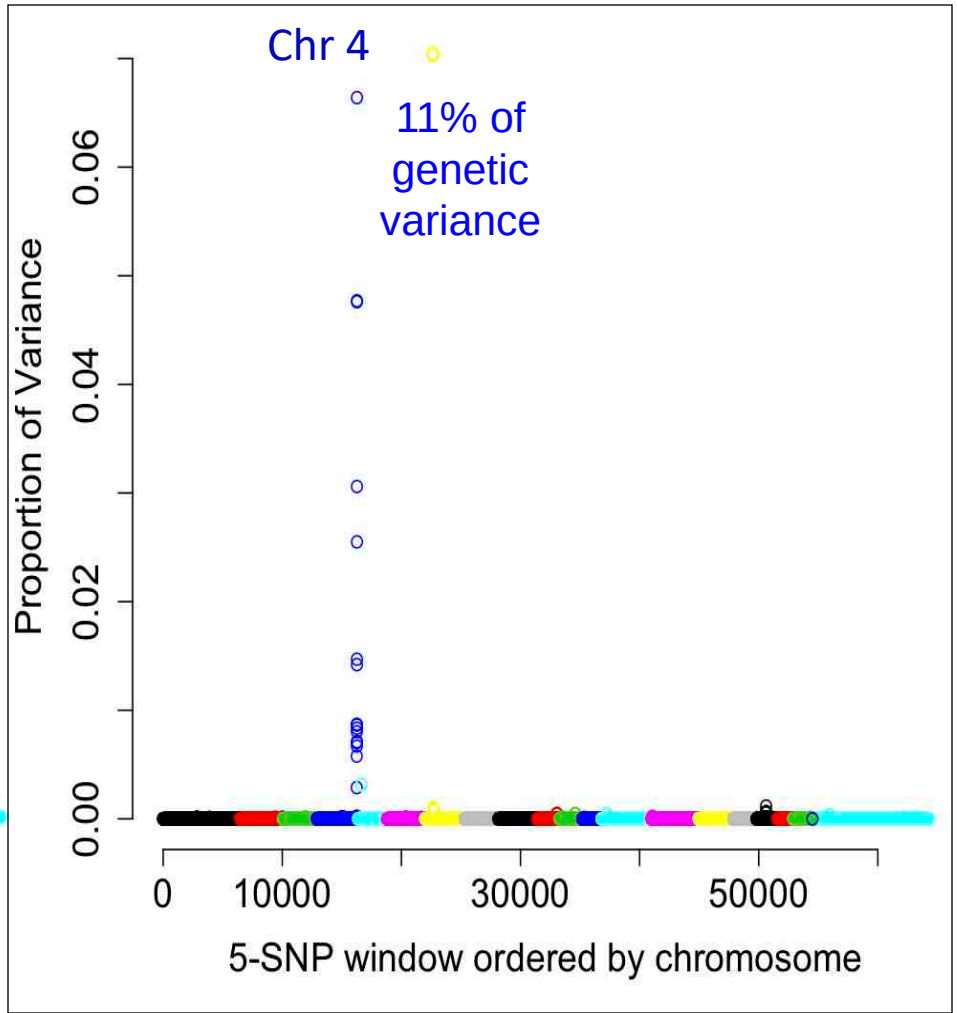
Results of Genomic Analyses

JAS 90: 1733

Viral Load

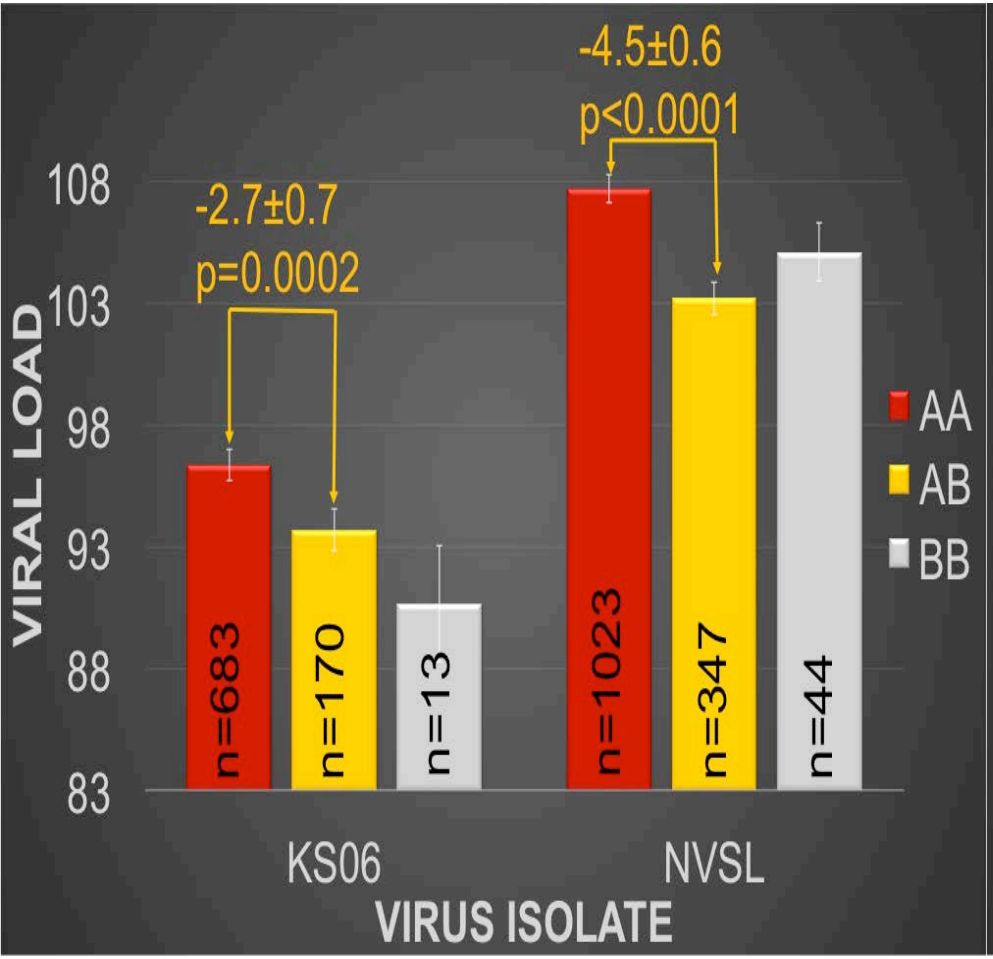


Weight Gain

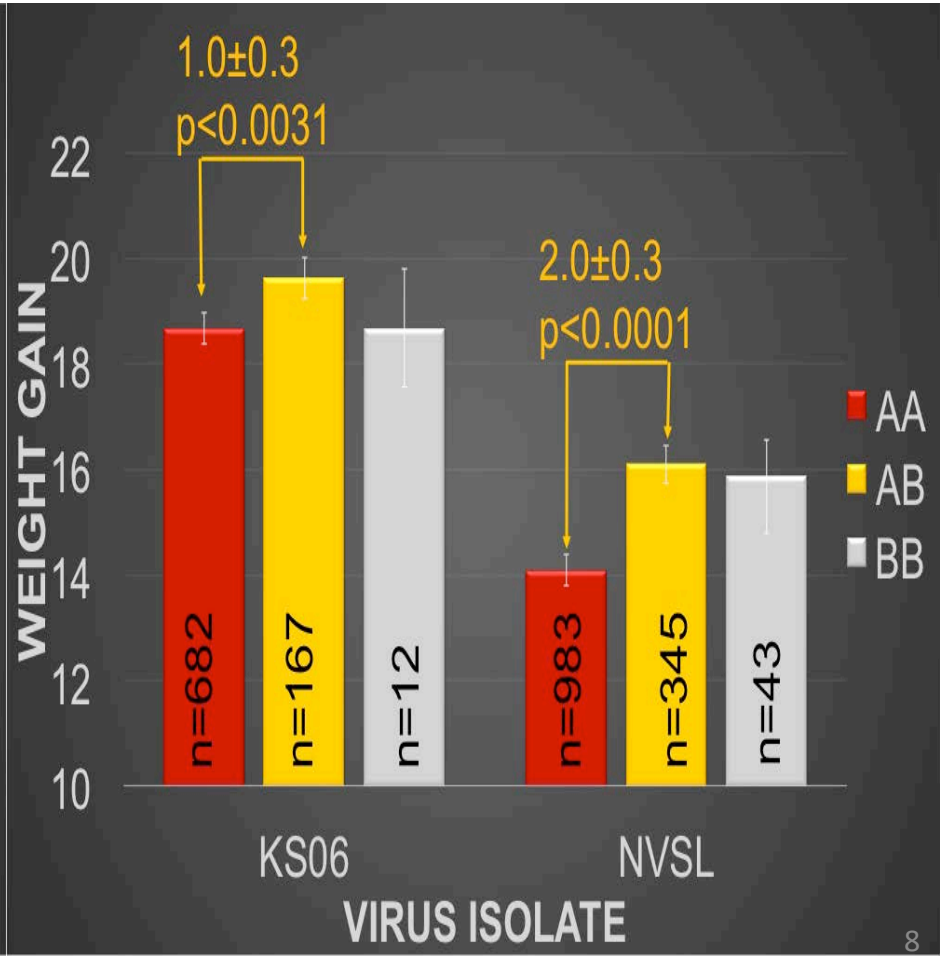


Effects of SSC4 SNP WUR10000125
in PHGC (NVSL) and Genome Canada (KS06) trials

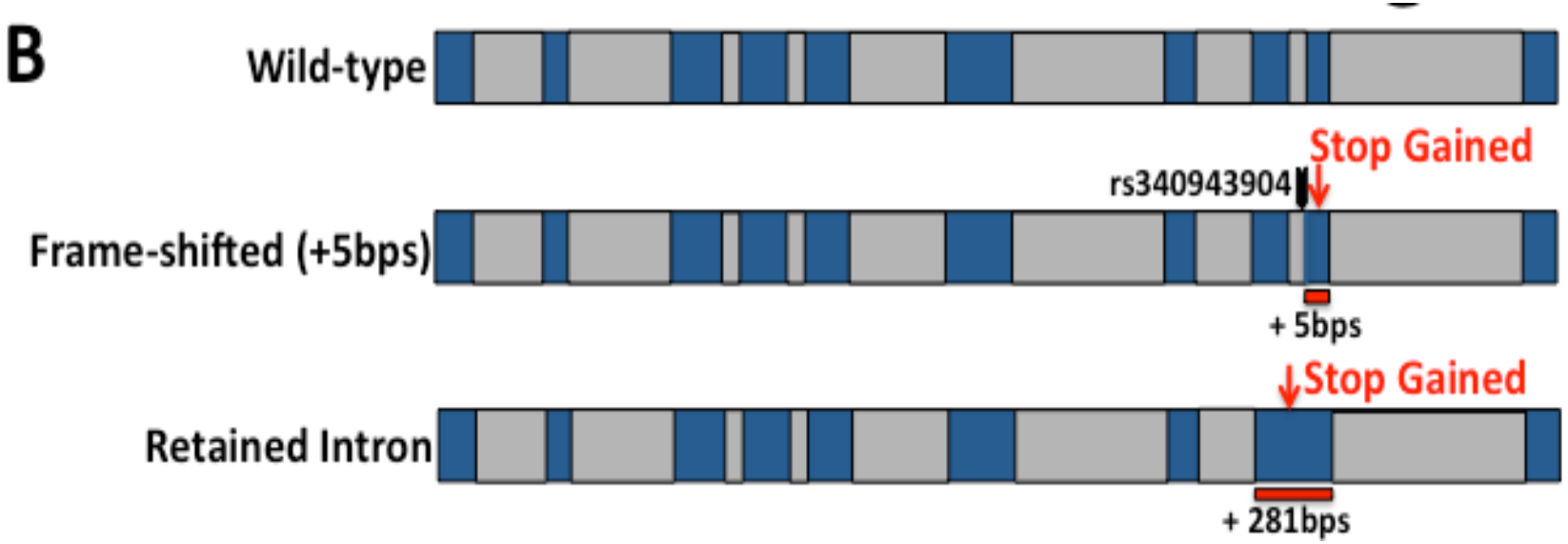
VIRAL LOAD



WEIGHT GAIN



A SNP introduces a new acceptor splice site, which causes five nucleotides to be added to the transcript (GBP5)

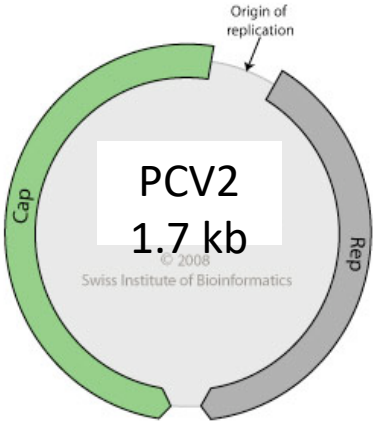


The 5bp transcript causes a frame-shift in the protein that introduced an early stop codon.

The susceptible genotype is missing the C-terminus of the protein

Koltes et al. *BMC Genomics* **16**:412.

Porcine Circovirus 2



- - Circular single stranded DNA virus
- Infect monocytes, macrophages and dendritic cells
- Two main clusters of strains exist: PCV2a and PCV2b
- PCV2 is environmental stable and resistant to common disinfectants
- Pigs become seropositive between 6 -18 weeks of age
- Vaccines available (2008)

