

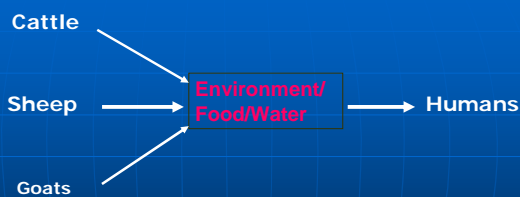
Functional genomics of *Escherichia coli* in cattle and poultry

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Subsets of pathogenic *Escherichia coli*

- ETEC – Diarrhoea in both humans and animals
- EPEC – Infantile diarrhoea in developing world
- EHEC – Significant food-borne related illnesses in humans
- exPEC {
 - UPEC – Urogenital infections in humans
 - APEC – Recalcitrant disease of poultry – colibacillosis
 - NMEC – Neonatal meningitis
 - EAEC – Acute diarrhoeal illness?
 - EIEC – dysentery-like diarrhoea with fever in humans
 - DAEC – diarrhoea in humans

Ruminants are the major reservoir of EHEC



Mechanisms that promote carriage in ruminants and/or survival in the environment are poorly understood.

Reduction in carriage in ruminants is likely to lower incidence of human infections

EHEC pathogenesis in the post-genomic era

- Complete genomes exist for EHEC O157:H7 (2), O26:H11, O103:H2 & O111:H⁻
- *In silico* analysis reveals pathovar-specific and conserved traits
- The role of only a fraction of *E. coli* genes in pathology and protection is known

Genomes on their own cannot tell us which genes are essential for carriage and virulence in the host

Bovine oral challenge model

A bovine oral challenge model and genome-wide mutagenesis strategy are crucial in unravelling genes important for carriage in the host.

Molecular approaches:

1. Signature tagged mutagenesis
2. Targeted mutagenesis

A combination of these strategies revealed the following information in EHEC

1. EHEC O157 colonises using distinct mechanisms from EHEC O26

Tissues from EHEC O157 and EHEC O26 infected calves were analysed by

1. Confocal Microscopy
2. Transmission microscopy

Differences were observed in colonisation pattern on mucosa and type of pedestal despite comparable numbers being shed in faeces

2. The LEE-encoded T3SS plays a vital role in intestinal colonisation of calves

Structural components of T3SS were identified to play key roles in colonisation, among them **EscN**

Secreted components, **EspA** and **Tir** (Translocated Intimin receptor)

3. Other EHEC Type 3 secreted effectors play little or inapparent roles in intestinal colonisation of calves.

NleD = non-LEE encoded secreted protein

TccP = Tir cytoskeleton coupling protein

Map = mitochondria associated protein

4. EHEC secreted serine protease influences colonisation dynamics in calves

1. PssA in EHEC O26:H-

2. EspP in O157:H7

EspP is 99% similar to PssA at amino acid level

5. A conserved fimbrial locus (*loc8*) is required for persistence of EHEC O157 and O26 in calves

Vaccination of calves with EHEC O157 rEspA

Based on faecal shedding data and evidence from literature that convalescent patients contain antibodies against EspA, we developed and tested a recombinant EspA vaccine in calves, but found it not protective

APEC infections in poultry

Bacteria reach the bloodstream from the **respiratory tract/intestinal tract** to establish septicaemia that may lead to acute death or localised inflammation in multiple organs.

Host and bacterial factors influencing/mediating carriage and systemic translocation are poorly understood.

Identification of such factors could yield insights into the development of intervention strategies.

The genome sequence of APEC O1 is available, but this again cannot tell us which genes are important for carriage and systemic translocation in poultry.

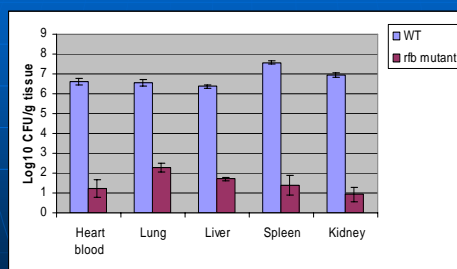
Development of model for induction of APEC infection in turkeys

To determine the route of inoculation, tissue and time point suitable for collecting a reliable output pools of signature-tagged bacteria ($>10^4$ CFU).

Routes of inoculation

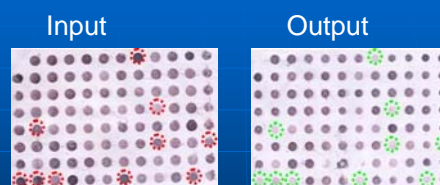
1. Intra-tracheal
2. Intra-airsac

Intra-airsac inoculation with APEC or Δrfb mutant & recovery of bacteria from various tissues



24h post infection

Screening of a pilot pooled STM mutants by intra-airsac route



Turkey liver after 24 h

Attenuated mutants: 13A8, 13D9, 13E2, 13F9, 13H1, 13H2, 13H3, 13H7, 13H11

Ongoing and future work

1. Screening the STM library in turkeys by intra-airsac route and identifying attenuated mutants.

1200 mutants screened in turkeys, 1) lung and airsac and 2) liver recoveries made.

2. APEC chi7122 genome is being sequenced at Sanger Institute.
3. Once library is screened, targeted mutagenesis will be undertaken in selected genes to validate STM findings.

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