Proud to be University of the Year for Graduate Employment

The University of Nottingham

UNITED KINGDOM · CHINA · MALAYSIA

PROHEALTH

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)

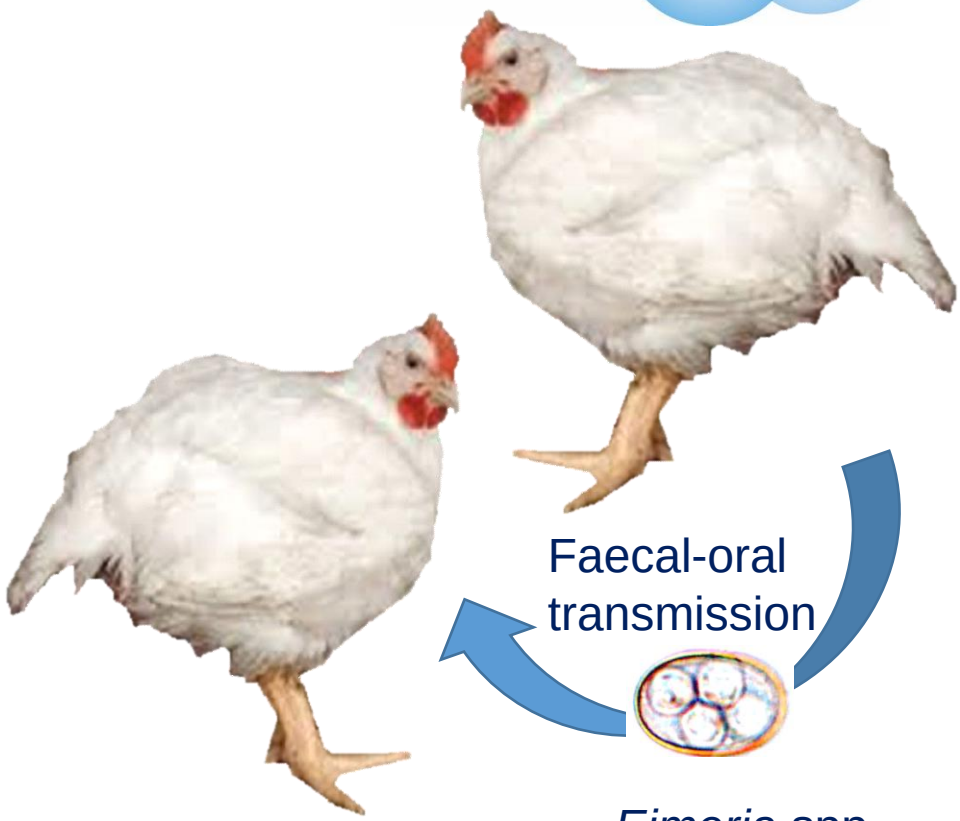
Illuminaseq (deep sequencin of V3/V4 regions of 16S rRNA)

Gene/gene pathways (Biomarkers) which profile production disease

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Coccidiosis in chickens

High intensity farming



Eimeria spp. oocysts

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

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

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)

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

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

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

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.