Previous evidence suggested that host genetics influences PCVAD

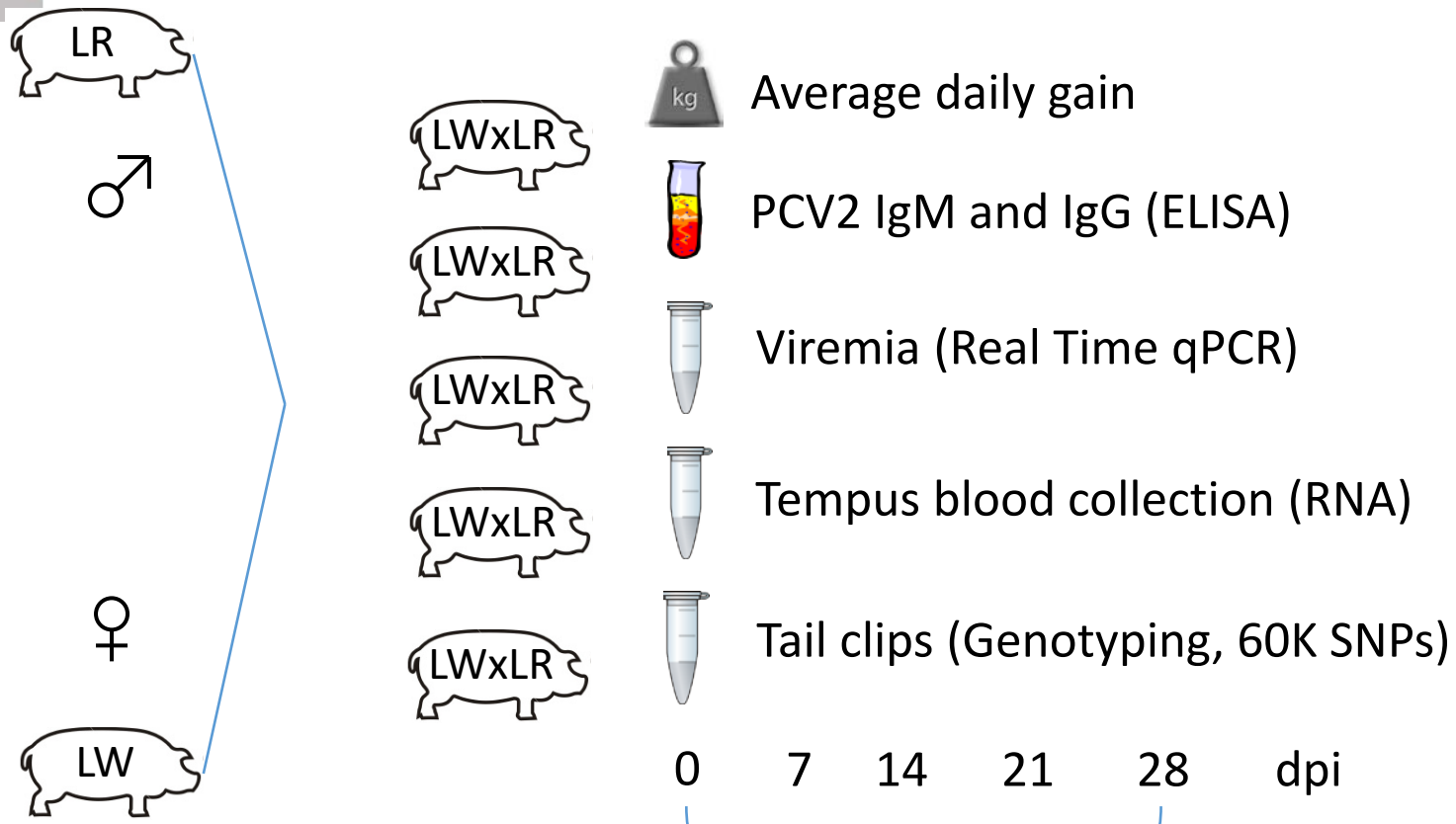
- Zhou et al., 2006- Sero-prevalence in 46 farms in Zhejian (China) was higher in Landrace than in Yorkshire and Duroc sows
- Opriessnig et al., 2006 - Landrace developed more severe lymphoid lesions than Duroc and Large White in experimental challenges
- Opriessnig et al., 2009 - Landrace had significantly more severe PCV2-associated lesions than Pietrain in experimental challenges
- Bates et al., 2009 - Variation in PCV2 immune response has an important host genetic contribution

Genome Canada project: Application of genomics to improving swine health and welfare

GenomeCanada

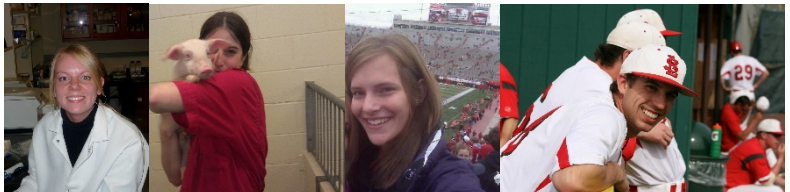
N ~ 1,000
Genetic lines (n = 14)
Genetic programs (n = 7)

PCV2b experimental challenge – 28 days (n ~ 1,000)



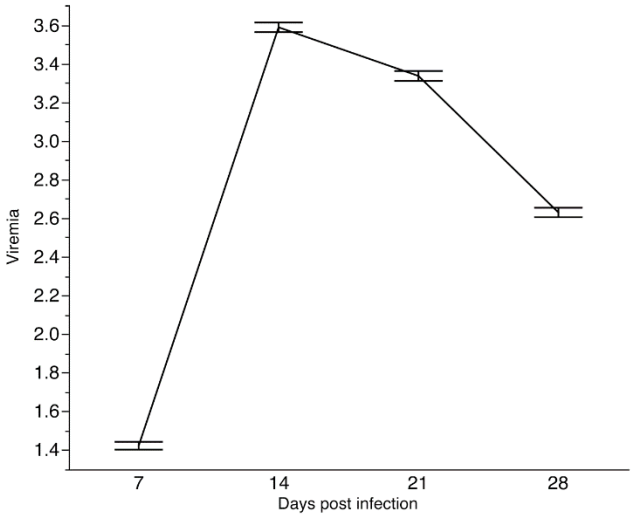
LW: Large White
LR: Landrace

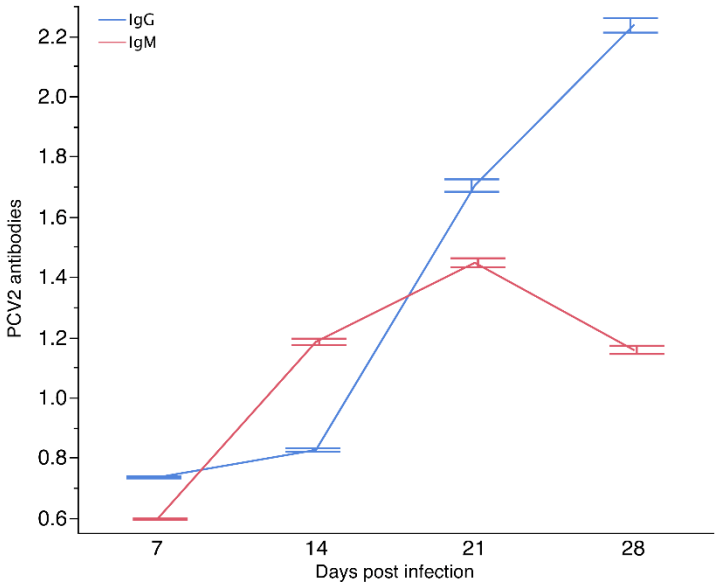
Ciobanu et al.



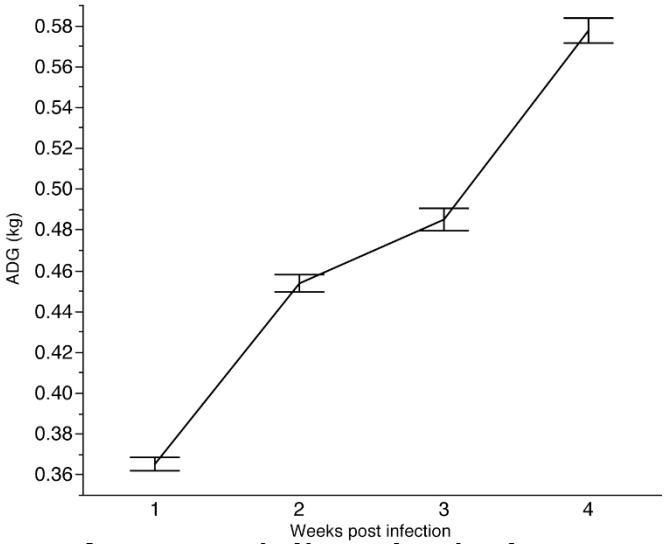
Indicators of PCVAD susceptibility (n=974)

