Using the pan-genome of *H. parasuis* to develop new molecular diagnostics

Kate Howell

PhD Student, Department of Veterinary Medicine
• Glässer’s disease is a systemic disease of pigs caused by *Haemophilus parasuis*.

• Disease caused by *H. parasuis* has been on the increase, with larger outbreaks seen since the adoption of higher health herds and multi-site practices.

• The only tests currently available in the UK are able to detect presence/absence of the bacterium.

• Serotyping is available in Europe but is expensive and can only type 80% of isolates.

• Vaccines are available but only effective against 2/15 serotypes.
Main objectives:

- Identification of new virulence associated genes by comparing whole genome sequence data of disease-associated and non-associated strains.
- Investigation of genetic determinants of serotype
- Design and validate molecular tests using the new biomarkers from the bacterial genomics analyses
HPS has a significant impact of disease on mortality, productivity and welfare of pigs globally.

The proposed diagnostic would fill a hole in the market by offering molecular serotyping in the UK and information on the chance of a strain causing disease.

It would be a cheap and fast diagnostic to allow fast turn around of results.
PhD Methods summary

- Creation of isolate collection
- Draft Genome Sequencing
- Data Purity Checking
- Genome Assembly
- Gene Prediction
- Homology group analysis
- Collection of clinical data
- Mobile element analysis
- Recombination analysis
- Capsule analysis
- Pan-genome analysis
- Identification of SNPs
- GWAS analysis
- Regression analyses
- Shortlist of genes associated with phenotypes
- Molecular serotyping assay development
- Optimisation and validation of these assays
- Virulence predicting assay development
H. parasuis isolate collection

Population of 212 isolates, 117 of which have been serotyped. 143 strains from a clinical background and 43 non-clinical.
From capsule genetics to molecular serotyping assay

Howell et al. 2013 – J. Bacteriology
Identification of a recombination barrier

Figure 4: Heat-map of the shared accessory genes between strains. A clear separation can be seen between the clades, and an association can also be seen between the BAPS groups.
Identifying clinically associated SNPs and genes

Bacterial GWAS using DAPC (Jombart et al. 2010)

PCA represents variation in the data using linear combinations of SNPs or gene presence/absence.

DAPC takes the linear component that best maximises the variation between two or more groups (here shown in blue and pink).
From GWAS to a user friendly pathotyping assay

48 new virulence associated genes were identified

9 were used to build a multiplex PCR

Now we are creating a GUI to run the model and output results

Howell et al. 2014 – BMC Genomics. Accepted with minor revisions
Conclusions

- Identified HPS as a diverse species based on the genomics results
- Molecular serotyping and pathotyping assays have been validated on 250 AHVLA isolates and have 87% and 82% accuracy compared to existing methods.
- Both diagnostic assays are able to detect HPS in DNA extracted from tissues and swabs
- Patent applications are underway.
- PhD intended hand in date – 28th November.
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