In silico analysis of Vi-positive *Salmonella* Dublin genomes

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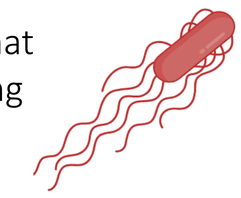
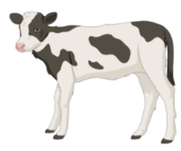
Salmonella enterica has >2600 serovars that cause diverse clinical symptoms depending on host and bacterial factors.



S. Dublin is a **host-restricted** serovar strongly adapted to cattle, causing **enteritis, systemic disease** and **abortions**.



S. Typhi, an **obligate human pathogen** with no other known reservoirs, causes **typhoid fever**.

The **Vi** capsular polysaccharide is **an important virulence factor** for ***S.* Typhi** and is occasionally found in ***S.* Dublin** strains.

The role of the Vi antigen ***in vivo*** has historically been researched using ***S.* Typhimurium** in **murine** models.

The presence of the Vi antigen in *S.* Dublin provides a **natural host-microbe model** to study the function of this virulence factor.

Aim

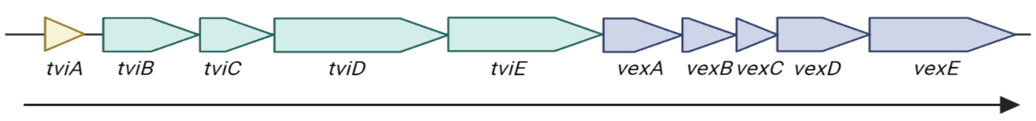
The research aim was to compare genomes of **Vi-positive *S.* Dublin strains** to study genome features

Strains

Strain	Isolation Source	Year
931	Human sputum	1964
934	Bovine placenta	1972
937	Bovine	1988

1. The *viaB* locus

Vi antigen synthesis and transport requires the ***viaB*** locus



<u>The Vi antigen:</u>

- Confers resistance to **complement-mediated killing**
- Confers resistance to **phagocytosis**
- Masks **lipopolysaccharide** from Toll-like receptor 4
- Prevents **neutrophil chemotaxis**

2. Genome structures in *Salmonella*

Salmonella shows **variation** in genome structure. Homologous recombination can occur around **long-repeat sequences of ribosomal operons** (~5kb in length), causing large genome fragments to **change orientation and/or position** in the genome. This results in **unique genome structures** (GSs).[2]

Variation on this scale can be identified using **long-read sequencing.** Reads can be produced which span the long-repeat sequences of ribosomal operons to **assemble complete genomes** which can then be used to identify GSs.

3. Methodology for long-read sequencing

The work flow to determine GSs from high molecular weight (HMW) DNA with long-read sequencing (Oxford Nanopore Technologies, ONT)

HMW DNA extraction
FireMonkey (Revolugen)

→ **Library Preparation**
96-plex, native ligation

→ 

→ **PromethION**

→ **Fitlong**
Data trim

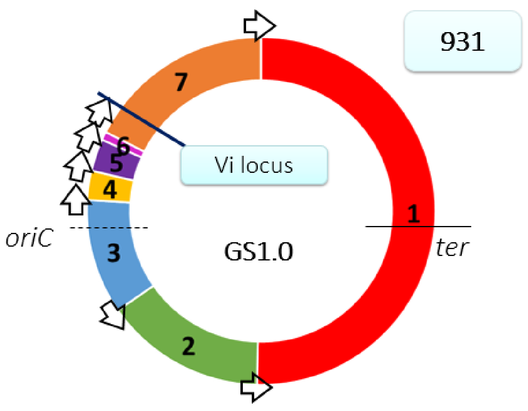
→ **Flye**
Long read assembly

→ **1x Racon, 1x Medaka**
Polishing the long read assembly

→ **Pilon**
Hybrid assembly

→ **Socru/Prokka/ MLST**
GS and MLST determination

4. Genome structures of Vi-positive *S.* Dublin strains



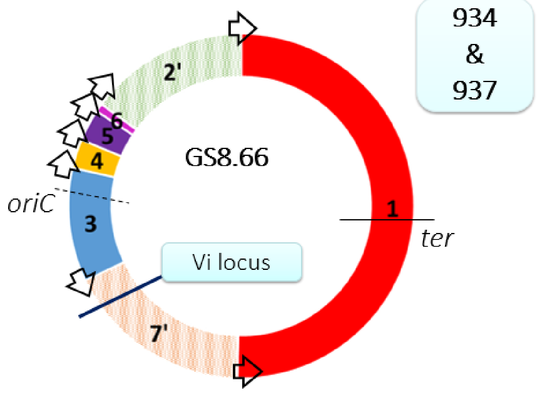


Figure 1. *In Salmonella, 7 fragments, numbered 1-7 relative to the conserved GS of S. enterica GS1.0, are separated by ribosomal operons (arrows). Inverted fragment orientations are denoted prime (') with striped colours. The location of the Vi locus is labelled. oriC denotes the origin and ter denotes the terminus of replication.*

Figure 2. *- Slide agglutination confirming the Vi+ phenotype of the S. Dublin strains 931 and 937 with E. coli XL1 as a negative control.*

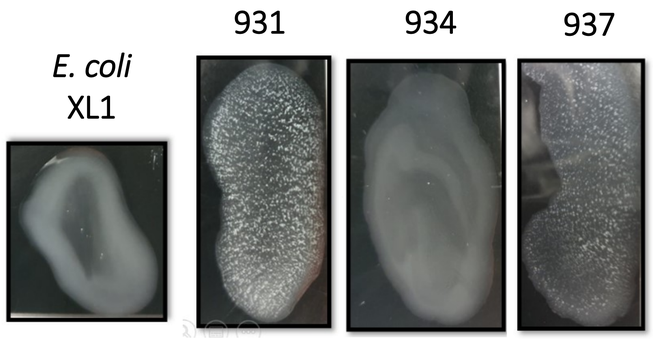
931 shows GS1.0, which is the main genome structure seen in *S.* Dublin, while **934 and 937** both show **GS8.66**.

