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Sequencing of PRRSV isolates, a useful tool in epidemiology

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PRRS

- Emerged North America 1987 found in Europe 1990
- Sows – reproductive failure
- Piglets – respiratory problems and poor growth
- Rarely fatal – fatalities generally associated with secondary infection
- One of the most economically important diseases of swine worldwide
- Vaccines available but not very efficacious

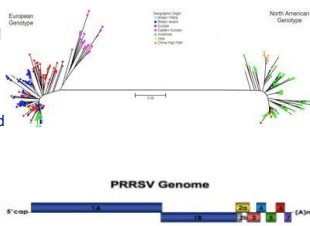


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PRRSV

- Enveloped, single-stranded RNA virus
- Arterivirus – EAV, LDV and SHFV
- Replicates in myeloid cells
- Immunomodulatory effects
- 2 genotypes, European and North American
- Highly diverse making efficient vaccine production difficult
- 15kb genome, 9 ORFs
- PRRSV associated with cases of PHFD in China



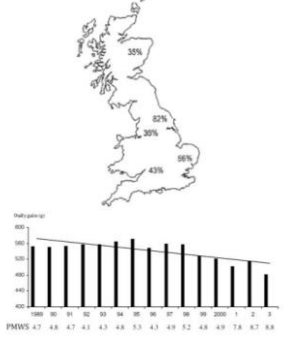
PRRSV Genome

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PRRSV in Britain

- Minimal epidemiological data for PRRSV in Britain
- Serology study (2004) – 56% (366 herds) seropositive for PRRSV
- More recent figures required
- Since PRRSV entered Britain in 1991 growth weight has fallen and PMWS has increased



PMWS cases per year

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Role of Sequencing in Epidemiology and Control

- Sequence data useful for both researchers and vets/producers
- Current strains present in the UK
- Diversity of British strains – vaccine production
- Distinguishing between infected and vaccinated animals
- Outbreaks – re-emergence of an earlier strain? Multiple strains present on the same farm?
- Transmission – same strain found in outbreaks in other farms?

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ORF 5 Sequencing

- Work aims to utilise ORF 5 sequence data requested by vets to aid in epidemiological investigations
- Encodes GP5
 - Viral attachment
 - Main immunogenic epitope
- Why use ORF 5?
 - Most commonly used for sequencing worldwide
 - Data also available for ORF7, but less variable than ORF5

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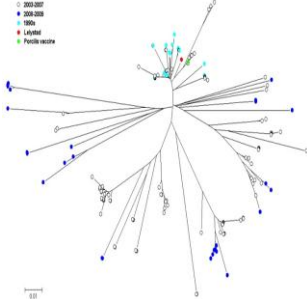
Methods

- RNA extracted from clinical submissions, tested for PRRSV by QPCR
- cDNA generated from QPCR positive samples using random hexamers or specific reverse PCR primer
- ORF 5 amplified by conventional PCR using primers generated from ORF 4 (forward) and ORF 6 (reverse)
- Large proportion of negative samples – primer design?
- DNA extracted from gel sequenced using ABI Prism TM310 Genetic Analyzer
- Sequences aligned and phylogenetic trees composed

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Results



- 121 sequences obtained so far
- Vaccine-like
- Majority showed no close association
- Of 31 farms 14 had multiple strains present
- Some very similar strains found on different farms

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Results

- Farm A
 - Coughing gilts and neonates
 - 2 different strains
 - Similar to a nursery finisher owned by the same company
- Farm B
 - Breeder finisher using live vaccine
 - 2006 – Abortions and neonatal death. Virus isolated from vaccinated sows
 - 2007 Pneumonia in 18 week finishers – virus found to be similar to 3 other breeder finisher farms

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Results

- Farms C and D
 - Neighbouring breeders
 - Farm C stillbirths and weak neonates
 - Farm D abortions, stillbirths and weak neonates. Sows vaccinated with live vaccine
 - Related to earlier isolate from another farm owned by the same company
 - Related to a case of pneumonia in 8 week old pigs from a distinct breeder finisher

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Conclusion

- UK PRRSV strains becoming more diverse – implications on vaccination
- Discovery of a vaccine-like strain from a clinical submission highlighting dangers of using live-vaccines
- Revealed epidemiological links between outbreaks on separate farms – confidentiality issues
- Still not enough data
 - Problems with PCR – is ORF 5 best choice???
 - Need farmers to submit more samples

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.....or is it PRRS???