PCV2b viremia following experimental challenge





PCV2 antibody response following PCV2b challenge

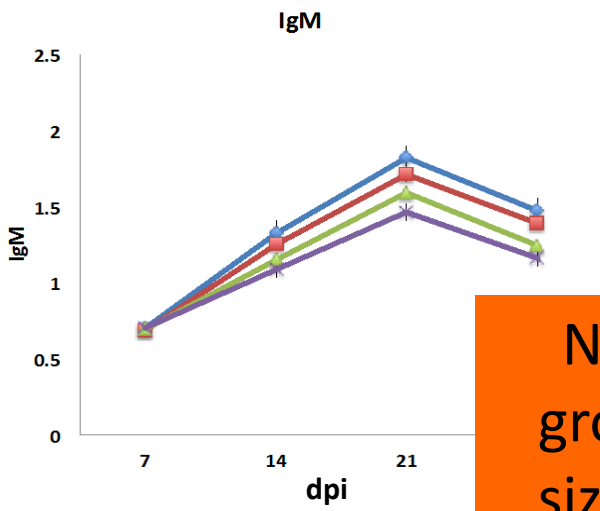


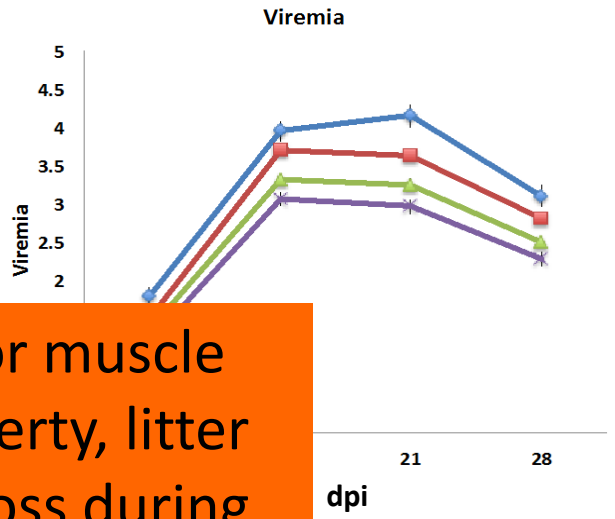
Average daily gain during PCV2b challenge

SSC7 and SSC12 explained ~ 15% of genetic variance

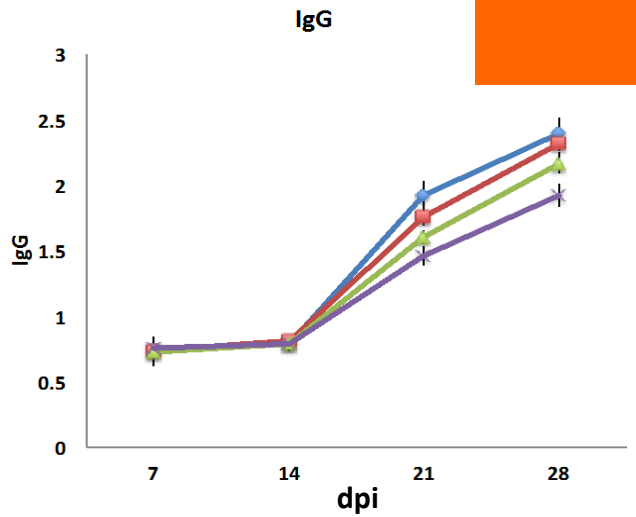
* Kachman, 2014

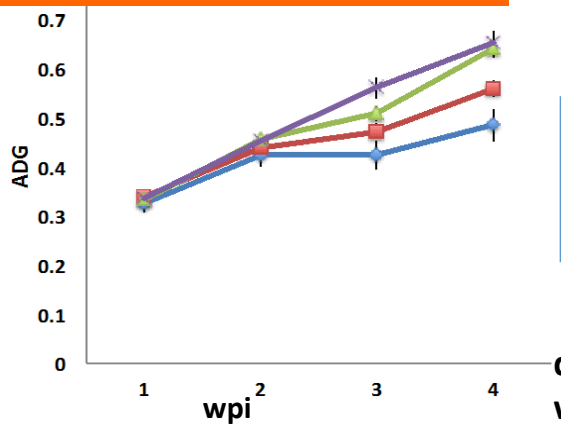
Alleles from 2 major SNPs influence viremia, immune response and growth during PCV2b infection

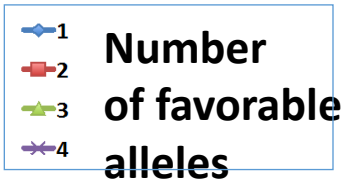




No effects on fat or muscle growth, age at puberty, litter size traits, weight loss during lactation etc., in non-challenged pigs.

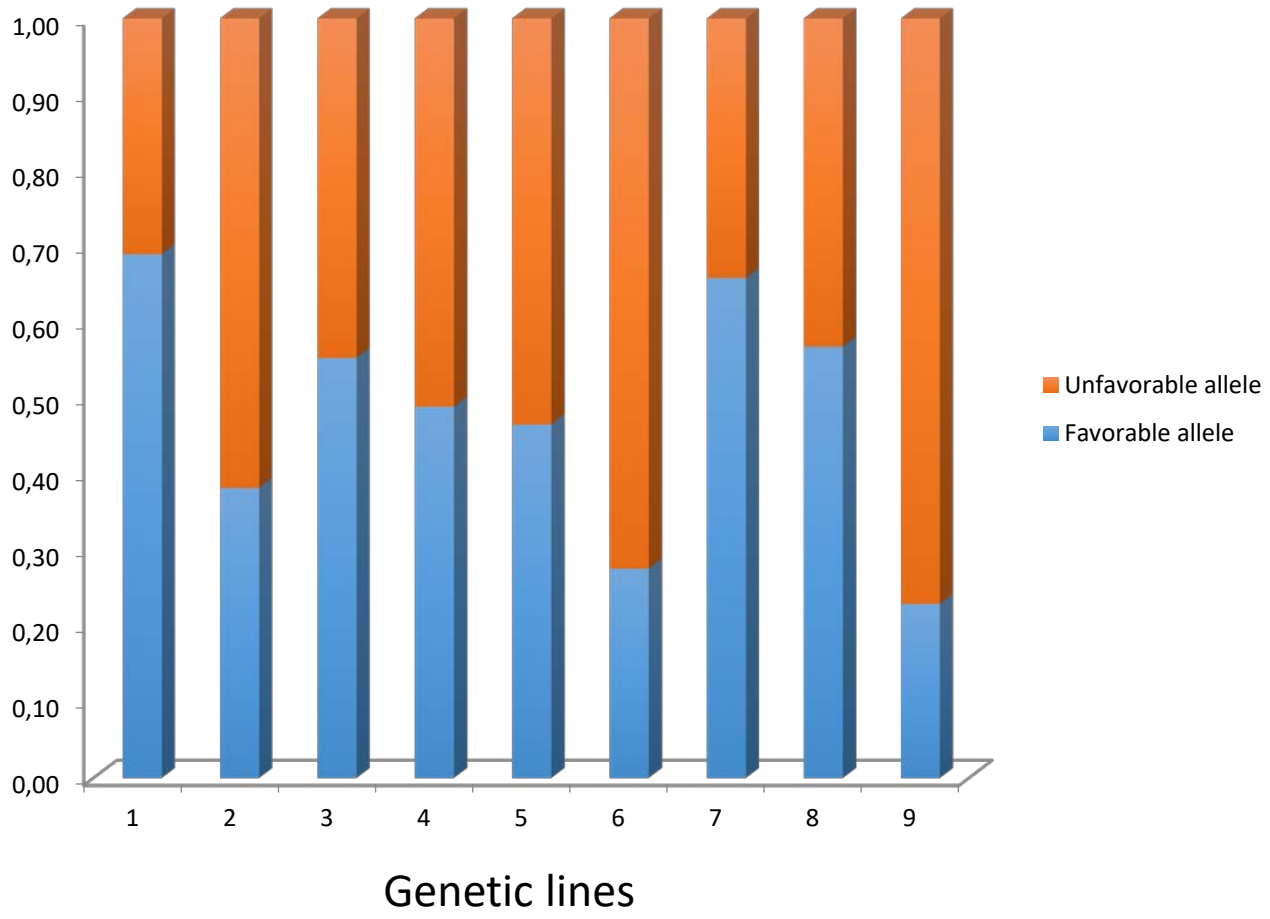






dpi: days post infection
wpi: weeks post infection

Frequency of favorable allele for the SNP located on SSC12 provides opportunities for Marker Assisted Selection



Genomic Differences Between Pre-weaning Survival and Mortality of Piglets Following PEDV Outbreaks

