Title of PhD project / theme	**Functional Characterisation of the *Campylobacter jejuni* Type VI Secretion System**
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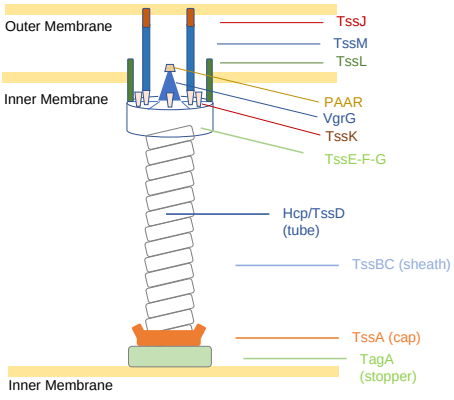
Brief description of project / theme	***Campylobacteriosis and routes of infection.*** *Campylobacter* is the leading cause of bacterial gastroenteritis worldwide and is abundant within the chicken gut. Consumption and handling of meat products, particularly poultry, is the primary foodborne route of transmission to humans. *Campylobacter jejuni* is the most prevalent species in poultry and responsible for over 80% of human infections. Although considered a commensal of the chicken gut, *Campylobacter* has recently been demonstrated to be pathogenic in chickens, depending on the genetics of both the host and bacterial strain. *Campylobacter* infection can cause bloody diarrhoea, fever and abdominal pains in humans. In low-resource regions, *Campylobacter* infections are common in young children and correlate with stunted growth and life-long physical and cognitive deficiencies. In high-resource regions, an estimated 1 in every 100 individuals develop a *Campylobacter*-related illness each year. ***Campylobacter and the Type VI Secretion System.*** The limited space of the gut and intestinal environments are sites of fierce competition for essential nutrients such as metal ions. Enteric pathogens have developed strategies to co-exist or compete with other bacteria to survive the host environment. The Type VI Secretion System (T6SS), present in ~25% of gram-negative bacteria, is a contractile secretion nanomachine that injects toxins into bacterial or eukaryotic cells (Fig. 1). Injected effectors can typically modify host cell functions, where upon recognition of host cells can swtich the type of secreted protein (1).  **Figure 1. Hypothetical structure of the *C. jejuni* T6SSs.**

In recent years, our group has identified T6SS-positive *Campylobacter* strains isolated from chickens (2). Though widely studied in other enteric pathogens, the T6SS within *C. jejuni* has been poorly studied to date. Our group has shown that the T6SS is associated with the oxidative stress response, where mutation of TssD (tube structure) reduced *C. jejuni* ability to survive oxidative stress (3). We demonstrated that presence of the T6SS increased *C. jejuni* interactions with host cells and promoted the invasion of chicken primary intestinal cells (Fig. 2A, B), thereby supporting the colonisation of chickens (Fig. 2C). Recently, we have performed a T6SS secretome analysis of *C. jejuni* (Fig. 3), using Liquid Chromatography Mass Spectrometry (LC-MS) and identified novel putative effectors potentially deleterious to other bacteria and host intestinal cells. Results from this preliminary study indicate that there are a number of proteins that are putative effectors involved in *C. jejuni* virulence. ***Importantly, we currently do not understand how these C. jejuni T6SS effectors function.***

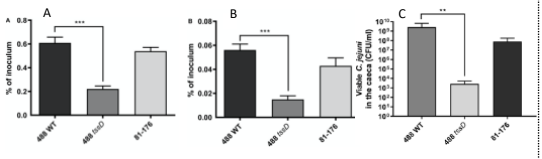


Figure 2. Interaction (A) with and invasion (B) of primary chicken intestinal cells with *C. jejuni* 488 wild-type strain, 488 T6SS *tssD* mutant or *C. jejuni* 81–176 wild-type strain. Primary chicken intestinal cells were infected with *C. jejuni* strains at a MOI of 1,000:1. **(C) Infection of broiler chickens** with *C. jejuni* 488 wild-type strain, 488 T6SS tssD mutant or *C. jejuni* 81–176 wild-type strain. **Method as described in** (3).

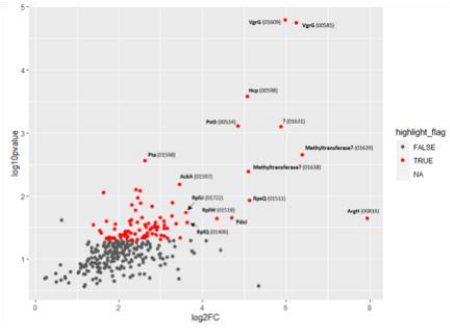


Figure 3. Comparative secretome analysis of a *C. jejuni* wild-type (T6SS-positive) with a *C. jejuni tssBC* (a T6SS secretion mutant). Points in red have a p-value < 0.05 and fold change > 2.

T6SS and microbial ecology: In the host gut, the T6SS can mediate bacterial competition, possible damage to host cells, and also interfere with nutrient availability. The potent T6SS antibacterial activities of invading pathogens can lead to dysbiosis, disrupting the host immune response and ultimately lead to host disease (4). In contrast, commensal members of the microbiota bearing a T6SS form a symbiotic relationship with the host, based on symbiotic metabolic cooperation. For *Campylobacter*, farming intensification may have led to the expansion of selective clonal lineages that give a competitive advantage when colonising chickens or persisting in poultry (5). ***Importantly, we currently do not understand (i) the effects of T6SS effectors from C. jejuni on the chicken gut microbiome, (ii) the consequences of these strains' prevalence on overall poultry health, and (iii) the risk they pose to animal and public health.***

Depending on the direction the doctoral student wishes to explore, the aims of the project can be one or more of the following using molecular, phenotypic and/or omics-based methods: -

1. **Investigate the *C. jejuni* T6SS role in bacterial antagonism.** The role of the *C. jejuni* T6SS in relation to damage to other bacteria is currently unknown. The conditions and cellular mechanisms of attack or defence of *C. jejuni* harbouring a T6SS can be further explored with competition-based assays.
2. **Investigate the *C. jejuni* T6SS role in interaction with host cells.** The role of the *C. jejuni* T6SS in relation to damage to eukaryotic host cells is currently unknown. How *C. jejuni* subverts host cell compartments for its own benefit is unknown?
3. **Investigate the *C. jejuni* T6SS in relation to survival.** Although the mechanisms are unknown, we have identified a number of secreted putative T6SS effectors associated with iron and zinc uptake which may have a potential role related to survival.
4. **Investigation of the *C. jejuni* T6SS effectors.** We have identified a number of novel effectors that are 'low hanging fruit', ready to be characterised using genotypic and phenotypic and omics-based technologies.
5. **Investigate the impact of the *C. jejuni* T6SS on the chicken gut microbiome.** The impact of the *C. jejuni* T6SS on chicken gut microbiome population structure and chicken health is unknown. Here, we can explore this using chicken infection model experiments and omics-based approaches such as metagenomics and bioinformatics.

This study will build on the recent *C. jejuni* T6SS research at the LSHTM and Nagasaki University.

References (own in bold):

1. **Tandhavanant** et al., 2018, ***mBIO***, . mBio 9:e01366-18.
2. **Ugarte-Ruiz** et al., 2015, ***Zoon Pub Health***, 62(7):497:500.
3. **Liaw** et al., 2019, ***Front Microbiol***, 10:2864.
4. Wood et al., 2019, ***Front Cell Infect Microbiol***, 10:587948.
5. **McKenna** et al., 2020, ***Microbiome***, 8:128.

The role of LSHTM and NU in this collaborative project	The primary supervisor (OG) is currently leading a diverse set of PhD students at the LSHTM investigating *Campylobacter* pathogenesis. The PhD candidate will join a vibrant team with expertise in molecular microbiology and omics-based methods (e.g. genomics, metagenomics). The doctoral student will have the opportunity to develop core molecular microbiology skills, learn how to grow *Campylobacter*, how to create defined isogenic mutants, and how to perform a number of different phenotypic assays depending on the direction the doctoral students wishes to drive the project. All aims have the option to also integrate omics-based techniques that can allow bioinformatics and statistical skills to be developed. The Co-Supervisor (TK) at the Nagasaki University will provide expertise in the functional characterisation of effectors that will link to damage of other bacteria, damage of host-eukaryotic cells and survival. TK has extensive experience in effector characterisation from a range of bacteria and secretion systems. In collaboration with NU, the doctoral student can develop core molecular microbiology skills to characterise the *C. jejuni* T6SS. The doctoral student will also be able to have a work placement in the laboratory of TK (NU) and also have the option to collect new *C. jejuni* isolates for genome sequencing and T6SS characterisation.
Particular *prior* educational requirements for a student undertaking this project	The doctoral candidate should have completed an undergraduate and postgraduate degree related to microbiology.
Skills we expect a student to develop/acquire whilst pursuing this project	*Molecular Biology* – The doctoral student will have the opportunity to develop core molecular microbiology skills, learn how to grow *Campylobacter*, how to create defined isogenic mutants, and how to perform a number of different phenotypic assays depending on the direction the doctoral student wishes to drive the project. *Bioinformatics* – All aims can also utilise omics-based techniques (e.g. transcriptomics and metagenomics) allowing the opportunity to run and develop bioinformatic pipelines and statistical analyses methods using UNIX, python and R for 16S microbial survey profiles and also shotgun metagenomics. As well as key scientific skills, development of core transferrable skills are core tenets of the LSHTM doctoral programme. The doctoral student will join the Campylobacter research group and be provided with world-class training that will lead to them becoming an independent scientist. They will have the option to attend MSc modules which may be relevant to their research project. In addition, the doctoral student will attend relevant courses/workshops to understand the value of knowledge transfer and the value of intellectual property (IP). The doctoral student will

	be able to take part in numerous courses provided by the Teaching and Education Development (TED) team based within the School. Meetings and social events are organised at intervals throughout the year to encourage students to get to know each other and to develop a supportive environment.