# Cathelicidins modulate the host immune response and the microbiota in *Salmonella* Typhimurium infection

by

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## Abstract

*Salmonella enterica* serovar Typhimurium is a prevalent incitant of enteritis in human beings and swine. The historic use of antibiotics at non-therapeutic concentrations for growth promotion, and at therapeutic doses for group and individual treatment of pigs has been linked to development of antimicrobial resistance in *Salmonella* Typhimurium. This has resulted in restrictions on the use of antibiotics in swine production, necessitating the identification of non-antibiotic strategies to eliminate pathogens and mitigate their impact on the host (e.g. inflammation). It has been proposed that host defense responses incited by *Salmonella* allow the bacterium to overcome colonization resistance. To address this, piglets were orally inoculated with *S. enterica* serovar Typhimurium DT104, and the host responses and microbiota changes were temporally examined at the acute (2 days post-inoculation [dpi]), subacute (6 dpi), and recovery (10 dpi) stages of salmonellosis. At the acute stage of disease, body temperatures were elevated, and feed consumption and weight gain were reduced. Densities of *Salmonella* associated with mucosa decreased over time, with higher densities of the bacterium in the ileum and the large intestine. Moreover, substantive histopathologic changes were observed as a function of time, with prominent epithelial injury and neutrophil infiltration observed at 2 dpi. Correspondingly, a variety of host metrics were temporally affected in piglets with salmonellosis (e.g. *TNFα, IFNγ, PR39, βD2, iNOS, IL8, REGIIIγ*). The enteric microbiota was characterized using culture-independent and -dependent methods in concert, and taxon- and location-specific changes to the microbiota were observed in infected piglets. *Bacteroides* spp. (e.g. *B. uniformis, B. fragilis*), *Streptococcus* spp. (e.g. *S. gallolyticus*), and various *Gammaproteobacteria* were highly associated with inflamed tissues, while bacteria within *Ruminococcaceae* and *Veillonellaceae* were mainly associated with healthy mucosa. In conclusion, the findings showed that *S.* Typhimurium incited temporal and spatial modifications to the swine autochthonous microbiota, and to host defense responses, that were consistent with overcoming

colonization resistance to incite salmonellosis in swine. This included upregulation of the cathelicidin and host defense peptide PR39.

Host defense peptides, also known as antimicrobial peptides, have been shown to protect the host via a variety of mechanisms. In mice, the cathelicidin, murine cathelicidin-related antimicrobial peptide (mCRAMP) has been demonstrated to impair the proliferation of *S.* Typhimurium *in vitro*. However, the impact of mCRAMP on host responses and the microbiota following infection by this pathogen has not been determined. To address this, mCRAMP knockout mice (mCRAMP$^{-/-}$) and wildtype mice (mCRAMP$^{+/+}$) were administered the broad-spectrum antibiotic, streptomycin (ST+) or water alone (ST-). Furthermore, they were orally inoculated with *S.* Typhimurium DT104 (SA+) or buffer alone (SA-), and impacts on the host and enteric bacterial communities were temporally evaluated. Higher densities of the pathogen were observed in cecal digesta and associated with mucosa in SA+/ST+/mCRAMP$^{-/-}$ mice than in SA+/ST-/mCRAMP$^{-/-}$ mice at 24 hpi. Both SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST-/mCRAMP$^{-/-}$ mice were more susceptible to infection exhibiting greater histopathologic changes (e.g. epithelial injury, leukocyte infiltration, goblet cell loss) at 48 hpi. Correspondingly, immune responses in SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST-/mCRAMP$^{-/-}$ mice were affected (e.g. *Ifnγ, Kc, Inos, Il1β, RegIIIγ*). Systemic dissemination of the pathogen was characterized by metabolomics, and the liver metabolome was affected to a greater degree in SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST-/mCRAMP$^{-/-}$ mice (e.g. taurine and cadaverine). Treatment-specific changes to the structure of the enteric microbiota were associated with infection and mCRAMP deficiency, with a higher abundance of *Enterobacteriaceae* and *Veillonellaceae* observed in infected null mice. The microbiota of mice that were administered the antibiotic and infected with *Salmonella* was dominated by *Proteobacteria*. The absence of mCRAMP was observed to modulate both host responses and the enteric microbiota enhancing local and systemic infection by *Salmonella* Typhimurium, implicating cathelicidin as an important regulator of salmonellosis. Given the protective role provided by cathelicidin, this HDP may be an effective therapeutic alternative to antibiotics for use against *Salmonella*

enterocolitis. Moreover, the mCRAMP knockout model may advance elucidation of mechanisms of salmonellosis toward effective mitigation of this important disease.