Francesca Bertolini, PhD,
Department of Animal Science,
Iowa State University

Graham Plastow

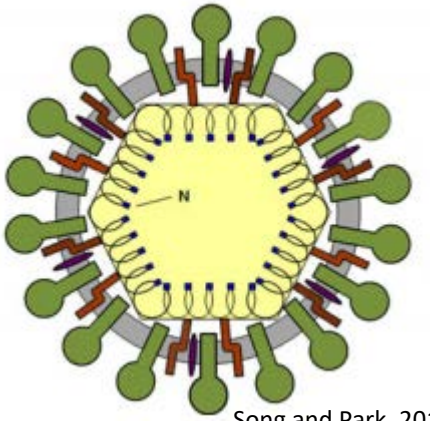
John Harding

Benny Mote

Max Rothschild

Porcine Epidemic Diarrhea (PED) virus

Coronavirus



Song and Park, 2012

Infects the cells lining the small intestine

- **Watery Diarrhea**
- **Vomit**
- **Dehydration**

The incubation period is approximately 2 days and diarrhea lasts for 7 to 14 days

Rapid spread across all breeding and growing pigs with almost 100% morbidity (pigs affected) within 5 to 10 days

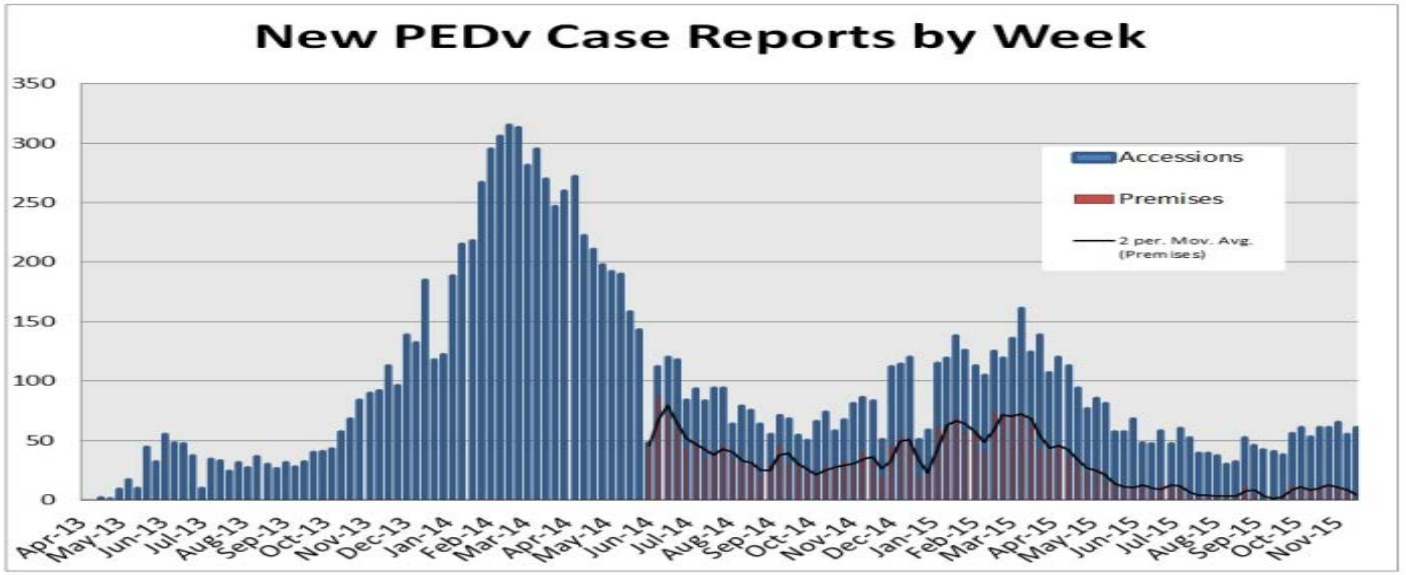
PEDV DIFFUSION

First reported in Europe in the 1970s (England and Belgium) and later in Asia, 1980s

First diagnosed in the United States in mid-May 2013

Reported in Canada in January 2014

PLEASE NOTE: the chart below now also contains the number of confirmed + presumptive positive premises by week since June 5, 2014.

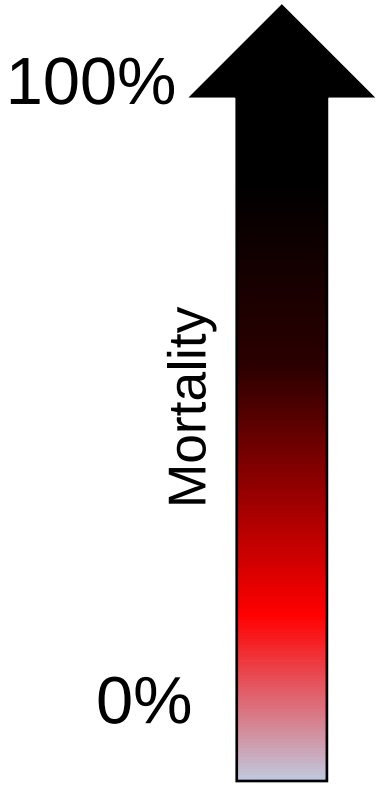


Source: USDA

MORTALITY/RESILIENCE

AGE-DEPENDENDENT PEDV RESILIENCE

(Shibata et al. 2000)

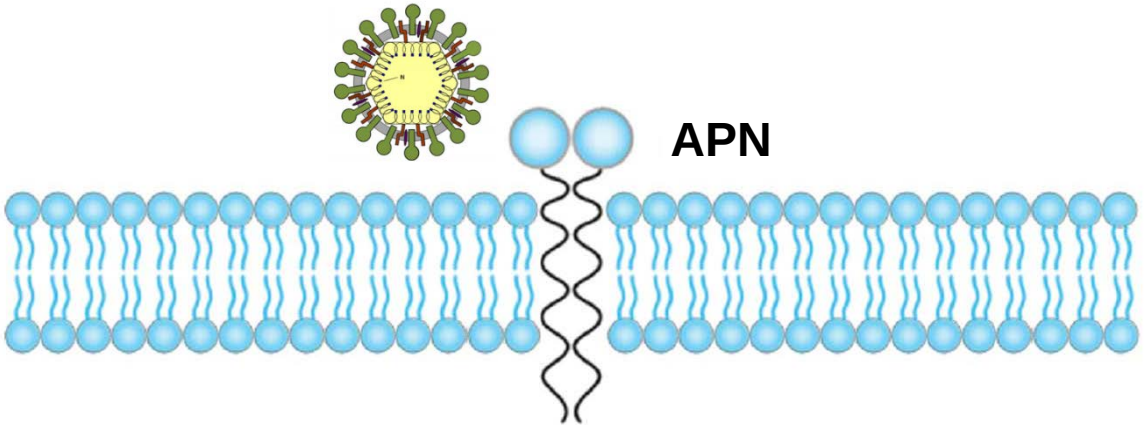


PRE-WEANED PIGS

POST-WEANED PIGS

ADULT PIGS

Alanyl (membrane) aminopeptidase

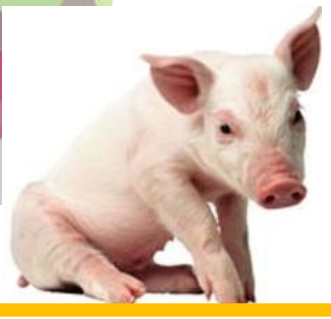


Antibodies against pAPN block the infection (Sik et al. 2003; Li et al. 2007)

Immunofluorescence assays (Li et al. 2007)

Other coronaviruses such as TGEV, HCoV-229E and FeCoV all use APN as their cellular receptor (Delmas et abbbbbl., 1992; Yeager et al., 1992; Tresnan et al., 1996; Tresnan and Holmes, 1998; Kolb et al., 1998)