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

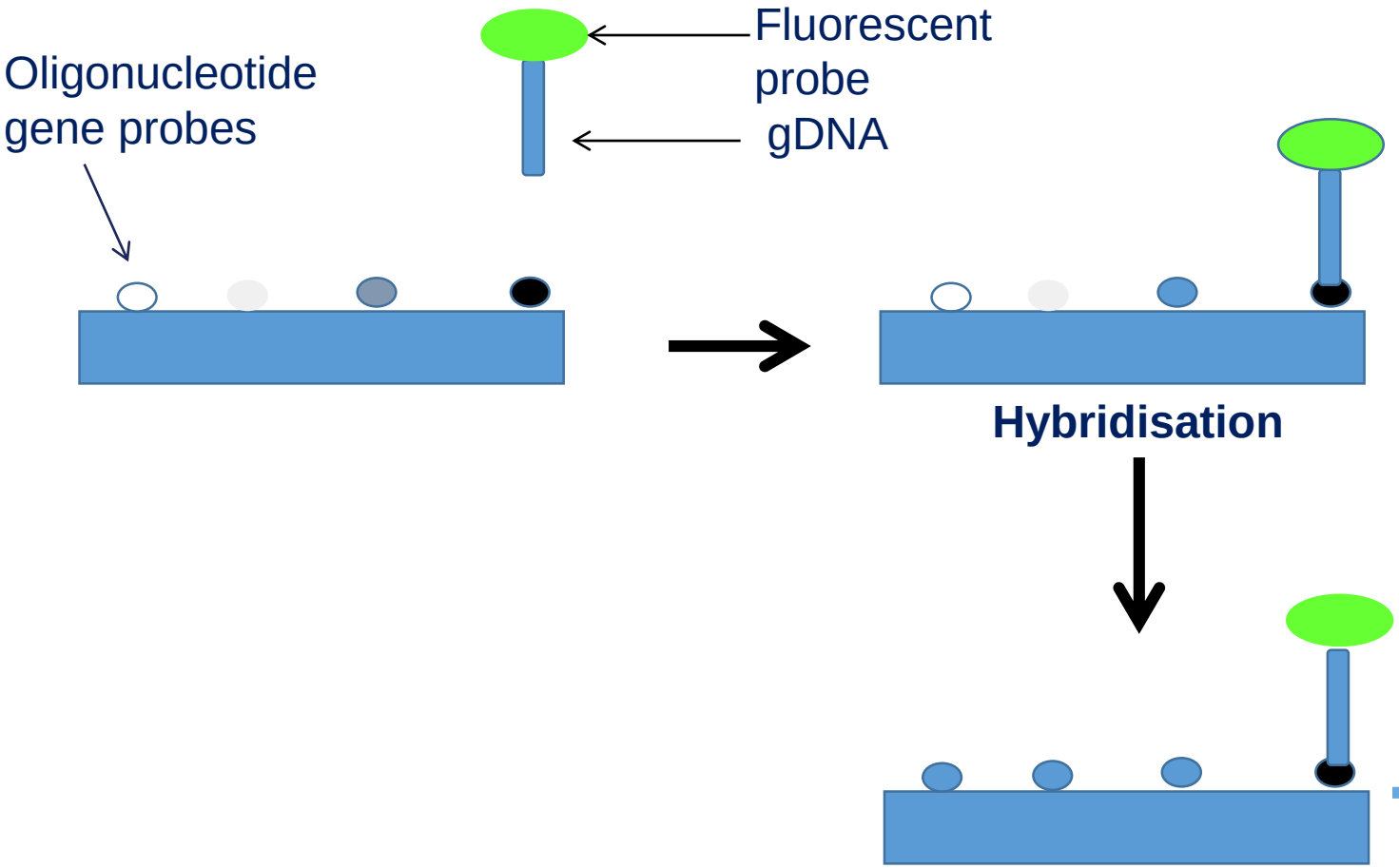
Gene Expression Microarrays

- 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

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Microarray



This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

