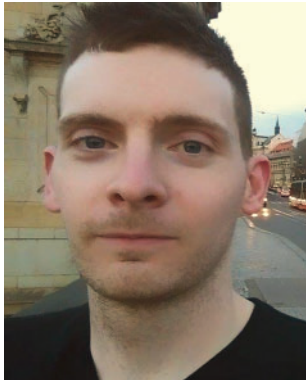


# Salmonella in Australia: understanding and controlling infection



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The bacterium *Salmonella* causes a spectrum of foodborne diseases ranging from acute gastroenteritis to systemic infections, and represents a significant burden of disease globally. In Australia, *Salmonella* is frequently associated with outbreaks and is a leading cause of foodborne illness, which results in a significant medical and economic burden. *Salmonella* infection involves colonisation of the small intestine, where the bacteria invades host cells and establishes an intracellular infection. To survive within host cells, *Salmonella* employs type-three secretion systems to deliver bacterial effector proteins into the cytoplasm of host cells. These bacterial effectors seek out and modify specific host proteins, disrupting host processes such as cell signalling, intracellular trafficking, and programmed cell death. This strategy of impairing host cells allows *Salmonella* to establish a replicative niche within the cell, where they can replicate to high numbers before escaping to infect neighbouring cells, or be transmitted to new hosts. While the importance of effector protein translocation to infection is well established, our understanding of many effector proteins remains incomplete. Many *Salmonella* effectors have unknown function and unknown roles during infection. A greater understanding of how *Salmonella* manipulates host cells during infection will lead to improved strategies to prevent, control, and eliminate disease. Further, studying effector proteins can be a useful means for exploring host cell biology and elucidating the details of host cell signalling.

## Salmonella infections in Australia

Australia experiences an unusually high number of reported *Salmonella* infections, comprising a significant baseline of

individually acquired infections, combined with a string of well-publicised foodborne outbreaks<sup>1</sup>. Typically, outbreaks are associated with bacterial contamination of livestock and animal products in farms and factories, which are then carried on through to consumers. Several high-profile outbreaks have been linked to contamination of animal products: the outbreak at the Langham hotel in Melbourne in 2015 was linked to raw egg mayonnaise<sup>2</sup>, while chicken and pork products were linked to an outbreak at a Sydney bakery in 2016<sup>3</sup>. Interestingly, several recent outbreaks have been linked to contaminated fruits and vegetables: *Salmonella* Anatum in bagged salads<sup>4</sup>, *Salmonella* Saintpaul on beansprouts<sup>5</sup>, and *Salmonella* Hvitittingfoss on rockmelons<sup>6</sup>. These outbreaks translate to a significant economic impact to the affected industries, in addition to the medical burden caused to the affected individuals<sup>7,8</sup>.

Australia enjoys a high standard of public health surveillance, and *Salmonella* infections are notifiable and publicly reported through the National Notifiable Diseases Surveillance System. These reports indicate more than 17 000 cases of *Salmonella* infection were reported in 2016<sup>9</sup>, and already more than 10 000 reported cases in 2017 at the time of writing<sup>10</sup>. These numbers are derived from a symptomatic person presenting to a healthcare professional, and so the true number of infections is likely to be significantly higher<sup>11</sup>. *Salmonella* therefore represents a significant public health concern.

## Salmonella causes a range of disease states

At first glance, the phylogeny of *Salmonella* appears relatively simple, with the genus comprising only two species. *S. enterica* is the species most frequently associated with disease states in humans and other animals, while *S. bongori* lacks several important virulence factors and consequently rarely causes disease in humans<sup>12</sup>. Within *S. enterica* however lies an astonishing variety of serologically distinct serovars, some of which have undergone selective pressures resulting in host adaptation and acquisition of additional virulence factors. Serovars within *S. enterica* can cause drastically different disease states: *S. enterica* serovars Typhimurium and Enteritidis are frequently the causative agents of gastroenteritis outbreaks, while *S. enterica* serovar Typhi causes typhoid fever, a systemic infection with broadly different clinical outcomes<sup>13</sup>. Even within serovars, variation and selection can result

in strikingly different strains. For example, the globally disseminated *S. enterica* Typhimurium strain ST19 causes self-limiting gastroenteritis, while the genetically similar *S. enterica* Typhimurium strain ST313 is causative of bloodstream infections resulting in significant mortality rates in Sub-Saharan Africa<sup>14</sup>.