AIM



INVESTIGATE THE GENETIC DIFFERENCES BETWEEN SURVING AND DEAD PRE-WEANING PIGLETS THAT CAN BE LINKED TO PEDV RESILIENCE

MATERIALS AND METHODS

Animals

Dead and surviving neonatal pigs during the acute phase of a PED outbreak before the development of maternal antibody

	DEAD	ALIVE	TOT
USA1	12	13	25
USA2	71	20	91
CANADA	25	25	50
GERMANY1	25	25	50
GERMANY2	23	23	46
TOTAL	**156**	**106**	**262**

DATA ANALYSES

Genotyping with the 80K SNPchip

Retaining SNPs in autosomes and call rate >0.90

1Mb windows

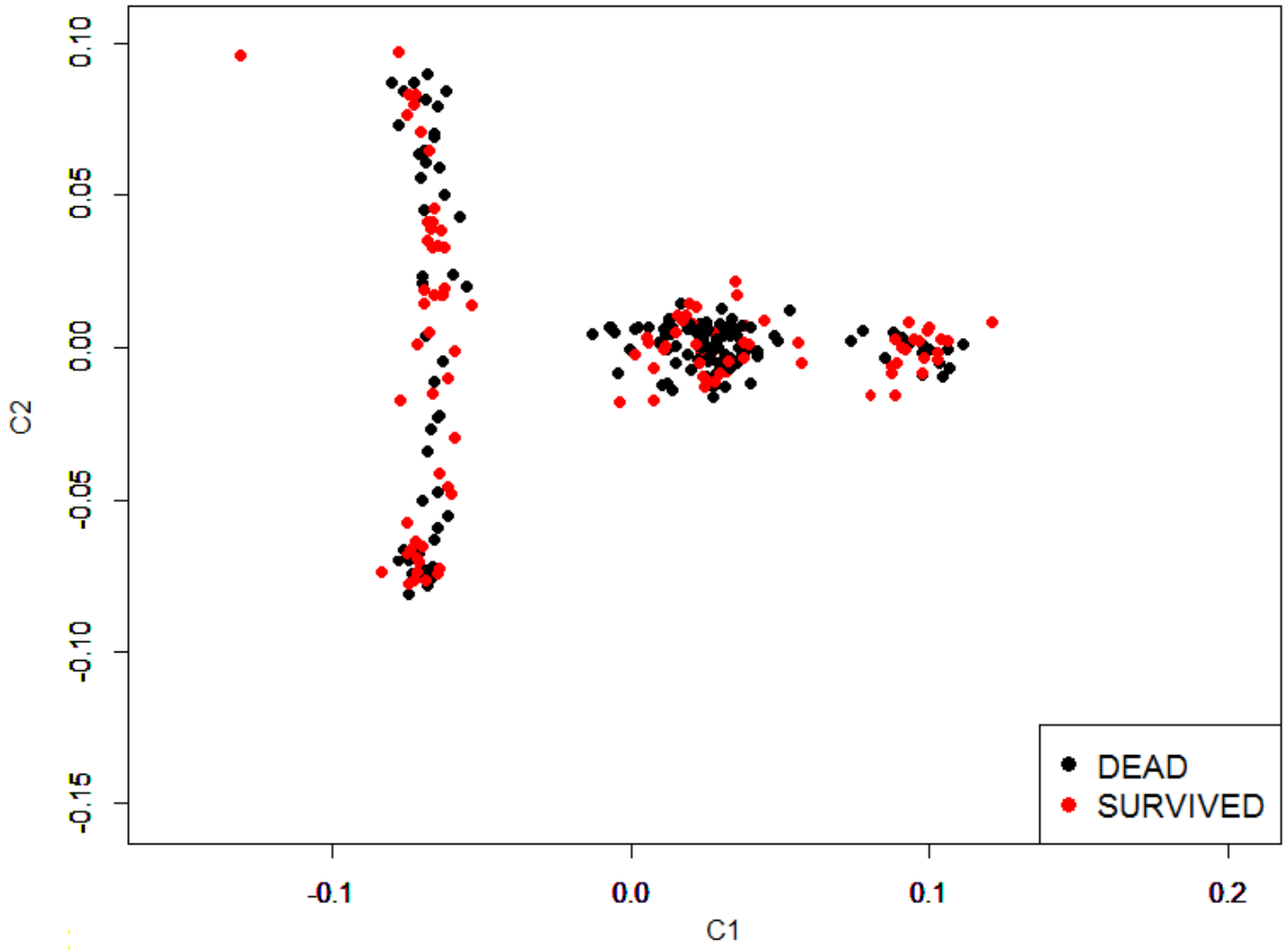
Mean Fst of each window

Normalization of the mean Fst value

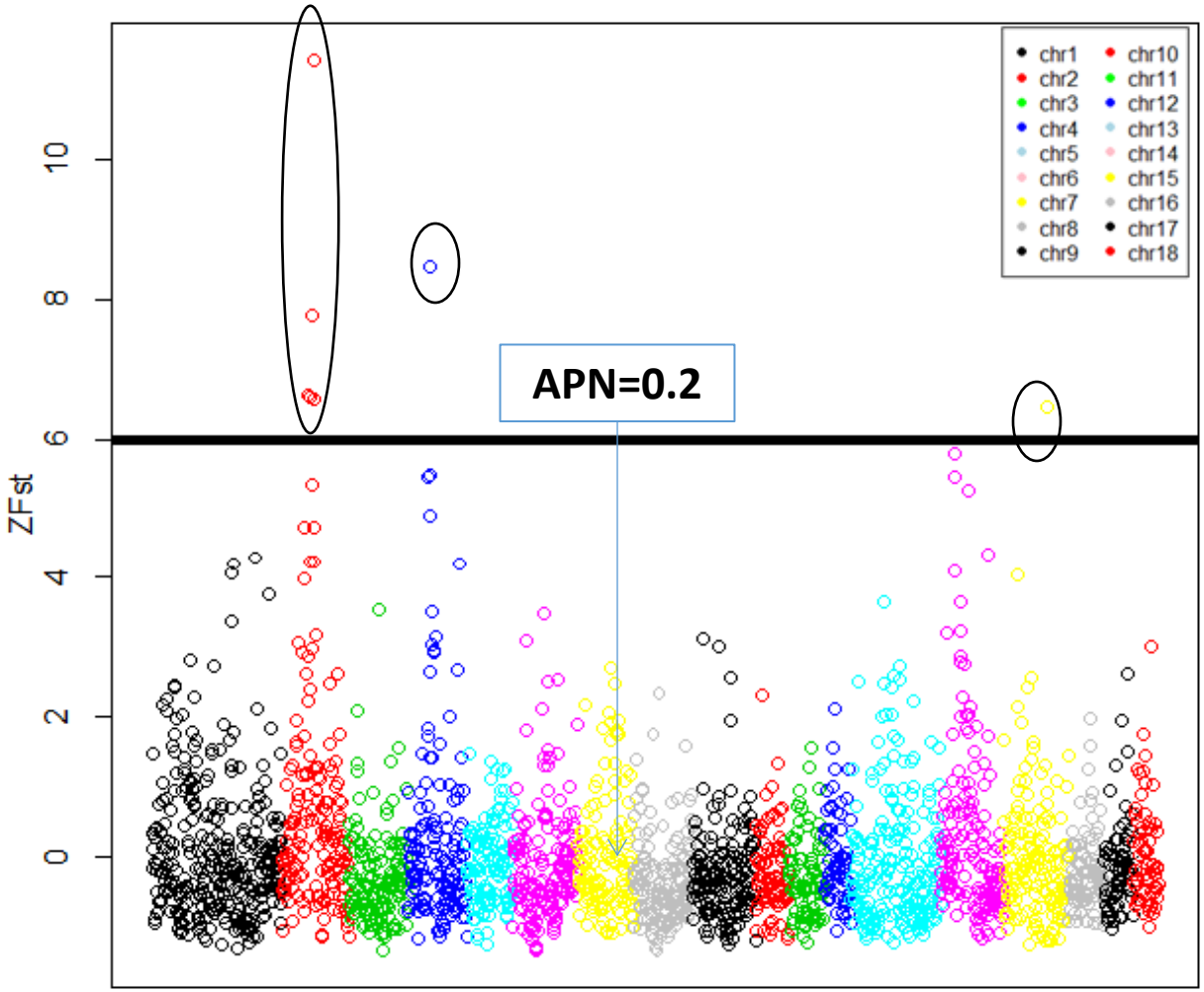
GOrilla and Enrichr

RESULTS

Samples distribution



Normalized Fst Plot



162 ANNOTATED GENES

GENE ENRICHMENT

GORILLA

COMPONENT	N. GENES
Golgi apparatus	7
cell projection part	4
Golgi membrane	4

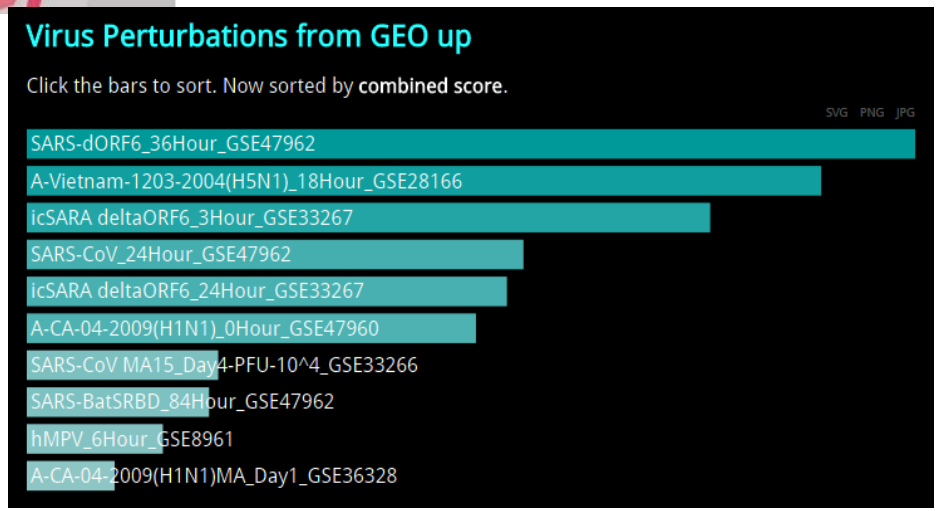
GENE ENRICHMENT