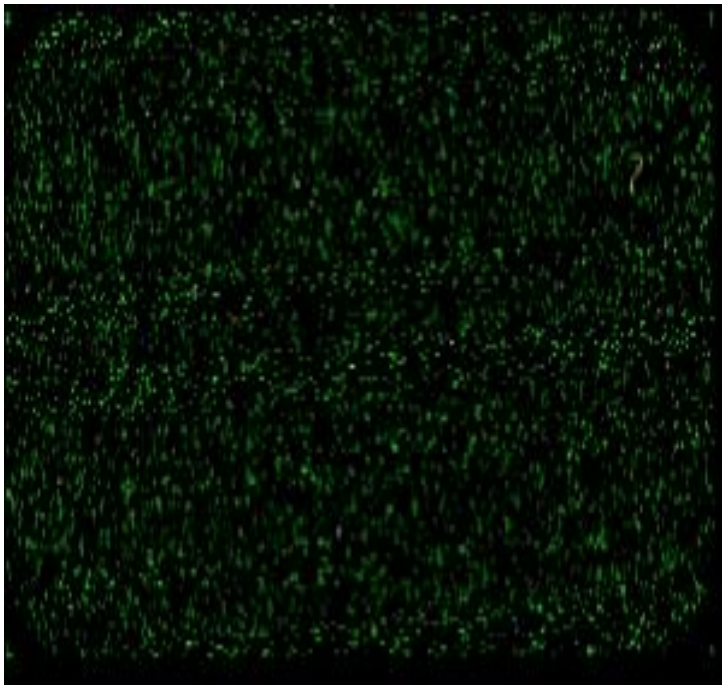
Infection/sampling protocol

- Ross 308 broilers inoculated on d18, 19 and 20 with 5 x 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

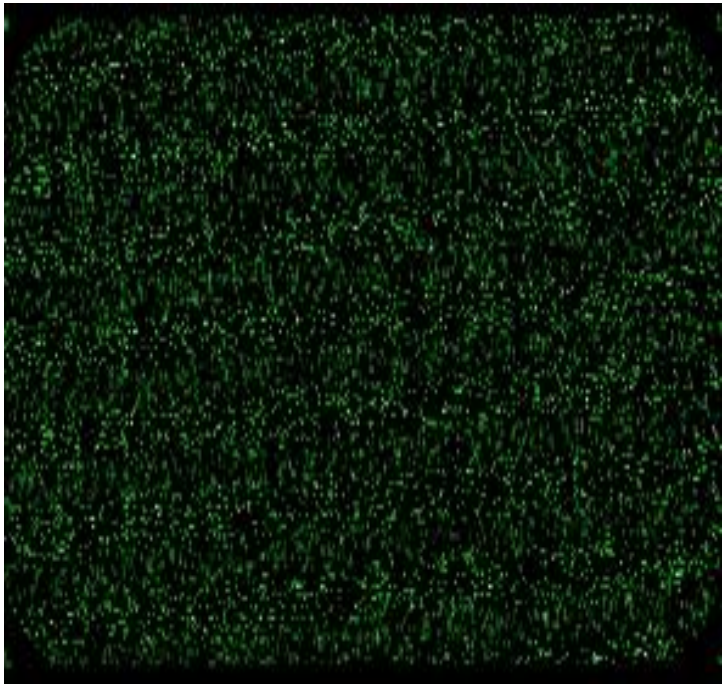
This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Read-out and comparison