In Australia, the burden of disease arises from a variety of *Salmonella* serovars. Individually acquired infections and outbreak-associated infections are most typically caused by serovar *S. Typhimurium*<sup>15</sup>, which is a generalist strain capable of infecting different animals. Some serovars are specialised to infect certain livestock animals: *S. Gallinarum* and *S. Pullorum* are adapted to chickens, while *S. Choleraesuis* is associated with pigs. Occasionally, outbreaks are associated with rarer serovars, such as *S. Anatum* and *S. Hvitittingfoss*. Nearly all cases of *S. Typhi* infection in Australia occur in travellers that have contracted the bacteria while overseas<sup>1</sup>.

Ultimately, the diversity of *Salmonella* serovars and spectrum of diseases that it causes demonstrate a bacterium that is adept at persisting through the food supply chain, from contamination of farms and factories to restaurants and household kitchens, despite stringent food safety standards and public health surveillance.

### **Salmonella subverts host cells to achieve infection**

Infection by *Salmonella* spp. follows ingestion of live bacteria, which survive passage through the stomach and attach to the gastrointestinal epithelium. The infection strategy of *Salmonella* involves the invasion of host cells (either by localised disruption of the membrane of non-phagocytic epithelial cells, or by endocytic uptake into professional phagocytes) followed by the establishment of an intracellular replicative niche<sup>16–18</sup> (Figure 1). These activities are heavily mediated by the use of type-three secretion systems (T3SS), which function as molecular syringes to penetrate host cells and deliver bacterial effector proteins into the host cytoplasm (Figure 2). These effector proteins typically recognise specific host proteins, and disrupt the normal function of these proteins often by catalysing post-translational modifications<sup>19,20</sup>.

*Salmonella* employs two distinct type-three secretion systems at different stages of infection. These secretion systems translocate distinct cohorts of effector proteins into the host cell to achieve different goals for the bacterium. At early stages of infection, *Salmonella* uses the SPI-1 secretion system (encoded on the genomic region termed *Salmonella* pathogenicity island 1) to achieve uptake into non-phagocytic epithelial cells. Effector proteins translocated by this secretion system manipulate the host