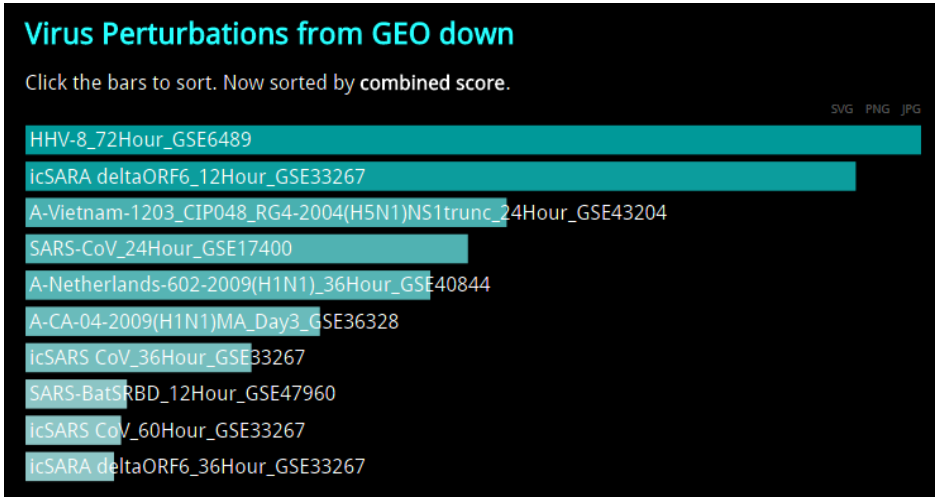
GORILLA

FUNCTION	N. GENES
transporter activity	11
ATPase activity	5
substrate-specific transporter activity	3
ATPase activity, coupled to movement of substances	3
ATPase activity, coupled	3

GENE ENRICHMENT

Enrichr





GENES under or over expressed in several virus infections, including Coronaviruses.

CONCLUSIONS

Found evidence of several genes that can be linked to pig resilience

-Genes linked to Golgi apparatus
-Genes involved in the regulation of ER
-Genes involved in ion transports

Further studies to confirm and investigate the role of each gene in pre-weaning pig resilience

PEDV Study 2
viral inoculum - ~50% survival rate Case and Controls

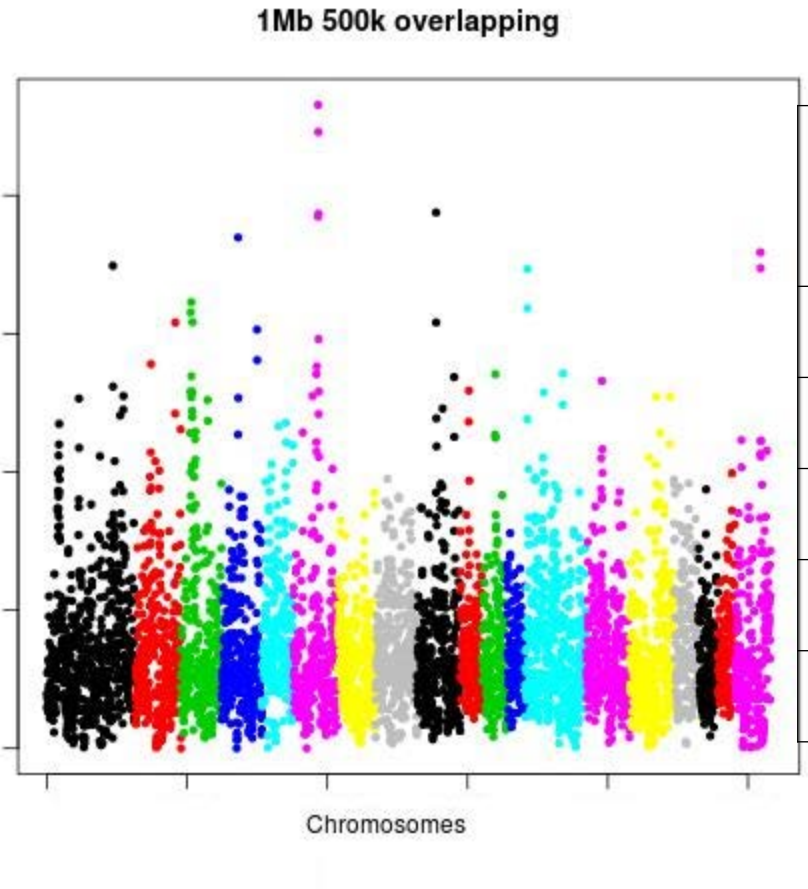
- **3 farms**
- **VD**= found dead with diarrhea
- **VM**= terminated pig for welfare reasons before it died naturally
- **VS**= survived for 7 days
- **We considered both VM and VD as DEAD**

