PROJECT REVIEW

NAME: Georgina Crayford	
INSTITUTE: University of Liverpool	**FULL TIME** **Final Year**
TITLE: The infection biology of pig-associated *Salmonella*	

AIMS & OBJECTIVES:

1. Determine the phenotype of monophasic and biphasic strains of *Salmonella* Typhimurium phage type DT193
2. Characterise the interaction between DT193 isolates and a porcine intestinal epithelial cell line (IPEC-1) including adhesion and invasion
3. Determine the physiological response of host IPEC-1 cells to DT193 infection

Overall objective is to add to the current limited knowledge of the pathogenicity of monophasic *S*. Typhimurium DT193 isolates and determine the reasons behind their rapid and worldwide spread in recent years.

KEY MILESTONES:	**TARGET DATE:**	**ACHIEVED DATE:**
Complete literature review	October 2011	September 2012
Characterise phenotype of DT193 isolates	December 2012	October 2013
Fluorescence staining of host cell-bacteria interaction	February 2013	February 2013
Quantitative real-time PCR to determine IPEC-1 gene expression	April 2013	June 2013
Flow cytometry to analyse host cell death	September 2013	September 2013
Invasion assays into other cell lines (human colonic and murine macrophage)	November 2013	November 2013
Murine intestinal explant infection and analysis by confocal microscopy	December 2013	Ongoing

PROJECT REVIEW AND COMMENTARY:

Little is currently known about monophasic strains of *Salmonella* and the fitness benefit conferred by loss of second phase flagellin expression. This project has gone some way to characterising their behaviour *in vitro*. Analysis of invasion of monophasic isolates into porcine intestinal epithelial cells, at the single cell level, has shown that DT193 isolates are moderately invasive, although there difference between the invasiveness of monophasic and biphasic isolates is not statistically significant. We have also performed assays to determine their motility, secretion of SPI-1 effector proteins, expression of virulence genes and ability to form biofilms. In all of these assays monophasic DT193 isolates showed no differences to biphasic DT193 isolates. Using real-time qPCR we measured the expression of the host cell receptor TLR-5 and the chemokine IL-8, which is responsible for the influx of neutrophils during infection. Once again there were no significant differences between monophasic and biphasic isolates in the level of up-regulation of these genes, except for one of the monophasic isolates which induced significantly higher up-regulation of both ($p>0.001$). It is possible that if similarly high levels of IL-8 were produced *in vivo* following infection with this particular monophasic isolate the host animal would experience severe inflammation of the gut leading to enteritis.

Overall, we have been unable to identify the reason for the success of monophasic isolates. However, the focus of this project has been on early infection into intestinal epithelial cells. It could be that monophasic isolates utilise pathogenic mechanisms that only come into play once breach of the intestinal epithelium has taken place. It should also be borne in mind that these results were obtained using a cell model, which is not wholly representative of the complex *in vivo* situation. What seems to be clear, however, is that monophasic isolates are somehow capable of maintaining pathogenicity despite their inability to phase variate due to the loss of second phase flagellin expression.

POTENTIAL BENEFIT TO INDUSTRY:

Monophasic isolates of *S.* Typhimurium are being isolated with increasing frequency from both humans and pigs. They are now the 2nd most common serotype isolated from pigs and the 3rd most common isolated from humans in Europe. There is increasing evidence from the veterinary profession that infection with monophasic *Salmonella* is causing clinical disease in pigs, unlike normal *Salmonella* infection in pigs which is usually asymptomatic. This is cause for concern to pig farmers whose herd health and productivity could be affected. There is also concern for public health given the zoonotic potential of these strains and their resistance to multiple antimicrobials.

Although this project has been unable to identify the reason for the epidemiological success of monophasic DT193 isolates, we have shown that their loss of *fljB* expression does not attenuate them during early infection and that they behave very similarly to normal biphasic isolates of the same phage type. Further work is necessary to learn more about these isolates in order to develop effective control strategies.

SUPERVISOR: Paul Wigley

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Notes from Seminar: