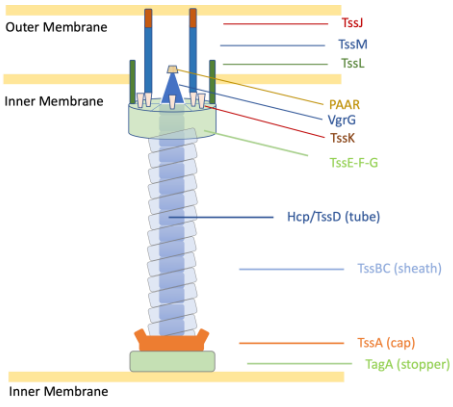
Title of PhD project / theme	**Functional Characterisation of the *Campylobacter jejuni* Type VI Secretion System**
Supervisory team	**LSHTM/Australia** Lead: Dr Ozan Gundogdu (Ozan.Gundogdu@lshtm.ac.uk, Faculty of Infectious and Tropical Diseases) Dr Abderrahman Hachani (University of Melbourne) **Nagasaki University** Dr Daniel Ken Inaoka (danielken@nagasaki-u.ac.jp) Dr Toshio Kodama
Brief description of project / theme	***Campylobacter, an important but poorly studied pathogen.*** *Campylobacter* is the most common bacterial cause of human gastroenteritis in the world, with the species *Campylobacter jejuni* responsible for over 80% of C*ampylobacter* infections (1). *C. jejuni* is abundant within the avian gut and the consumption and handling of poultry is the main route of transmission to humans. In humans, *C. jejuni* infection ranges from asymptomatic carriage to bloody diarrhoea, fever and abdominal pains as well as serious post-infectious sequelae such as the neuromuscular paralysis of Guillain-Barré syndrome. In high resource countries, an estimated 1 in every 100 individuals develop a *Campylobacter*-related illness each year, with elderly people most at risk to serious complications. With an ageing population, the consequences of *Campylobacter* infection are therefore bound to increase. ***Pathogenesis and Type VI Secretion System.*** Secretion systems play a central role in infectious diseases by enabling pathogenic bacteria to deliver virulence factors into target cells. 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). In addition to killing or reducing the fitness of their microbial competitors, T6SS effectors can also be used to subvert host cell processes by manipulating the cytoskeleton, evading host defence mechanisms, and modulating host inflammatory responses. T6SS effectors injected into competitors or host cells are typically located downstream of VgrGs (spike protein), and in the case of antimicrobial effectors, encoded in gene tandem with a cognate immunity coding gene to protect the bearer from self-intoxication, thus forming effector-immunity modules. The T6SS not only provides a competitive advantage for bacteria to survive and dominate in host intestinal niches, but also impedes host cell functions,  **Figure 1.** Hypothetical representation of the *C. jejuni* T6SS in extended state.

promoting immune evasion, and utlimately enabline successful infection of host (2).

Campylobacter and Type VI Secretion System. Though widely studied in other enteric pathogens, investigating the functions of the T6SS within *C. jejuni* have been limited. Our own studies of the genetic architecture of *C. jejuni* strains uncovered T6SS clusters that are highly conserved and syntenic between humans and chicken isolates (3, 4). We showed 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 the presence of the T6SS increased *C. jejuni* interactions with host cells and promoted the invasion of chicken primary intestinal cells (Fig. 2A, 2B), thereby supporting the colonisation of chickens (Fig. 2C)***. The current literature on* C. jejuni *T6SS functions is extremely limited.***

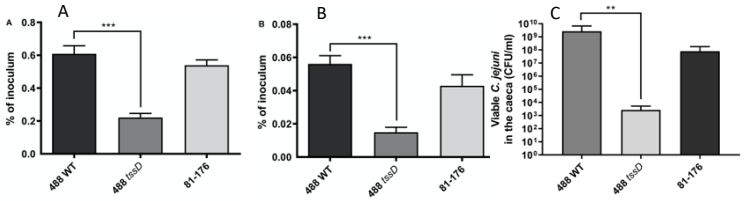


Figure 2. Interaction (A) with and invasion (B) of primary chicken intestinal cells with *C. jejuni* 488 wild-type strain, 488 T6SS *tssD* mutant or *C. jejuni* 81–176 wild-type strain. Primary chicken intestinal cells were infected with *C. jejuni* strains at a MOI of 1,000:1. **(C) Infection of broiler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

Bioinformatic analysis and effector prediction. Our recent study investigated the genetic architecture of *C. jejuni* strains and uncovered T6SS clusters that are highly conserved and syntenic between humans and chicken isolates (5). We then sought to perform a comprehensive bioinformatics study to further investigate the genetic architecture and putative effectors (Fig. 3) (5). We identified putative effectors up- and downstream of the T6SS locus including two canonical VgrG homologues, a putative DinJ-YafQ Type II toxin-antitoxin (TA) module, as well as several open reading frames functionally predicted to encode for nucleases, lipases, and peptidoglycan hydrolases. ***This comprehensive* in silico *study provides a framework for experimental characterisation of T6SS-related effectors and effector-immunity modules in* C. jejuni*.***

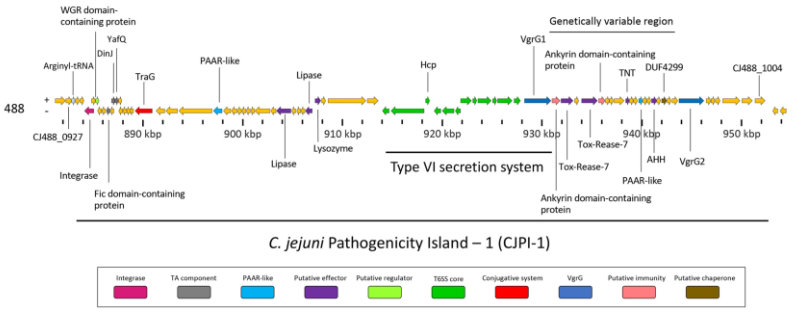


Figure 3. Genomic architecture of the PAI CJPI-1 in T6SS-positive *C. jejuni* 488 strain. Coloured coding domain sequences (CDS) represent proteins with inferred functions and are labelled. The scale under the CDS represents the nucleotide position [kilobase pair (kbp)] of the pathogenicity island in the genome of *C. jejuni* 488 strain. The location of the T6SS and genetically variable region are also denoted. The positive (+) symbol on the left-hand side indicates the sense strand in the genome of *C. jejuni* 488 strain, whilst the negative (–) symbol indicates the antisense strand. Genes sizes are not to scale. Genes are coloured according to predicted function.

	Bases on discussions with the doctoral candidate, the aims of the project can be one or more of the following using molecular, phenotypic and/or omics-based methods: - 1. **Characterise the *C. jejuni* T6SS and putative effectors in relation to bacterial fitness.** 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 will be further explored with competition-based assays. 2. **Characterise the *C. jejuni* T6SS and putative effectors in relation to host cell interactions.** The role of the *C. jejuni* T6SS effectors 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. **Characterise the *C. jejuni* T6SS and putative effectors in relation to survival.** We have identified several putative T6SS effectors associated with iron and zinc uptake which may have a potential role related to survival. 4. **Investigate the impact of the *C. jejuni* T6SS and effectors on the chicken gut microbiome.** The impact of *C. jejuni* T6SS and effectors on the chicken gut microbiome population structure and chicken health is unknown. Here, using an chicken infection model, we can explore this further using omics-based approaches such as metagenomics. This study will build upon the recent *C. jejuni* T6SS research at LSHTM and Nagasaki University. References (own in bold): 1. **Gundogdu and Wren** *et al*., 2020, ***Microbiology***, 166:230–232. 2. Hachani *et al*., 2016, ***Current Opinion in Microbiology***, 29. 3. **Liaw** *et al*., 2019, ***Frontiers in Microbiology***, 10:2864. 4. **Ugarte-Ruiz** *et al*., 2015, ***Zoon Pub Health***, 62(7):497:500. 5. **Robinson** *et al*., 2021, ***Frontiers in Microbiology***, 12:694824.
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 several different phenotypic assays depending on the direction the doctoral student 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. Co-Supervisor (TK) at 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. Co-Supervisor (DKI) at Nagasaki University will provide expertise in biochemical and structural biology characterisation of effector proteins of interest. If required, a high-throughput screening system can be developed to identify small molecules which can inhibit the function of effector proteins. In collaboration with NU, the doctoral student can develop core molecular microbiology skills to characterise the *C. jejuni* T6SS. The doctoral student can also have a work placement at NU developing novel complementary molecular microbiology techniques for the characterisation of the *C. jejuni* T6SS.
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 several 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., genomics 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.