VD	91
VM	52
VS	61

→

DEAD=143
SURVIVED=61

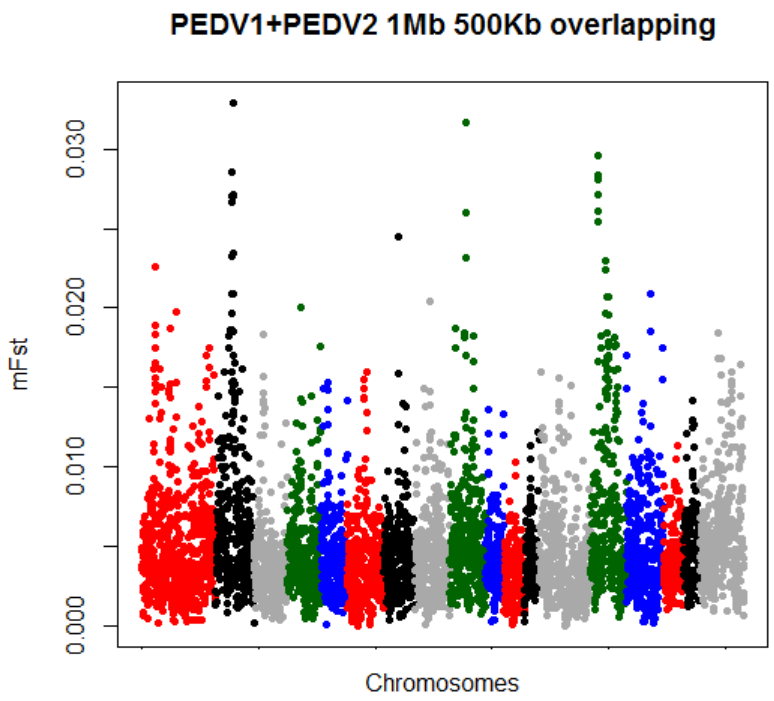
mFst analysis with 500kb overlapping windows



chr	start	End	N. SNPs	mFst
6	88000000	89000000	25	0.093
6	88500000	89500000	19	0.089
9	68000000	69000000	8	0.077
6	89000000	90000000	19	0.077
6	87500000	88500000	23	0.077

9: 68000000-69000000: common in the first analysis

mFst analysis with 500kb overlapping windows combining the 2 PEDV data sets



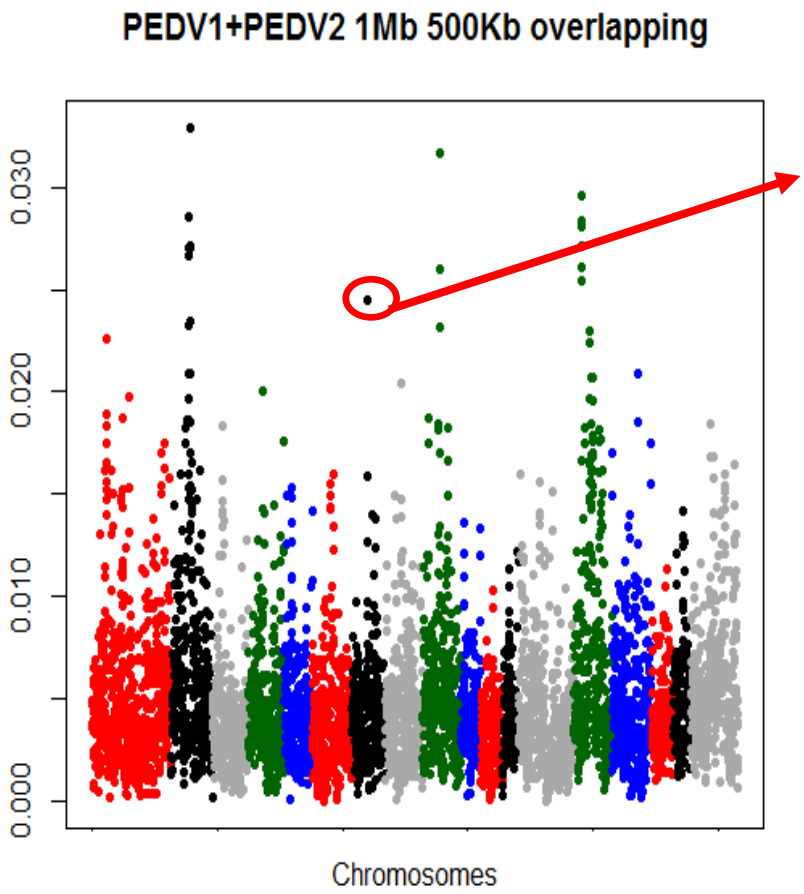
chr	start	end	N. SNPs	mFst
2	67000000	68000000	8	0.027039
2	68500000	69500000	6	0.028513
2	69000000	70000000	7	0.026674
2	76500000	77500000	7	0.027158
2	77000000	78000000	8	0.032892
2	77500000	78500000	10	0.027044
9	68000000	69000000	8	0.031661
9	68500000	69500000	11	0.026032
14	31500000	32500000	17	0.028339
14	32000000	33000000	18	0.026054
14	33000000	34000000	14	0.027162
14	33500000	34500000	21	0.02955
14	34000000	35000000	22	0.0281

Regions partially overlapp

Regions totally overlapping in both

New regions

DETAILS FOR CHROMSOME 7 AND ANPEP GENE



chr	start	end	mFst
7	61500000	62500000	0.016
7	61000000	62000000	0.025

ANPEP gene Window

chr	start	end	N.	mFst
7	60000000	61000000	18	0.005

A new approach:

Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus

Kristin M Whitworth, Raymond R R Rowland, Catherine L Ewen, Benjamin R Trible, Maureen A Kerrigan, Ada G Cino-Ozuna, Melissa S Samuel, Jonathan E Lightner, David G McLaren, Alan J Mileham, Kevin D Wells & Randall S Prather

Nature Biotechnology 34, 20–22 (2016)
doi:10.1038/nbt.3434
Published online 07 December 2015

http://www.nature.com/nbt/journal/v34/n1/full/nbt.3434.html

News > Science

'Pig 26': Can this little piggy win over the enemies of GM?

news

Apr 23, 2013

UK: African Swine Fever-resistant pig created

BREEDING

886 0

A disease-resistant piglet has been produced using new genetic engineering. It is the first animal created from 'gene-editing'.

The piglet was born four months ago at Edinburgh's Roslin Institute, and is known as 'Pig 26'. 'Gene-editing' involves researchers snipping the

According to researche
copy's a natural genetic
DNA had been artificial

'Pig 26': Can this little piggy win over the enemies of GM?

Live pigs produced from genome edited zygotes

Simon Lillico, Christopher Proudfoot, D. F. Carlson, Dana Stverakova, Claire Neil, Carol Blain, Tim King, WA Ritchie, Spring Tan, A Mileham, D Mclaren, Scott C Fahrenkrug, **Bruce Whitelaw** — 2013 — Scientific Reports *Vol: 3 Pages: 1-4*

Species-specific variation in RELA underlies differences in NF-κB activity: a potential role in African swine fever pathogenesis

Christopher J Palgrave, Linzi Gilmour, C Stewart Lowden, Simon G Lillico, Martha A Mellencamp, **C Bruce A Whitelaw** — Jun 2011 — Journal of Virology *Vol: 85 Pages: 6008-14*

Efficient TALEN-mediated gene knockout in livestock

Daniel F Carlson, Spring Tan, Simon G Lillico, Dana Stverakova, Christopher Proudfoot, Michelle Christian, Daniel F Voytas, Charles R Long, **C Bruce A Whitelaw**, Scott C Fahrenkrug — 2012 — Proceedings of the National Academy of Sciences of the United States of America - PNAS *Vol: 109 Pages: 17382-7*

Improving Sustainability

- Genomic identification and selection could be designed and applied to improve resistance in nucleus herds
  - Reduce the production cost
  - Increase robustness
  - Improve welfare
- Consider identifying gene targets for gene editing or designer breeding

Acknowledgments

- PIG GENOME COORDINATION PROGRAM
  (Chris Tuggle, Cathy Ernst)
- Genome Alberta
- PRRS consortium members (Joan Lunney, Bob Rowland, Jack Dekkers and colleagues)
- Andrea Ladinig, Univ. of Veterinary Medicine, Vienna
- Daniel Ciobanu and colleagues, University of Nebraska
- Graham Plastow, University of Alberta
- John Harding, University of Saskatchewan
- Benny Mote, Fast Genetics, University of Nebraska

Thank you