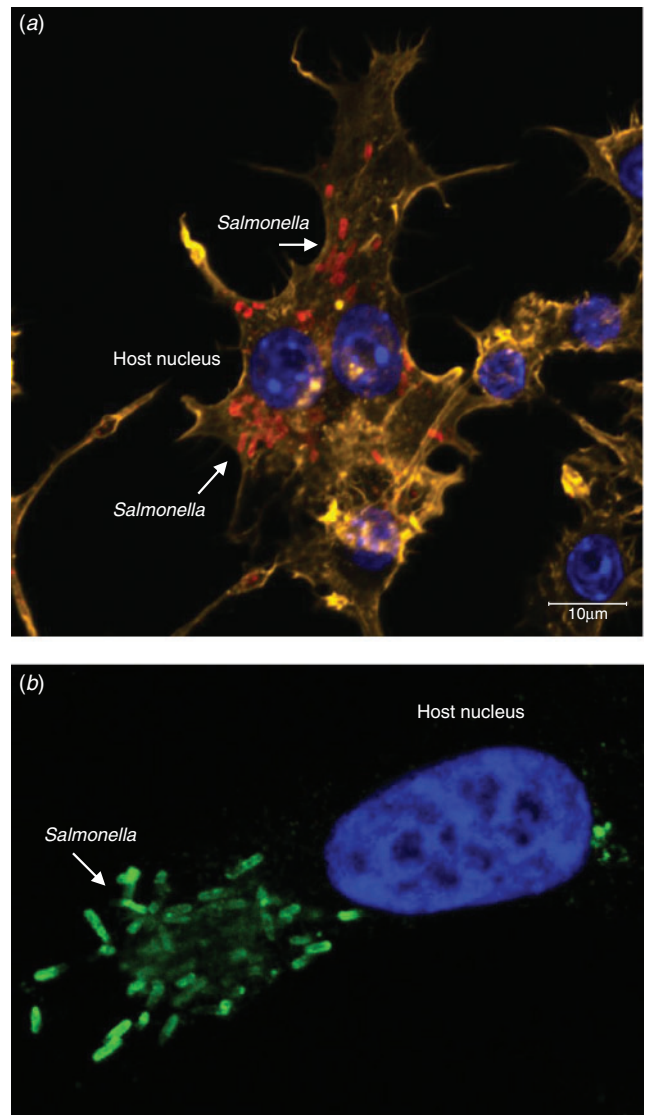


Figure 1. Immunofluorescence micrographs of *Salmonella* replicating within infected host cells. (a) RAW264.7 murine macrophages infected with *Salmonella* Typhimurium for 18 hours. (b) HeLa cell infected with *Salmonella* Typhimurium for 16 hours. Arrows indicate *Salmonella* (red in panel (a), green in panel (b)), as visualised with a polyclonal antibody to the *Salmonella* common structural antigens. Host cell nuclei visualised with Hoescht stain (blue). Host cells visualised in (a) with Phalloidin–Tetramethylrhodamine B isothiocyanate (yellow).

actin cytoskeleton to induce membrane ruffling, which enables bacterial uptake<sup>21</sup>. Shortly after invasion, the SPI-1 secretion system is downregulated and the bacterium is taken up into a phagocytic vacuole. Sensing the acidification of the vacuole, *Salmonella* then deploys the SPI-2 secretion system, which delivers another set of effectors into the host cytoplasm. Broadly, these effectors are responsible for remodelling the vacuole into a replication-permissive space (Figure 3), translocating and extending the vacuole along host microtubules, and interfering with the secretion of inflammatory cytokines<sup>22,23</sup>. Together, these activities enable *Salmonella* to survive within both epithelia and phagocytic cells, to replicate to high numbers, and to disseminate to adjacent cells and on to new hosts.

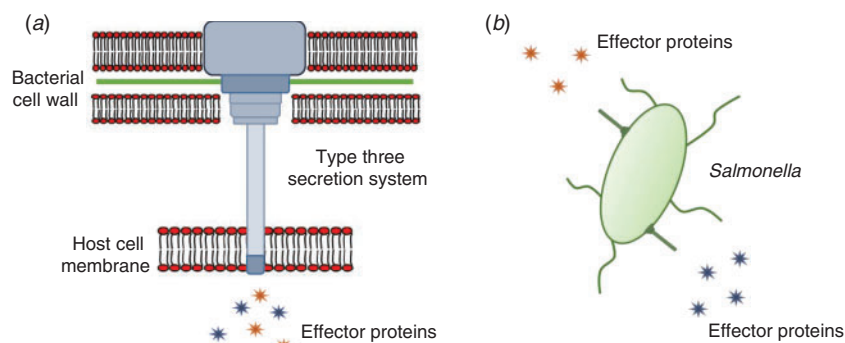


Figure 2. Schematic representation of the type three secretion system. (a) Representative structure of the type three secretion system, comprised of a basal body embedded in the bacterial cell wall, and a needle and tip complex which penetrates the host cell membrane. The complex forms a conduit from bacterial to host cytoplasm, allowing translocation of effector proteins. (b) Cartoon representation of *Salmonella* demonstrating translocation of distinct cohorts of effector proteins.

