Factors influencing MLVA loci stability in *Salmonella enterica* serovar Typhimurium

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Contamination of poultry products by *Salmonella enterica* species and in particular *S. enterica* serovar Typhimurium (*S.* Typhimurium) can lead to public health problems. Adverse, uninformed and sensational media coverage of food poisoning incidents has heightened consumer awareness of food safety. These reports can often unnecessarily affect retail consumption of implicated products. Tracing of *S.* Typhimurium involved in such outbreaks is paramount in order to protect the public and prevent further episodes. It is therefore of vital importance that the methods involved in tracing such outbreaks are rapid and accurate.

Classical phage typing has been the method of choice until recently for sub-typing S. Typhimurium isolates. Phage typing essential examines the ability of a panel of typing phages to plaque on the isolate under test. It is a simple and useful method, but suffers from a number of major deficiencies; often a particular phage type will predominate in a region, and if an outbreak is caused by that particular predominant phage-type it is impossible to distinguish it from other non involved isolates, secondly isolates can be non-typable (i.e. they do not plaque with any members of the phage typing panel) and thirdly it is highly dependent on the experience of the operator to interpret lysis patterns and assign a phage type.

MLVA (Multi locus VNTR (Variable Number of Tandem Repeat) Analysis – a widely accepted method for typing bacteria has received support as a potential replacement methodology for *S.* Typhimurium typing over phage typing. MLVA of S. Typhimurium is based on the number of tandem repeats found within five defined loci (STTR-3, STTR-5, STTR-6, STTR-9 and STTR-10) (Lindstedt et al., 2003; Lindstedt et al., 2004; Ross et al., 2009). Small fragments of repeat DNA are found in these loci, the number of repeats may vary between isolates, based on PCR and genotyping data a "profile" can be established for strains of interest. For an example of the use of both phage-typing and MLVA in an outbreak scenario in South Australia see Ross et al. (2011).

One of the most important aspects in any typing system is the stability of the parameters used in the typing process. Like all typing methodologies MLVA suffers from some deficiencies. It has been reported in the literature that some loci are "unstable" (Hopkins et al., 2007; Torpdahl et al., 2007). This means that the number of tandem repeats within a locus can change either in number or sequence. This has been observed in our own laboratory in a previous, but limited *in vivo* study using chickens. The MLVA loci tend to be perfectly stable in the laboratory because it is postulated that as the laboratory environment is relatively constant, there is no selective pressure that results in locus instability. However, in the changing environment of the chicken gut there is selective pressure for changes in some loci. It should also be noted we observed changes in phage type in recovered isolates from the environment of the chicken gut along with the changes in MLVA profiles. The dynamics of these changes are unclear and the main aim of the Honours project is to attempt to explain these changes.

The methodology for determining MLVA loci stability will involve conventional bacterial culture on selective growth media and basic molecular techniques such as PCR and DNA genotyping. Pre-typed *S.* Typhimurium isolates (made resistant to nalidixic acid by spontaneous mutation) from the poultry industry and human sources will be used. These bacteria will be fed to chickens and their persistence within the chicken gut will be monitored by collecting faeces over the duration of the experiment. MLVA will be undertaken on isolates recovered from faeces. The influence of factors such as bacteriophages and antibiotics on this process will also be assessed.

Background reading:

Hopkins K.L., et al. (2007) Stability of Multiple-locus variable-number tandem repeats in *Salmonella enterica* serovar Typhimurium. Journal of Clinical Microbiology. 45: 3058-3061.

Lindstedt B-A., et al. (2003) DNA fingerprinting of *Salmonella enterica* subsp. *enterica* serovar Typhimurium with emphasis on phage type DT104 based on variable number of tandem repeat loci. Journal of Clinical Microbiology. 41: 1469-1479.

Lindstedt B-A., et al. (2004) Multiple-locus variable-number tandem-repeat analysis of *Salmonella enterica* subsp. *enterica* serovar Typhimurium using PCR multiplexing and multicolour capillary electrophoresis. Journal of Microbiological Methods. 59: 163-172.

Ross, IL.,. Parkinson IH and Heuzenroeder MW. (2009) The use of MAPLT and MLVA analyses of phenotypically closely related isolates of *Salmonella enterica* serovar Typhimurium and comparison with PFGE. International Journal of Medical Microbiology 299: 37-41

Ross IL, Davos DE, Mwanri L, Raupach J, Heuzenroeder MW. (2011) MLVA and phage typing as complementary tools in the epidemiological investigation of *Salmonella enterica* serovar Typhimurium clusters. Current Microbiology 62:1034-1038.

Torpdahl M., et al. (2007) Tandem repeat analysis for surveillance of human *Salmonella* Typhimurium infections. Emerging Infectious Diseases. 13: 388-395.