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SRF Vacation Scholarship Report – Victoria Carliell 2013

Background:
The zoonotic pathogen *Listeria monocytogenes* is a public health concern in the UK and is significant to the beef and dairy industry because it causes several diseases in cattle including abortion. In 2010, *L monocytogenes* was the 7th most common infectious cause of bovine abortion identified by the AHVLA (Weston et al., 2012); the economic significance of which is demonstrated by Cabell (2007) who estimates that each abortion costs a UK dairy herd £630.

L monocytogenes causes abortion in late gestation by infecting the placenta. Bacterial virulence factors enable this intracellular invasion; in particular Internalin A (InlA) and Internalin B (InlB) facilitate bacterial adherence and internalisation due to reorganisation of the local cytoskeleton. InlB assists with entry into a wide range of cell types, whereas InlA interacts specifically with intestinal cells and the caruncular epithelial cells of the bovine placenta. The role of virulence factors in enabling placental invasion and therefore abortion is species specific. Placental infection by *L monocytogenes* requires both InlA- and InlB-invasion pathways in humans, however in guinea pigs only InlA is required, and in mice only InlB is required (Pizarro-Cerdá et al., 2012). The role of InlA and InlB in bovine abortion has not yet been determined.

Aims:
This project aimed first to determine the ability of *L monocytogenes* isolates to invade and survive within bovine caruncular epithelial cell lines in comparison to human CaCo2 cells (which are routinely used as an in vitro model to test the virulence factors of intracellular pathogenic bacteria such as *Listeria monocytogenes)*. If successful, this would enable the project's second aim of investigating the bovine-specific role of InlA and InlB.

Methods:
Cultures of bovine caruncular cells (BCEC and BCECT) and CaCo2 cells were grown in Dulbecco's Modified Eagle's Medium nutrient mixture Ham's F12 with 10% Fetal Calf Serum, 2mM L-Glutamine and 1% Penicillin/Streptomycin. Five strains of *L monocytogenes* were used for the infection experiments: LM4 and LM6 were isolated from milk, LM23 from a clinical source, LM10403s is a reference strain commonly used in research laboratories, and LM3007 is the reference strain with a green fluorescent protein added. In a series of experiments, wells of confluent BCEC, BCECT and CaCo2 cells were incubated with *L monocytogenes* inoculum cultures for two hours (to determine infection) and for 24 hours (to determine viability). The experiments utilised a range of multiplicities of infection (MOI) from 10-500, and bacterial strains grown at two different temperatures (25 or 37 degrees). Control experiments were undertaken to ensure that the *L monocytogenes* strains survived within the cell culture medium and also within the cell lysis solution.

PCR primers for InlA and InlB were tested using *Listeria monocytogenes* DNA to ensure that they detected their targets correctly. RNA was isolated from *L monocytogenes* strains that had been incubated overnight or had been in contact with BCEC or CaCo2 cells. The primers would then have been used to determine the level of expression of InlA and InlB genes within the various *L monocytogenes* isolates via quantitative PCR; however it was not possible to undertake this step within the time constraints of the project.

The distribution of *L monocytogenes* invasion was investigated by growing cultures of BCEC, BCECT and CaCo2 cells on coverslips, incubating with LM3007 (which contains green fluorescent protein), fixing, staining and observing under a fluorescent microscope. This was undertaken in collaboration with another student project.

Results:
Colony forming units (CFU) of all five strains were recovered from BCECT and BCEC cells after both two hours and 24 hours of incubation, therefore it can be concluded that L *monocytogenes* isolates are able to invade and survive within bovine caruncular epithelial cell lines. However, it was found that BCECT cells are less readily infected than human CaCo2 cells (figure 1). It was also noted that LM3007 appeared to infect cells less readily than LM10403s, suggesting that the presence of the green fluorescent protein may have an effect upon the ability of *L monocytogenes* to invade cells.

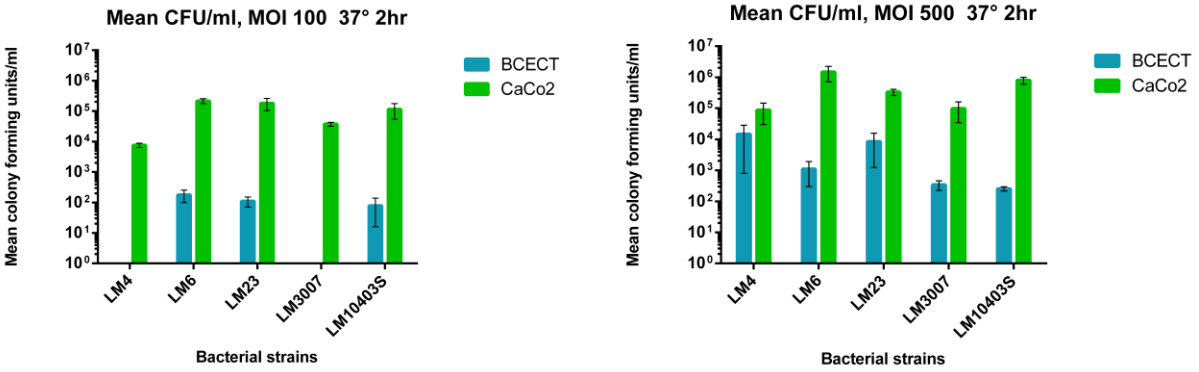

Figure 1 – Comparison between mean CFU/ml of the five *L monocytogenes* strains recovered from bovine caruncular cells and human CaCo2 cells, with standard error of the mean (SEM) bars.

A difference was observed between bacterial inoculum grown overnight at different temperatures, with inoculum grown at 25° appearing to infect CaCo2 cells more successfully than those grown at 37° (figure 2).

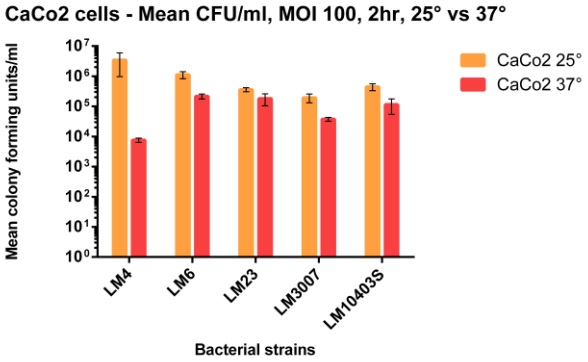

Figure 2 –Comparison between mean CFU/ml recovered from CaCo2 cells infected at MOI 100 with the five strains of *L monocytogenes* grown overnight at 25° or 37°, with SEM bars.

Conclusions:
The finding that *L monocytogenes* isolates are able to invade and survive within bovine caruncular epithelial cell lines, albeit less readily than human CaCo2 cells, enables BCEC and BCECT cells to be used in future work to investigate the bovine-specific role of InlA and InlB. This was subsequently investigated as the basis of an undergraduate research project at Nottingham University School of Veterinary Medicine and Science.

The results of the project also support the need for future research to be undertaken to investigate whether the green fluorescent protein has a detrimental effect on the ability of *L monocytogenes* strains to invade cells, and to investigate the optimum temperature for *L monocytogenes* strains to invade and survive within host cells.

References

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