One other published *S.* Dublin strain displays GS8.66, which is also Vi-positive.[3]

In *S.* Dublin **96.64%** of strains are **ST 10**.

931 is ST 1816 while **934 and 937** are both **ST 73**

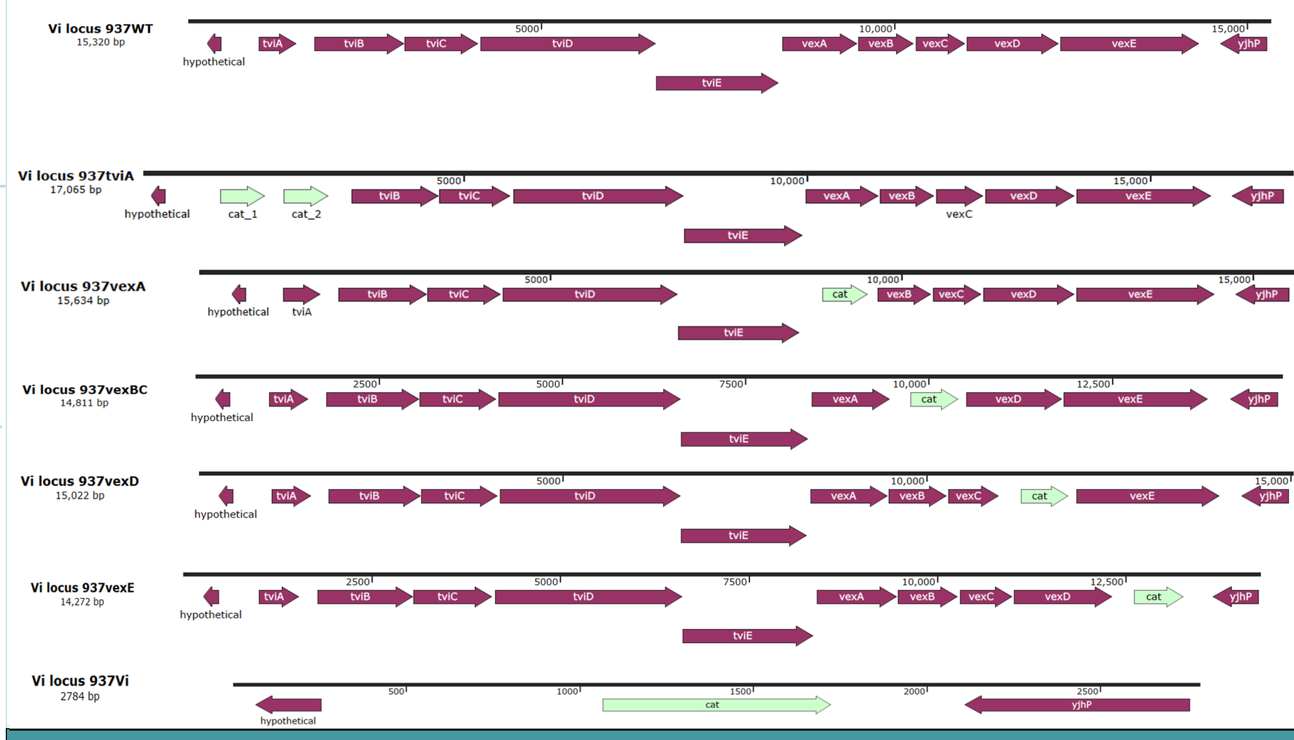
The Vi locus as been **inserted in the same location** of the genome, in fragment 7, in all 3 strains.



931 and 937 show a Vi+ phenotype by slide agglutination, while **934 appears to have lost expression**.

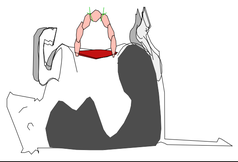
5. Construction of defined Vi mutants

S. Dublin 937 mutants lacking the *viaB* locus, in full or in part, were created using λ-red mutagenesis.[4]



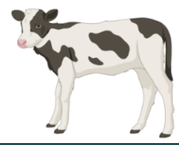
6. Next steps

- Place the Vi-positive *S.* Dublin strains into an evolutionary context
- Investigate the short-read data for the Vi-positive *S.* Dublin strains
- Phenotypic analysis in vitro of the Vi-positive *S.* Dublin strains and the mutants.



Long term plan:

Phenotypic analysis of the strains and Vi mutants in cattle



References

1. Parkhill *et al.* 2001. Nature 2001 413:6858 413, 848–852. **2.** Page, A. J., Ainsworth, E. V. and Langridge, G. C. 2020. *Microb Genom,* 6, 1-6. **3.** Waters, E. V. (unpublished). **4.** Datsenko, K. A, and Wanner B. L., 2000. PNAS, 97 (12), 6640-6645. Figures were created with BioRender.com

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