Healthy flock control

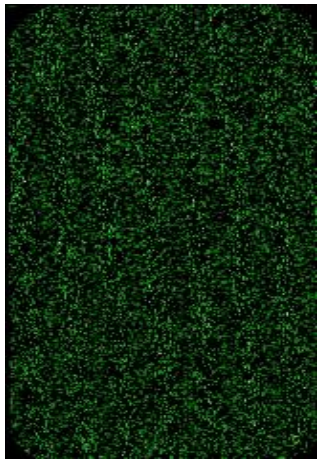


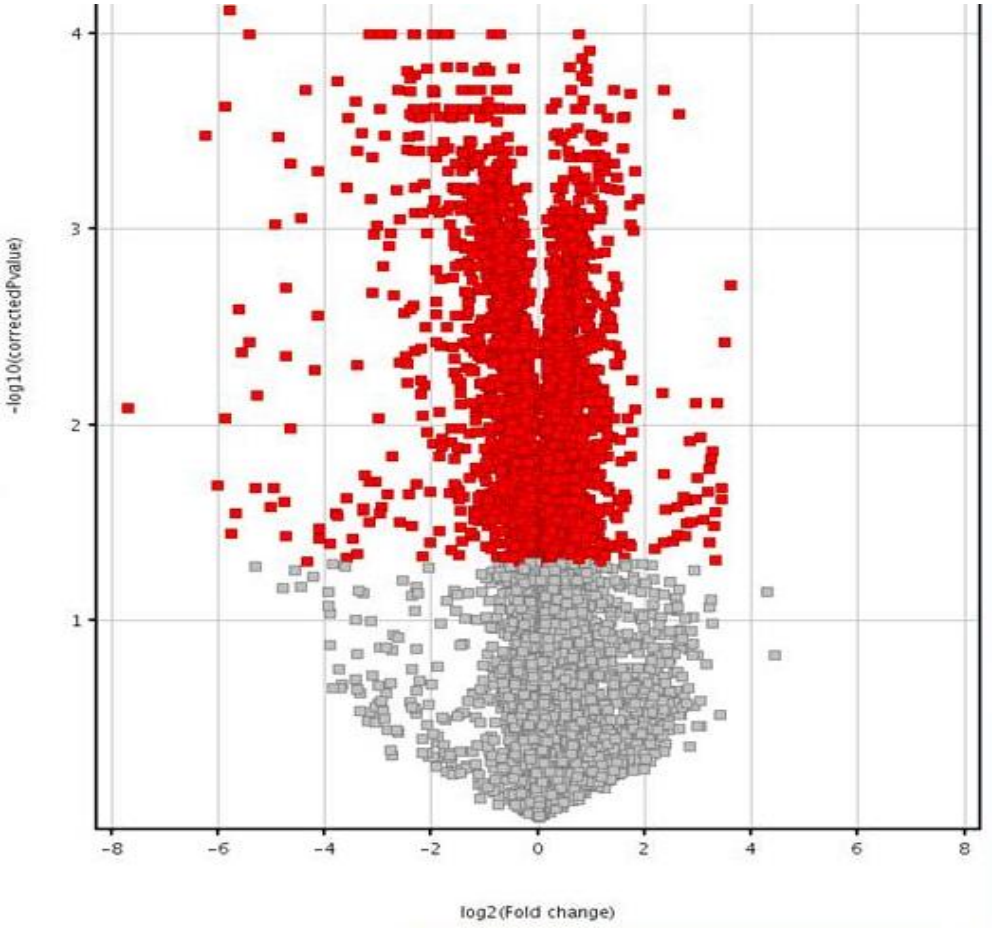
Necrotic Enteritis



This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Making sense of the dots (Genespring)