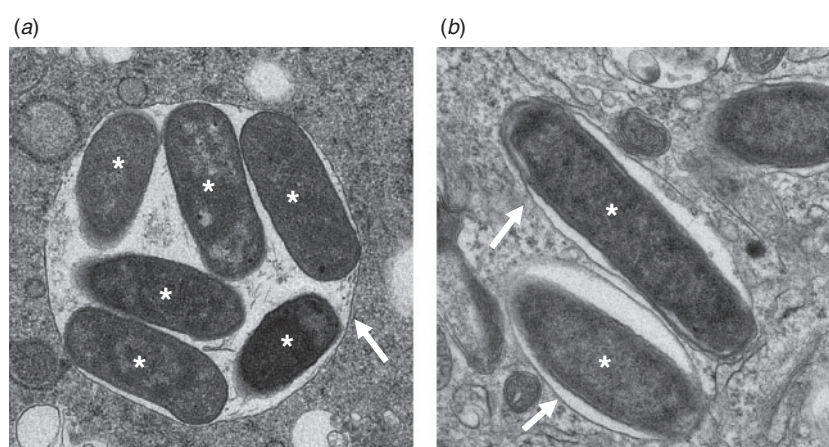


Figure 3. Electron micrograph of vacuole-bound *Salmonella* surviving within infected epithelial cells. Arrows denote the perimeter of the *Salmonella*-containing vacuole. Asterisks denote vacuolar *Salmonella*, residing in either (a) communal or (b) individual vacuoles. Images acquired by Vicki Bennett-Wood.

## Discovering the function of *Salmonella* effector proteins

It is well established that *Salmonella* relies on effector protein translocation to achieve infection – mutant strains lacking type-three secretion systems do not invade epithelia effectively, and are deficient in intracellular persistence<sup>24</sup>. However, the contribution of individual effector proteins is less well defined. Some effector proteins are relatively well described, others are poorly understood, and many have no known function and no known role during infection. Our research aims to characterise the poorly understood effector proteins of the SPI-2 secretion system.

Classical methods for identifying interactions between a given protein and its potential binding partner rely on the strength of the interaction between the two. Approaches such as yeast two-hybrid screening and immunoprecipitation have found success in identifying the host substrates of many *Salmonella* effector proteins. However, these approaches are unsuitable if the binding affinity between two proteins is weak or transient. For instance, effector proteins that are predicted to be enzymes are not likely to have strong or stable binding affinity for their host substrates.

Our research focuses on the enzymatic activity of a family of *Salmonella* effector proteins. These effectors are known substrates of the SPI-2 secretion system, and are predicted to be glycosyltransferases. Therefore, we have screened the proteome of infected host cells to detect changes in glycosylation activity. This approach combines custom immunoprecipitation techniques with highly sensitive mass spectrometry to detect glycosylated peptides during infection. Our approaches are not limited by the binding affinity of an effector for its substrate, and also allow for interrogating the activity of effector proteins without overexpression or ectopic expression. Preliminary results suggest *Salmonella* engages in a blockade of apoptotic cell death signalling, a finding that expands our understanding of how bacteria survive within host cells.

## Directions and applications

Antibiotic treatment is generally ineffective at reducing the impact of *Salmonella* infection, and vaccines that are effective and economically viable are still in development<sup>25</sup>. The successful control and prevention of *Salmonella* infection requires a more robust understanding of the interaction between the pathogen and the



host cell. Given that type-three secretion mutants are significantly attenuated during infection, it stands to reason that interfering with effector translocation represents a viable opportunity for developing novel anti-infective therapeutics. As we move towards a more complete understanding of the *Salmonella*–host interaction, it is likely that new opportunities will arise to antagonise the intracellular lifestyle of the bacteria. Ultimately, effective control and prevention of *Salmonella* infection will require a combination of human and animal vaccination, public health surveillance and food safety compliance, and effective therapeutic options.

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## Biography

**Joshua Newson** is a PhD student at the Doherty Institute, The University of Melbourne. His research involves identifying mechanisms by which *Salmonella* survive within infected immune cells, and elucidating the function of translocated effector proteins.