A genetic biomarker panel for diagnosis of necrotic enteritis and coccidiosis in broilers

Neil Foster
School of Veterinary Medicine and Science, University of Nottingham, UK

This project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.
Work Package 5 PROHEALTH Consortium

University of Nottingham, UK
Analysis of whole pig and chicken genome (intestine and lung)

INRA (Tours), France
Analysis of regulation of 96 immune genes in pigs and chickens (intestine)

Veterinary Research Institute, Czech Republic
Intestinal microbiota analysis in pigs and chickens

Microarray (44k gene analysis)
Fluidigm (High through-put PCR)
Illuminaseq (deep sequencing of V3/V4 regions of 16S rRNA)

Gene/gene pathways (Biomarkers) which profile production disease

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Coccidiosis in chickens

High intensity farming

- Highly infectious
- Reduced feed intake and feed conversion
- Significant economic loss (> $3billion)

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Eimeria

- A number of species/strains that can infect chickens

- Post mortem intestinal lesions, common species colonise different regions of the intestine

- Coccidiosis is known to be a predisposing factor for necrotic enteritis (NE)

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Diagnosis of coccidiosis

- Infection confirmed by Eimeria oocysts in faeces or intestinal scrapings (Number of oocysts poorly relates to severity)

- Location and appearance of lesions in the host and size of oocysts can determine *Eimeria* species

- Severity of lesions, morbidity, daily mortality, feed intake and growth rate can be used in diagnosis

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Necrotic Enteritis (NE)

Clostridium perfringens

- Gram positive anaerobe
- Ubiquitous in nature (found in healthy chickens also)
- NE associated with α toxin and necrotic enteritis B-like toxin (netB)

Wet litter can be used in diagnosis but very high mortality may occur before PM diagnosis.
Gene Expression Microarrays

• 4 X 44K in-situ synthesised oligonucleotide microarrays

• 60-mer oligonucleotide probes

• Prohealth_Pig_1
  Porcine (V2) Gene Expression Microarray 4x44K (G2519F-026440) per slide

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Strength of signal correlated with the amount of oligo/gDNA interaction.
Infection/sampling protocol

- Ross 308 broilers inoculated on d18, 19 and 20 with $5 \times 10^8$ CFU/ml *C. perfringens* strain 56

- Euthanised day 21

- Intestinal and lung tissues obtained by SOP (identical sites), placed into RNALater for transport to Nottingham

- 18s RNA gene used as reference

- Microarray performed on 6 Ross 308 with clinical signs of NE and 4 healthy controls
Read-out and comparison

Healthy flock control  Necrotic Enteritis

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Making sense of the dots (Genespring)

Volcano plot (+/- fold change)

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Differential gene expression in Chickens with NE versus healthy flock controls

Principal component analysis (PCA) measures multiple variants

Variation within group

Variation between groups

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Heat maps and hierarchical clustering of differentially expressed genes

Table 1 Giles et al

<table>
<thead>
<tr>
<th></th>
<th>Intestine</th>
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<th>Lung</th>
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<tbody>
<tr>
<td>Uninfected</td>
<td></td>
<td>Infected</td>
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<td>Infected</td>
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<table>
<thead>
<tr>
<th>Tissue</th>
<th>&gt; 2 Fold change in gene expression</th>
<th>&gt; 5 Fold change in gene expression</th>
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<tbody>
<tr>
<td></td>
<td>Up-regulated</td>
<td>Down-regulated</td>
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<tr>
<td>Intestine</td>
<td>1241</td>
<td>854</td>
</tr>
<tr>
<td>Lung</td>
<td>2142</td>
<td>823</td>
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Results so far:

- NE causes a global gene response in mucosal tissue
- Greater change in lung but more robust in intestine
- 89 genes significantly down-regulated in both the intestine and lung
- 59 genes significantly up-regulated in both intestine and lung

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Multiple test corrections:

• Assuming P-value set at 0.05 (5% chance of false positive) 5/100

• However when analysing genes we may be dealing with thousands

• MTC corrects the individual P-value for each gene to keep the overall error rate at <= 0.05

Bonferroni analysis → Relatively few genes remain significantly expressed (greatest DE)

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qPCR Biomarker panel

- 11 genes chosen out of those which survived MTC
- Genes chosen had functions which if altered would be consistent with NE
- 10 further infected/control chickens tested by qPCR (fast growing Ross 308 and slow growing Ranger Classic)

\[
\frac{\text{Value 1}}{\text{Value 2}} = \text{Fold change in expression infected versus uninfected}
\]

Ct gene A infected /Ct 18s reference gene (value 1)
Ct gene A uninfected /Ct 18s reference gene (value 2)
11 genes expressed in same direction in microarray and qPCR. 8/11 significant differential expression.
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<table>
<thead>
<tr>
<th>Ross 308 Necrotic Enteritis</th>
<th>Ross 308 Coccidiosis</th>
<th>Ranger Classic Coccidiosis</th>
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<tbody>
<tr>
<td>A Down</td>
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<tr>
<td>B Down</td>
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<td>C Down</td>
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Summary

• Isolated a panel of at least 6 genes which has potential to diagnose NE in Ross 308 and differentiate between Coccidiosis in same bird line

• How does the biomarker panel perform with NE in Ranger Classic?

• How does the panel perform in dual co-infection with C. perfringens and Eimeria?

• Continue to test specificity (different Eimeria spp. Different pathogens

• Continue to test sensitivity of expression

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Thank you for listening

Neil Foster
n.foster@nottingham.ac.uk

School of Veterinary Medicine and Science
University of Nottingham, UK
Tim Giles
Scott Hulme
Paul Barrow
Neil Foster

www.fp7-prohealth.eu

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