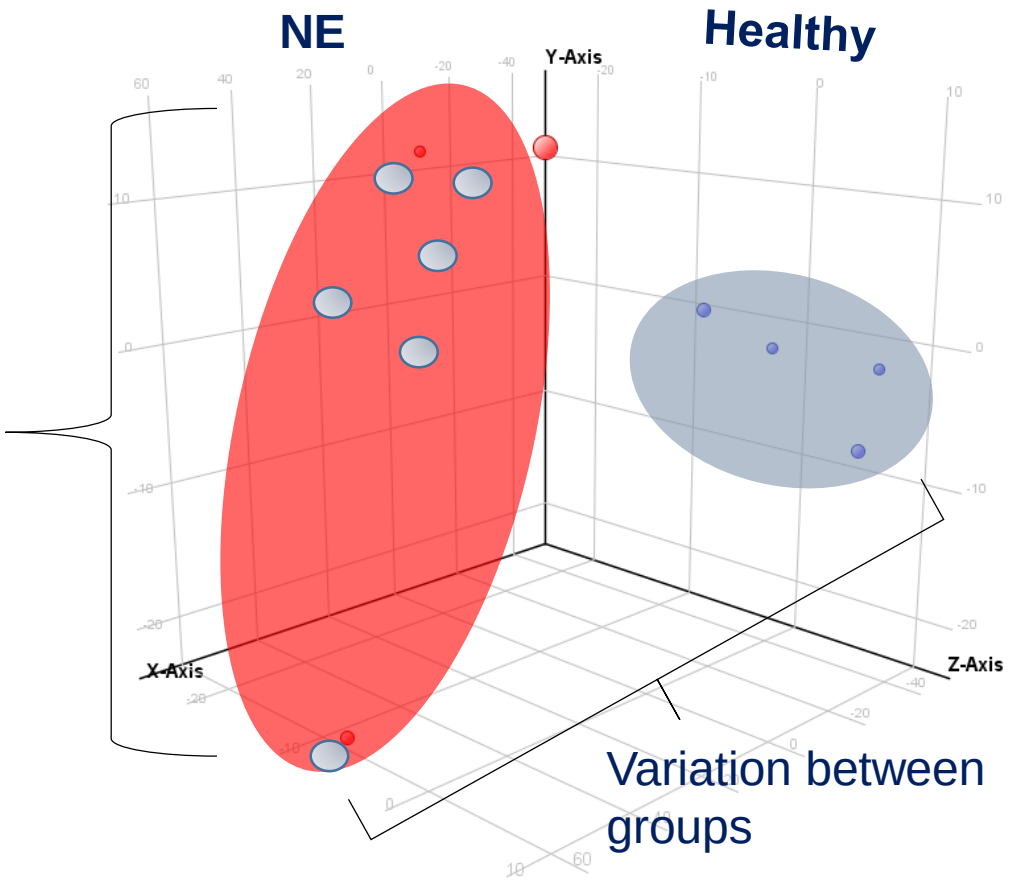


Volcano plot (+/- fold change)

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Differential gene expression in Chickens with NE versus healthy flock controls

Principal component analysis (PCA) measures multiple variants



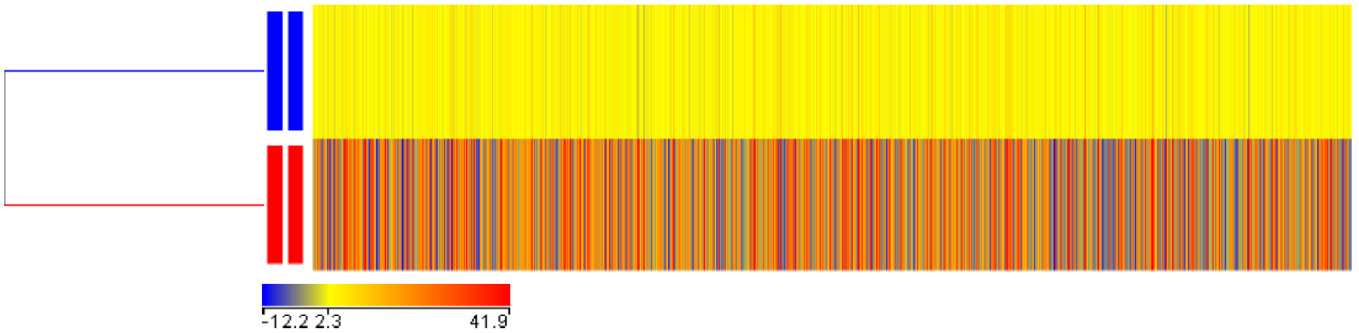
This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Table 1 Giles et al

Heat maps and hierarchical clustering of differentially expressed genes

PROHEALTH

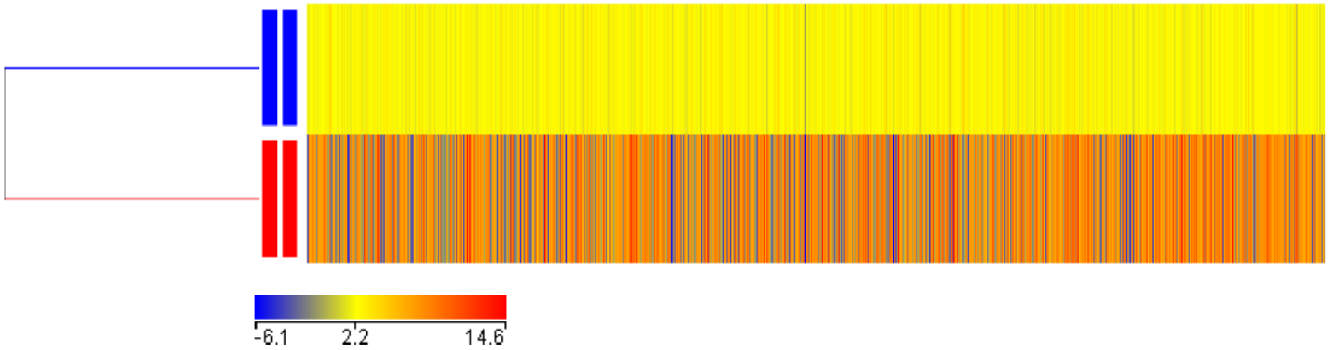
Intestine



Uninfected

Infected

Lung



Uninfected

Infected

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Tissue	> 2 Fold change in gene expression		> 5 Fold change in gene expression	
	Up-regulated	Down-regulated	Up-regulated	Down-regulated
Intestine	1241	854	229	131
Lung	2142	823	51	56

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

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

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

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)

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

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

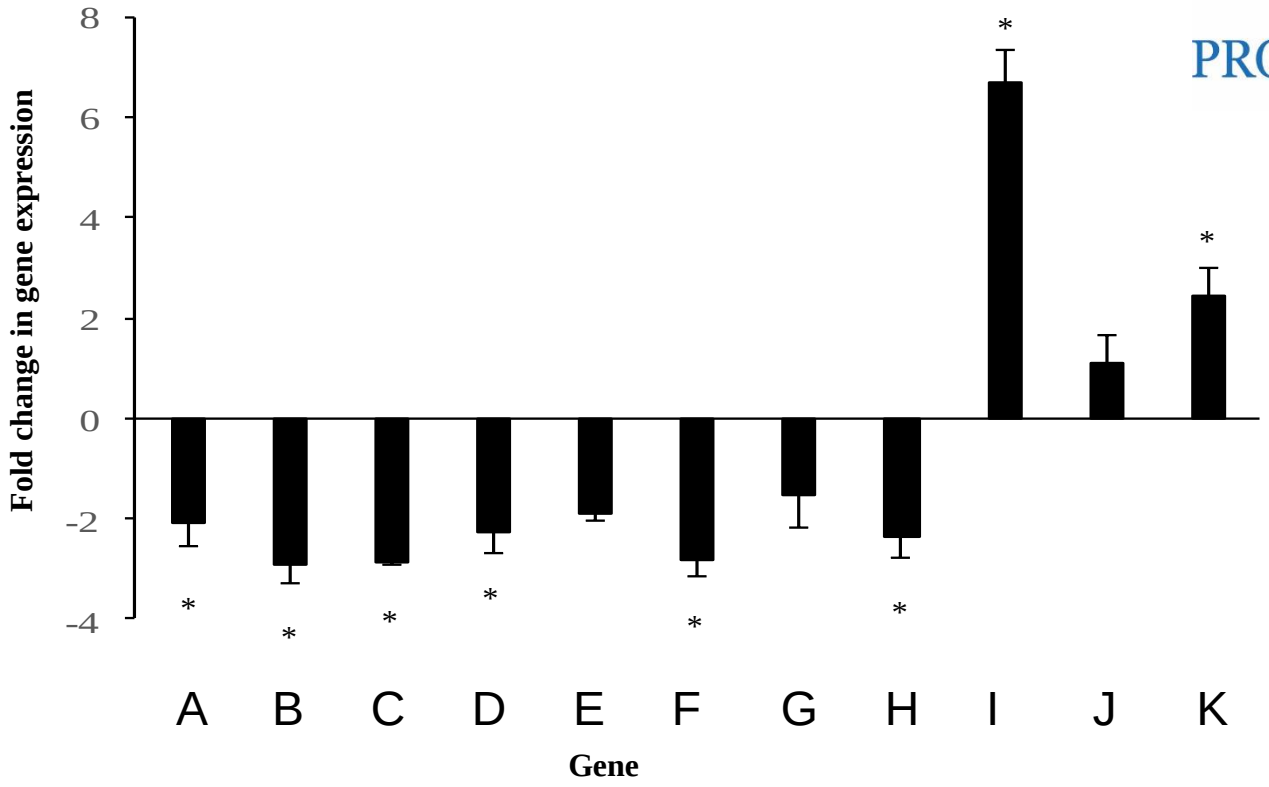
Ct gene A infected /Ct 18s reference gene (value 1)

Ct gene A uninfected /Ct 18s reference gene (value 2)

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

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Figure 2 Giles et al

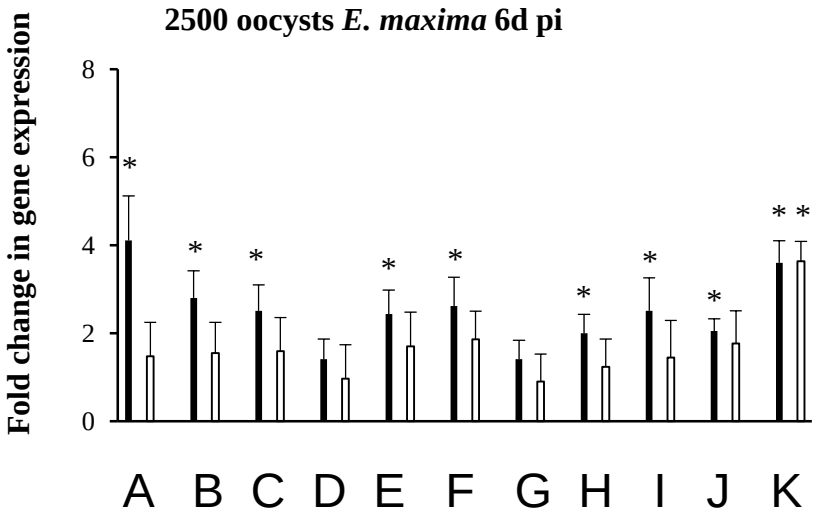


11 genes expressed in same direction in microarray and qPCR. 8/11 significant differential expression

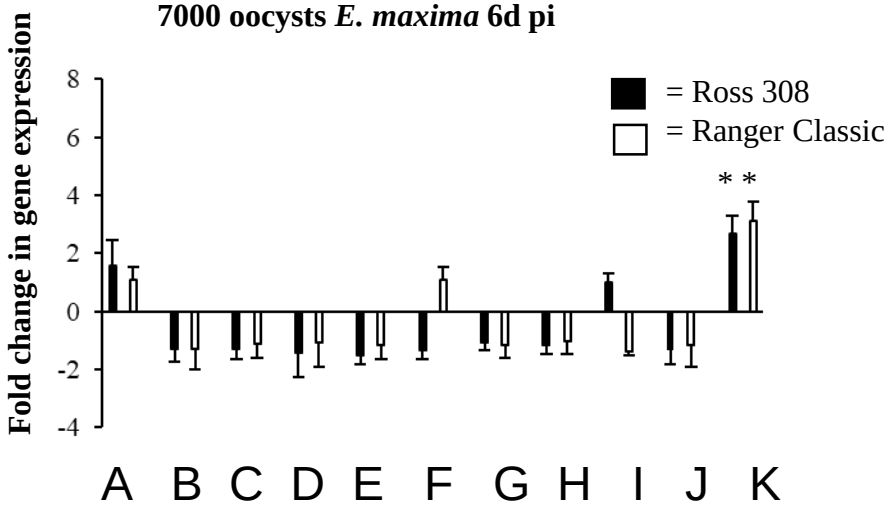
This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Peak Infection

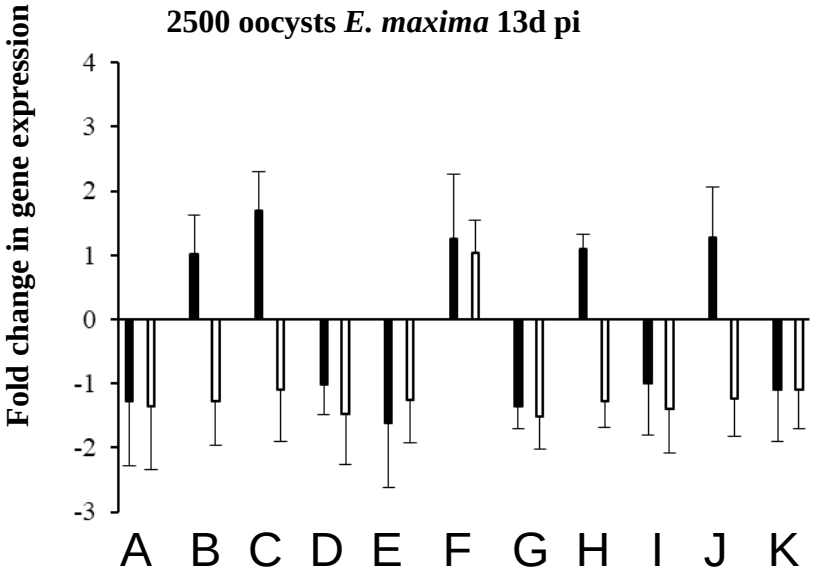
(A)



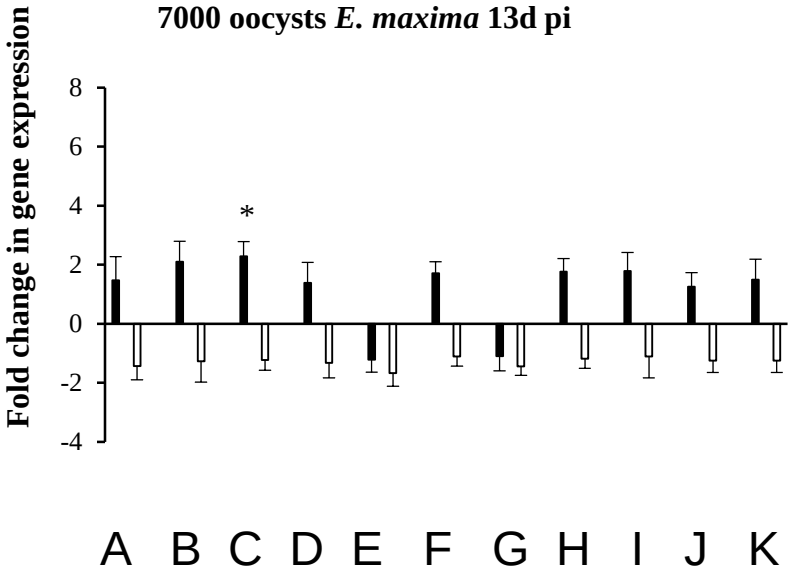
(B)



(C)



(D)



This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Figure 3 Giles et al

PROHEALTH

Ross 308 Necrotic Enteritis	Ross 308 Coccidiosis	Ranger Classic Coccidiosis
A Down	A Up	A -
B Down	B Up	B -
C Down	C Up	C -
D Down	D -	D -
E -	E Up	E -
F Down	F Up	F -
G -	G -	G -
H Down	H Up	H -
I Up	I Up	I -
J -	J Up	J -
K Up	K Up	K Up

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**

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement No. 613574.

Proud to be University of the Year for Graduate Employment

The University of Nottingham

UNITED KINGDOM · CHINA · MALAYSIA

PROHEALTH

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

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 613574.