## Preface

This thesis is an original work by Danisa M. Bescucci. The research project, of which this thesis is a part, received research ethics approval from the Animal Care Committee at Agriculture and Agri-Food Canada Lethbridge Research and Development Centre for the following projects: “Mitigation of enteric inflammation in pigs” (ACC Protocol 1512, Oct 2015- May 2016); and “Elucidation of the role that the murine cathelicidin-related antimicrobial peptide plays in colonization resistance: utilization of a streptomycin-*Salmonella enterica* Typhimurium infection model in mice” (ACC Protocol 1729, May 2018-Aug 2018).

Chapter 2 was a collaborative effort by myself and Paul Moote. Paul conducted culture-dependent methods, and he was in charge of the collection and analysis of data associated to culturomics. Dr. Richard Uwiera conducted scoring of tissues for histopathologic changes. I was responsible for the collection and analysis of the non-culturomics data, and for preparation of a scientific manuscript with Drs. Inglis and Uwiera. A version of the manuscript has been submitted to AEM (May 2020). For Chapter 3, Dr. Sandra Clarke conducted flow cytometry, including the analysis of data. Kate Brown and Tony Montina were responsible for conducting metabolomic analyses, and Dr. Valerie Boras completed scoring of tissues for histopathologic changes. With the exception of flow cytometry and metabolomics data, I was in charge of the collection and analysis of remaining data, and for the preparation of a scientific manuscript with Dr. Inglis. A version of Chapter 3 has been submitted to Gut Pathogens (May 2020).

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## List of Abbreviations

| | |
|---|---|
| AGPs | Antimicrobial Growth Promoters |
| AMR | Antimicrobial Resistance |
| ASVs | Amplicon Sequence Variants |
| βD | Beta-defensin |
| BGA | Brilliant Green Agar |
| BSA | Bovine Serum Albumin |
| CB | Columbia Broth |
| CBC | Complete Blood Count |
| CFU | Colony Forming Units |
| CR | Colonization Resistance |
| DSS | Dextran Sodium Sulfate |
| FPR | Formyl Peptide Receptor |
| GALT | Gut Associated Lymphoid Tissue |
| GIT | Gastrointestinal Tract |
| GM-CSF | Granulocyte-Macrophage Colony-Stimulating Factor |
| GUSβ | Beta-Glucuronidase |
| HACCP | Hazard Analysis and Critical Control Points |
| hCAP18/LL37 | Human Cationic Antimicrobial Peptide |
| HDPs | Host Defense Peptides |
| H&E | Hematoxylin and Eosin |
| HPRT | Hypoxanthine-guanine Phosphoribosyl Transferase |
| IBac | Intestinal Bacterial Collection |
| IECs | Intestinal Epithelial Cells |
| IFN | Interferon |
| IL | Interleukin |
| iNOS | Inducible Nitric Oxide Synthase |
| IVCs | Individual Ventilated Cages |
| KC | Keratinocyte-derived Chemokine |
| KO | Knock Out |
| LPS | Lipopolysaccharide |
| MA | MacConckey's Agar |
| mCRAMP | Murine Cathelicidin-Related Antimicrobial Peptide |
| MIP | Macrophage Inflammatory Protein |
| MLIA | Modified Lysine Iron Agar |
| MPO | Myeloperoxidase |
| Muc | Membrane bound Mucin |
| NGS | Next Generation Sequencing |
| NO | Nitric Oxide |
| PFGE | Pulse Field Gel Electrophoresis |
| PPIA | Peptidylprolyl Isomerase A |
| PR-39 | Proline-Rich antimicrobial peptide 39 |
| RDP | Ribosomal Database Project |
| REG | Regenerating islet-derived protein |
| ROS | Reactive Oxygen Species |
| SCFAs | Short-Chain Fatty Acids |
| SCV | *Salmonella* Containing Vacuole |

| | |
|---|---|
| SEM | Standard Error of the Mean |
| SGEF | $SH_3$-containing Guanine nucleotide Exchange Factor |
| sIgA | Secretory Immunoglobulin A |
| SPI | *Salmonella* Pathogenicity Island |
| T3SS | Type 3 Secretion System |
| TGF | Transforming Growth Factor |
| Th | T-helper cells |
| TLR | Toll-Like Receptor |
| TSIA | Triple Sugar Iron Agar |
| TNF | Tumor necrosis factor |
| ZO | Zonula occludens |

# Chapter 1: Literature review

## 1.1 Introduction

Swine (*Sus domesticus*) is an important global source of protein. Every year the value of Canadian pork exports exceeds $2.6 billion, and Canada is the fifth largest exporter of swine in the world, representing 8.5% of the total pork exportation (Brisson *et al.* 2014). The exponential increase in numbers of people has dramatically increased demand for pork products worldwide. *Salmonella enterica* is a Gram-negative facultative-anaerobic bacterial pathogen with a global importance. Over 2579 serovars have been identified, distinguishable serologically by antigenic properties of their lipopolysaccharide (LPS, O-antigen) and the protein subunits of their flagella (H-antigen) (Coburn *et al.* 2007; Uzzau *et al.* 2000). All *S. enterica* serotypes are considered to be pathogenic in human and non-human animals producing different syndromes according to the serotype and host affected (Uzzau *et al.* 2000). Following infection (i.e. salmonellosis), disease incited by the pathogen is localized to the gastrointestinal tract (GIT) (enterocolitis) or systemically manifested (septicemia) (Coburn *et al.* 2007). *Salmonella enterica* serovar Typhimurium is a zoonotic pathogen considered to be one of the main causes of gastrointestinal infections in human beings. Salmonellosis is a reportable disease in Canada, and infection rates (primarily due to infection by *S. enterica*) have remained relatively constant since 1959, averaging 21.9 cases per 100K over this period (https://dsol-smed.phac-aspc.gc.ca/notifiable/). Furthermore, *S.* Typhimurium is an important pathogen of swine, and pigs also serve as a non-human reservoir of the bacterium. Increasing domestic and international demand presents challenges for the sustainable and environmentally responsible production of pork. Currently, the only treatment available for diarrheic enterocolitis in swine is medical care (e.g. fluid therapy, antibiotic and anti-inflammatory therapy), which is expensive and not always effective (Boyen *et al.* 2008). Historically, antibiotics have been used at non-therapeutic levels for growth promotion (antibiotic growth promoters), and for therapeutic and metaphylactic treatment of enteric disease (Brown *et al.* 2017). The emergence of antimicrobial resistance (AMR) to antibiotics in *S. enterica* has become a primary concern for human health, and for the competitiveness and sustainability of Canadian agriculture (Haley *et al.* 2012). These challenges are compounded by increasing restrictions on the use of antibiotics in swine production ('Responsible use of Medically Important Antimicrobials in Animals' 2018). Since salmonellosis outbreaks in people primarily originate by ingestion of food contaminated with the pathogen, the utilization of effective surveillance programs in conjunction with the development and implementation of efficacious mitigation strategies are areas of current emphasis to reduce morbidity in people. In this regard, mitigation of *S.* Typhimurium in pigs is a priority for the swine sector worldwide.

### 1.2 *Salmonella* Typhimurium pathogenesis in human beings

*Salmonella enterica* serovar Typhimurium is responsible for causing an enterocolitis syndrome in human beings characterized by self-limiting gastroenteritis, abdominal pain, diarrhea with or without blood, nausea, and vomiting (Coburn *et al.* 2007; Chirullo *et al.* 2015). Histopathologic characteristics of disease include mucosal edema and polymorphonuclear infiltrate (Santos RL *et al.* 2001). Although *S.* Typhimurium infection normally remains localized in the intestine and mesenteric lymph nodes in healthy human beings, the systemic spread of the pathogen can occurs in the elderly, infants, and immunosuppressed individuals, leading to severe complications and sometimes death (Agbor *et al.* 2011; Boyen *et al.* 2008). The principal reservoirs of *S*. Typhimurium are contaminated water and food of animal origin such as, beef, pork, dairy products, and poultry meat and eggs (De Freitas Neto *et al.* 2010; Santos RL *et al.* 2001).

Ingestion of a threshold level of at least 50,000 bacterial cells is required for infection, which commences with the colonization of the intestine. This multistep process involves the following steps: (i) adhesion to epithelial cells; (ii) activation of Type III secretion system (T3SS); (iii) delivery of effector proteins; and (iv) re-arrangement of the actin cytoskeleton and internalization (Ly *et al.* 2007). *Salmonella* adherence to the epithelial cells consists of a reversible attachment mediated by Type I fimbriae (Fim) (Althouse *et al.* 2003), and an irreversible binding by T3SS (Misselwitz *et al.* 2011). The T3SS is the main virulence mechanism known for *S. enterica,* which is encoded within the *Salmonella* Pathogenicity Island (SPI), and especially within SPI-1 and SPI-2 (Lostroh *et al.* 2001; Hensel 2000). This complex system is composed of 20-30 proteins that form the supramolecular injection apparatus required for the delivery of bacterial effectors into host cells (Ly *et al.* 2007; Agbor *et al.* 2011). Each island encodes different protein effectors, which confer different functions during the infection process. For example, the protein effectors encoded in the SPI-2 are associated with intracellular survival of *S.* Typhimurium, while the SPI-1 effectors are involved in bacterial entry.

Penetration through the epithelium can be both transcellular and paracellular, with the latter achieved via modification of tight junctions (Agbor *et al.* 2011; Boyle *et al.* 2006). The effector proteins encoded in SPI-1 (SipA, SipC, SopB, SopE, and SopE2) mediate the process of endocytosis by non-phagocytic cells including intestinal epithelial cells (IECs). SipA and SipC promote the formation of actin filaments and prevent filament disassembly by host factors (e.g. gelsolin and cofilin), inducing in this way, the formation of membrane ruffling, which results in engulfment of the bacterium (Agbor *et al.* 2011; Lhocine *et al.* 2015; Ly *et al.* 2007). SopE and SopE2 also participate in membrane ruffling and bacterial engulfment via activation of Rho-GTPases (e.g. Rac-1 and Cdc42), which participate in the actin

bundling, polymerization, and cytoskeletal rearrangements (Hardt *et al.* 1998). SopB contributes to the remodeling of actin by indirectly activating the RhoG factor through the activation of SH3-containing guanine nucleotide exchange factor (SGEF) (Patel *et al.* 2006). Once *S. enterica* is internalized within the epithelial cell, the actin cytoskeleton returns to the normal organization through the inactivation of Rac-1 and Cdc42 by the protein effector SptP (Fu *et al.* 1999).

After successful entry into epithelial cells, *Salmonella* is kept intracellularly in a specific organelle called the *Salmonella* containing vacuole (SCV) (Steele-Mortimer *et al.* 1999), whose formation and maturation is accomplished by the effector SopB. Formation of the SCV organelle is essential for *Salmonella* intracellular niche creation. There are some effectors derived from SPI-2 that are important for bacterial survival within the SCV, including SifA, SseJ, SseF, SseG, SopD2 and PipB2 (Srikanth *et al.* 2011). SifA promotes the formation of tubules in the SCV, which in association with SseF and SseG, localizes the SCV to the perinuclear region close to Golgi apparatus, a crucial location within the IEC to access nutrients through a dynein-mediated transport system (Agbor *et al.* 2011).

After internalization within IECs has occurred, an inflammatory cascade is triggered (Coburn *et al.* 2007). This is characterized by neutrophil infiltration into the intestinal lamina propria, which can be intiated by different routes. Firstly, *Salmonella* uses some of the effectors mentioned above to induce migration of neutrophils, such as SopE, SopE2, and SopB (Hobbie *et al.* 1997). These proteins, with the activation of Rac-1 and Cdc42, trigger the Mitogen-activated kinase (MAPK) pathway, which mediates the initiation of factors AP-1 and NF-kB transcription resulting in the release of pro-inflammatory chemokines such as interleukin-8 (IL-8) (Hobbie *et al.* 1997). IL-8 acts as a neutrophil chemoattractant stimulating the migration of neutrophils from the bloodstream to the lamina propria of the intestinal tract. Release of IL-8 has also been observed after recognition of *Salmonella* flagellin by Toll-like receptor 5 (TLR5) in the basolateral surface of enterocytes (Gewirtz, Simon, *et al.* 2001). Neutrophil migration can also be stimulated by activation of Hepoxilin A3 via the protein effector, SipA (Wall *et al.* 2007). Migration of neutrophils has also been associated with the production of interleukin-23 (IL-23) and IL-18 by infected antigen presenting cells (APCs). Both of these cytokines stimulate T-cells to produce interleukin-17 (IL-17) and interleukin-22 (IL-22) in the intestinal mucosa, resulting in the influx of neutrophils to the mucosa (Liu *et al.* 2012). *Salmonella* can also colonize the intestinal wall by disrupting tight junctions via the protein effectors SipA, SopB, SopE, and SopE2 (Boyle *et al.* 2006), thereby promoting bacteria translocation and neutrophils transepithelial migration. The inflammatory response triggered after invasion by *S. enterica* is the main cause of the characteristic symptoms observed during the course of the disease. Thus, the enterocolitis syndrome in human beings develops

after an incubation period of less than 1 day, characterized by bloody diarrhea, nausea, vomiting, intestinal cramping, and fever (Glynn *et al.* 1992). Microscopically, human enterocolitis possesses focal and diffuse neutrophil infiltrate, crypt abscesses, epithelial necrosis, edema and fluid secretion (Giannella *et al.* 1973) affecting the caudal ileum, cecum, and the proximal colon.

### 1.3 *Salmonella* Typhimurium in pigs

Although there are many serovars of *S. enterica* capable of causing disease in pigs, *S. enterica* Typhimurium is the second most frequently isolated (Kim *et al.* 2009). Dissemination of this pathogen is mainly achieved by the fecal-oral route. Although infected pigs can shed up to $10^7$ colony-forming units of *S*. Typhimurium during the acute stages of the disease (Gutzmann *et al.* 1976), asymptomatic carriers can continuously or intermittently eliminate the pathogen in feces (Wood *et al.* 1989). Shedding of the pathogen can be exacerbated by mixing, transportation, and food deprivation, all stressors that are normally present during the production cycle. It is noteworthy that stressors that influence shedding of *S. enterica* late in the production cycle are of particular importance to foodborne transmission of the bacterium. The risk of consumption of pork contaminated with *S.* Typhimurium depends on many factors, ranging from the level of infection in the pig herd to the handling of pork by the consumer (Boyen *et al.* 2008). Although transmission of *S.* Typhimurium to pigs normally occurs via the fecal-oral route (e.g. via contaminated food), aerosol generation (Fedorka-Cray *et al.* 1995), and wild animals (Henzler *et al.* 1992) (e.g. rats, mice, cats) have also been shown to play an important role in the persistence and dissemination of the pathogen.

Swine salmonellosis caused by *S. enterica* serotype Typhimurium develops in a similar fashion to human enterocolitis; thus, pigs have been used as an animal model to study the pathogenesis and molecular mechanisms of this disease (Chirullo *et al.* 2015). *Salmonella* Typhimurium affects piglets between 6 to 12 weeks-of-age, and symptoms/signs of disease include watery diarrhea, lack of appetite, fever, and lethargy (Scherer *et al.* 2008). Studies in which piglets were experimentally infected with *S.* Typhimurium revealed that fecal shedding and persistence in tonsils or lymph nodes varied according to the age of infected pig, the dose with which pigs were orally inoculated, and by the *S.* Typhimurium strain used (Wood *et al.* 1989; Fedorka-Cray *et al.* 1995). Phage typing of S. Typhimurium has been applied as a tool for surveillance and outbreak investigation (Baggesen *et al.* 2010). The method involves differentiating *S.* Typhimurium subtypes based on their susceptibility to infection by bacteriophages (Rabsch 2007); however, whole genome sequence analysis has supplanted phage typing to subtype the bacterium in recent years ('FoodNet Canada annual report 2017' 2018). Phage typing of *S. enterica* serovar Typhimurium has identified more than 300 different definitive phage types (DT). The severity of

salmonellosis is often associated with specific phage types, and phage type DT104 is a highly virulent subtype that is also resistant to multiple antibiotics (Helms *et al.* 2005). The incidence of phage type DT104 has increased in the last decades with infections occurring globally (Helms *et al.* 2005). This phage type is of additional concern due to its association with human beings, its broad range of food animals including pigs, and its ability to rapidly acquire genetic determinants that confer resistance to antibiotics of importance in human medicine (Helms *et al.* 2005).

### 1.3.1 Pathology of salmonellosis in swine

Although the macroscopic lesions can extend from the ileum to the large intestine in swine, the large intestine is normally the most affected site (Wilcock, Armstrong, and & Olander 1976). In this regard, the spiral colon presents with diffuse catarrhal colitis, focal hemorrhages and superficial erosions typically covered with a fibrinonecrotic membrane. When the lesions progress, deep coalescing ulcers and necrotic debris appear in the colon and sporadically in the rectum (Wilcock, Armstrong, and Olander 1976). Mesenteric lymph nodes are normally enlarged, but the spleen and liver do not exhibit lesions in pigs (Wilcock, Armstrong, and Olander 1976). Microscopic lesions are characterized by infiltration of leukocytes into the lamina propria and submucosa, with necrosis of crypts and epithelial erosion (Chirullo *et al.* 2015). These alterations in the mucosa layer can extend from the ileum to the large intestine, and have been described as the mechanism that triggers the development of watery diarrhea (Arguello *et al.* 2018).

### 1.3.2 Immune response in swine

*Salmonella* Typhimurium infection in pigs is primarily limited to the intestinal tract. Early invasion in the ileum of piglets has been observed to depend on M cells, enterocytes, and goblet cells (Meyerholz *et al.* 2002). Similarly to human beings, intestinal invasion of the pathogen is mediated by Type I fimbriae adhesion (Althouse *et al.* 2003) and SPI-1 effector proteins (e.g. SipA, SipC, SseC) delivered by T3SS (Boyen *et al.* 2006). After invasion, the inflammatory response triggered by the pathogen has been observed to vary according to the intestinal site affected (Collado-Romero *et al.* 2010). In this regard, a strong inflammatory response is observed in the large intestine, but not in the ileum (Collado-Romero *et al.* 2010). The inflammatory response mounted by the host is strictly directed to eliminate the pathogen. *Salmonella* LPS has been described as the most potent effector of the immune response (Chirullo *et al.* 2015). After recognition of LPS is carried out by transmembrane TLR4 of enterocytes, activation of transcription factor NF-κβ occurs, which leads to expression of several pro-inflammatory cytokines and chemokines (Broz *et al.* 2012). Recognition of *S. enterica* flagellin by basolateral expressed TLR5 has also been described to induce a pro-inflammatory response to the pathogen (Gewirtz, Navas, *et al.* 2001).

Similarly to human enterocolitis incited by *S. enterica*, the pro-inflammatory response consists of the release of chemoattractants (e.g. IL8 and MIP1α) to recruit neutrophils to the site of infection (Collado-Romero *et al.* 2010). Intestinal colonization by the pathogen induces a Th1 type response with ensuring elevation of TNFα and IFNγ (Collado-Romero *et al.* 2010). Activation of this type of immune response is essential for the elimination of intracellular pathogens (Meurens, Berri, *et al.* 2009). In contrast to mice in which induction of Caspase-1 and release of *Il1β* and *Il18* have been shown to play an important role in *S. enterica* infection (Raupach *et al.* 2006), up-regulation of these cytokines was not observed in piglets (Meurens, M. Auray, *et al.* 2009). Arrival of neutrophils to the site of infection is associated with an increase of reactive oxygen species (e.g. NO, iNOS), and the release of antimicrobial peptides (e.g. βD-2) due to their bactericidal activity (Meurens, Berri, *et al.* 2009). This normally triggers an anti-inflammatory response to avoid secondary damage to the host (Toms *et al.* 2001). Thus, increased expression of *IL10* and *TGFβ* is observed in infected piglets (Meurens, Berri, *et al.* 2009). If *S. enterica* is able to invade the intestinal wall, phagocytosis of bacterial cells by macrophages and dendritic cells occurs (Kyrova *et al.* 2014). One of the main differences between human and swine salmonellosis is the capability of the latter to act as an asymptomatic carrier. However, the mechanisms by which the pathogen can survive in pigs, and establish a persistent infection are not well understood at present (Verbrugghe *et al.* 2015). Expression of HtpG by *S. enterica*, a homologue of the eukaryote heat shock protein, has been proposed to play an essential role in the survival of the pathogen under stressful conditions (Verbrugghe *et al.* 2015).

**1.3.3 Salmonellosis control in the porcine sector**

Since pigs are one of the main reservoirs of *S. enterica* infecting human beings, several surveillance programs are carried out to decrease the possible contamination of the food chain. Most of the studies conducted to evaluate prevalence of the pathogen have focused on the last stage of the production cycle (finishing pigs) (Wilkins *et al.* 2010). However, studies conducted along the production continuum have demonstrated that infection by the pathogen is present in every stage of swine production (Letellier *et al.* 1999; Wilkins *et al.* 2010). A variety of on-farm mitigation strategies have been attempted with the goal of decreasing the contamination of carcasses and pork. In this regard, reducing animal densities, improving hygiene of the barn, establishing effect pest control programs, testing *Salmonella* in incoming animals, ensuring correct storage of food, applying hazard analysis and critical control point (HACCP) programs in abattoirs, among others, are some of the measures carried out on farms to reduce transmission of the bacterium to food (Berends *et al.* 1996). Other control strategies that have been evaluated include acidification of water (Van der Wolf *et al.* 2001) or food (Creus *et al.*

2007) with the goal of reducing the pH in the stomach. In addition, the administration of coarsely ground meal to increase fermentation in the large intestine, thereby increasing concentrations of short chain fatty acids (SCFAs) has been attempted (Canibe *et al.* 2005). Numerous attempts using probiotics to competitively exclude *S.* Typhimurium in pigs have also been reported (Casey *et al.* 2007). However, the identification of single species or mixtures of microorganisms that are able to persistently and reproducibly colonize the swine intestine excluding the bacterium has not been achieved to date, frustrating the use of probiotics to mitigate *S. enterica* in pigs. Since none of the mitigation strategies evaluated to date have been demonstrated to completely eliminate *S. enterica* from the food chain, several programs of surveillance are carried out in Canada to detect the presence of the bacterium in pork products (Parmley *et al.* 2013). Concerns about *Salmonella* contamination of pork products is compounded by the development of antibiotic resistance (Haley *et al.* 2012). Thus, surveillance programs for *S. enterica* are not only directed to detect the source of contamination along the food chain, but also to track temporal and regional trends in the use of antibiotics and the phenotypic resistance to antibiotics along with carriage of antimicrobial resistance genes (Parmley *et al.* 2013). Since the use of antibiotics in the swine industry is considered to be a potential point of selection and proliferation of cells resistant to antibiotics, the development of new non-antibiotic strategies to mitigate enteric disease has become a priority for the porcine sector.

### 1.4 *Salmonella* Typhimurium pathogenesis in mice

*Salmonella* Typhimurium infection in normal flora mice mimics typhoid-like fever of human beings (Zhang *et al.* 2003; Santos RL *et al.* 2001). The infection is characterized by elevated temperature 4-8 days after oral inoculation without the development of diarrhea (Zhang *et al.* 2003). Pathologic changes in the GIT include diffuse enteritis in the small intestine with a mononuclear leukocyte infiltrate (Zhang *et al.* 2003), edematous and shorter villi, enlarged Peyer's patches, and thicker ileal mucosa (Santos RL *et al.* 2001). After ingestion, *S.* Typhimurium colonizes the intestine of mice via M cells or uptake by dendritic cells, which allows the bacterium to reach the blood stream (Coburn *et al.* 2007; Jones *et al.* 1994). Moreover, the bacteria can be disseminated by $CD18^{+}$ phagocytes residing in the SCV (Coburn *et al.* 2007). Systemic dissemination of *S. enterica* is characterized by colonization of liver, spleen, and mesenteric lymph nodes; internalization of the bacterium within macrophages with the consequent formation of granulomas is accompanied by hepatomegaly and splenomegaly (Santos RL *et al.* 2001). Damage to the liver is the main cause of death in mice with salmonellosis (Santos RL *et al.* 2001).

### 1.4.1 Murine model of *Salmonella* enterocolitis

In order to study human enterocolitis caused by *S.* Typhimurium, normal flora mice need to be administered a broad-spectrum antibiotic (e.g. streptomycin sulfate) to cause a dysbiosis in the autochthonous microbiota, thereby reducing colonization resistance and allowing the pathogen to colonize the intestinal tract (Que *et al.* 1985; Hapfelmeier *et al.* 2005; Barthel *et al.* 2003; Kaiser *et al.* (2012). In dysbiotic mice, *S.* Typhimurium is able to colonize the large intestine 24 h after inoculation. The disease caused by this pathogen starts in the cecum within 8-12 h after the pathogen reaches a density $\geq 10^8$ CFU $g^{-1}$ within digesta in the intestinal lumen (Barthel *et al.* 2003). Animals pretreated with streptomycin excrete higher numbers of *S.* Typhimurium in feces than animals that are not administered the antibiotic (Bohnhoff *et al.* 1954). Mice administered streptomycin and infected with the *S*. Typhimurium present a smaller, pale, and exudative cecum (Barthel *et al.* 2003), and histopathologic analysis show that these mice exhibit similar pathologic alterations to that in pigs and human beings with *Salmonella* enterocolitis (Kaiser *et al.* 2012). In this regard, the cecum of infected animals presents edema of the submucosa, erosion of the epithelial layer, crypt irregularity with elongation, loss of goblet cells, and infiltration of neutrophils that extends through the intestinal wall (Barthel *et al.* 2003; Stecher *et al.* 2007). Although infected mice pretreated with streptomycin exhibit many of the characteristics of human *Salmonella* enterocolitis, some differences exist. For instance, mice with enterocolitis do not present high fluid excretion in the lumen in contrast to other species (Tsolis *et al.* 1999). Additionally, salmonellosis in human beings is limited to the GIT, whereas in mice pretreated with streptomycin, a systemic infection can occur in addition to enterocolitis (Barthel *et al.* 2003). Thus, streptomycin pretreated mice infected with *S*. Typhimurium show higher bacteria loads in the mesenteric lymph node and liver than either pigs or people, which increases over time (Barthel *et al.* 2003).

### 1.4.2 Immune responses in mice

Characterization of the immune response in mice infected with *S.* Typhimurium has been extensively studied (Broz *et al.* 2012; Spees *et al.* 2014; Stecher *et al.* 2007). One of the first barriers *S.* Typhimurium encounters is the mucus layer covering the intestinal epithelium, which is thought to consist of an inner and thinner tightly adherent layer, and an outer loosely adherent layer (Sansonetti *et al.* 2004) (Figure 1.1). A recent study demonstrated that intestinal inflammation in mice pretreated with streptomycin varied along the large intestine, which corresponded to the thickness of the mucus layer. This study proposed that a higher level of inflammation may occur in the cecum due to the reduced thickness of the loosely adherent mucus layer, thereby providing easier access to the intestinal epithelium for the pathogen (Furter *et al.* 2019). Additionally, *S.* Typhimurium is motile, which can facilitate penetration of

both mucus layers in the distal colon of mice, and this may explain the delayed inflammation observed at this site (Furter *et al.* 2019). After *S.* Typhimurium reaches the epithelium in the large intestine, the bacterium uses SPI-1 T3SS to induce an exacerbated inflammatory response (Barthel *et al.* 2003). In this regard, animals inoculated with *S.* Typhimurium mutants that are unable to translocate effector proteins into the host cells induce a mild inflammatory response in the cecum (Barthel *et al.* 2003). These results also demonstrate that the presence of LPS and flagellin are not sufficient to trigger *Salmonella* enterocolitis in mice, which occurs in other species (Everest *et al.* 1999). The pro-inflammatory response triggered in dysbiotic mice after colonization of the intestinal tract by *S*. Typhimurium does not differ substantively from that in pigs (Broz *et al.* 2012). However, since animals pretreated with streptomycin can also present septicemia, several studies have been conducted to determine the role that M cells (Clark *et al.* 1994), CD18 phagocyte/dendritic cell sampling (Rescigno *et al.* 2001), and gastrointestinal-associated lymphoid tissue play in the development of salmonellosis in mice. Some studies have shown that M cells are the first site of attachment and invasion of *S.* Typhimurium (Jones *et al.* 1994) leading to systemic disease in mice. As early as 30 minutes after oral inoculation, *S.* Typhimurium is intimately associated with epithelial cells of the small intestine followed by destruction of the epithelium (Vazquez-Torres *et al.* 1999). Others have postulated that CD18-expressing phagocytes opposed to M cells are primarily responsible for systemic dissemination of the pathogen in mice (Vazquez-Torres *et al.* 1999). It has been demonstrated that lymphoid tissue associated with the intestine does not play an important role in the development of enterocolitis in streptomycin pretreated mice (Barthel *et al.* 2003). In this regard, LTβR null mice that do not form Peyer Patches, colonic associated lymphoid tissues, or lymph nodes, develop *Salmonella* enterocolitis when pretreated with streptomycin without showing any differences compared to wild type animals (Barthel *et al.* 2003).

**1.5 Colonization resistance**

The GIT is an ecosystem with complex interactions among the host and the microbiota that are essential to the health of the host. The GIT harbours microorganisms at densities that range from $10^3$ cells $ml^{-1}$ in the stomach to $10^{11}$ cells $g^{-1}$ in the colon (Xu *et al.* 2003). Thousands of different species are located in the GIT being mainly dominated by bacteria within the *Firmicutes* and *Bacteroidetes* (Donaldson *et al.* 2016). In the small intestine, the microbiota is predominated mainly by facultative anaerobic bacteria (e.g. *Enterobacteriaceae* and *Lactobacillaceae*) that are adapted to growing in acidic pH, high concentrations of bile acids, antimicrobial peptides, and higher levels of oxygen, which allows them to outcompete many obligate anaerobic bacteria for simple carbohydrates (e.g. oligosaccharides) (Zhang *et al.* 2018). In contrast, the large intestinal microbiota is dominated by fermentative obligate

anaerobic bacteria (e.g. *Bacteroidaceae* and *Clostridiaceae*) that are responsible for the breakdown of complex polysaccharides (Donaldson *et al.* 2016). The composition of the microbiota varies along the GIT, and in proximity to mucosa according to their ecological adaptation (Donaldson *et al.* 2016). There are two broad groups of microorganisms found in the GIT, which are allochthonous and autochthonous microorganisms. The former group are microorganisms that originate from a place other than where they are found, and are considered transients. The latter group are microorganisms that are resident in the place they are identified, and comprise microaerobic, facultative anaerobic, and obligate anaerobic bacterial taxa. These autochthonous bacteria may be intimately associated with mucosa/digesta and also transiently present in the gastrointestinal lumen (Xu *et al.* 2003). Whereas, allochthonous taxa are normally not intimately associated with mucosa. Importantly, the enteric autochthonous microbiota plays an essential role in the provision of nutrients, development of gastrointestinal-associated lymphoid tissue, maturation of the intestine, and protection against pathogens (Xu *et al.* 2003; Sansonetti *et al.* 2004; Lawley *et al.* 2013).

The defensive mechanism that the microbiota confers to the host is termed “colonization resistance” (CR), and it is the ability of autochthonous microorganisms to preclude pathogens from effectively colonizing tissues of the GIT (Lawley *et al.* 2013). Although the mechanisms of CR are enigmatic they have been broadly divided into direct and indirect mechanisms in an attempt to better understand their function (Figure 1.2A) (Sassone-Corsi *et al.* 2015). Direct mechanisms of CR include competition for nutrients, occupation of niches including colonization of adhesion sites, metabolic exclusion by production of SCFAs and oxygen consumption, and bactericidal/bacteriostatic function via the production of bacteriocins and delivery of antimicrobial effectors by means of type VI secretion system (Iacob *et al.* 2018; Lawley *et al.* 2013; Jana *et al.* 2019). Competition for niches related with the mucus layer are particularly important (Sicard *et al.* 2017). The intestinal mucus is composed of glycoproteins that act as adhesions sites and a carbon source for microorganisms (Sicard *et al.* 2017). Dominance of different bacterial species in the outer loosely adherent mucus layer depends on their metabolic function, ability to assimilate minerals, and utilization of phospholipids from shed host cells (Li *et al.* 2015). Thus, the presence of ‘commensals’ in the outer mucus layer is essential to inhibit/prevent colonization of enteropathogens (Sicard *et al.* 2017). It has also been observed that bacteria located in the mucus layer are normally coated by secretory IgA (SIgA). The natural secretion of this mucosal antibody has been described as an essential mechanism to maintain enteric homeostasis at the mucosal layer (Mathias *et al.* 2011). The mechanism by which SIgA induces homeostasis is still under study; however, it has been proposed that SIgA would coat commensal bacteria to control sensing of the

commensals by IECs, and thereby preclude triggering a pro-inflammatory response (Mathias *et al.* 2011). Direct competition with pathogens is also performed by commensals via the production of bacteriocins. Bacteriocins are peptides that are either bacteriostatic or bactericidal (Hammami *et al.* 2013). The production of these antimicrobials has mainly been described from Gram-positive bacteria, although some Gram-negative taxa can produce them as well (Hammami *et al.* 2013). The mechanism by which these peptides kill bacterial cells is associated with pore formation in the cell membrane, thereby altering its permeability (Hammami *et al.* 2013). After the pore is formed, the death of the pathogen is triggered by different mechanisms including efflux of ions and metabolites, leakage of cell contents, degradation of DNA, and inhibition of protein or peptidoglycans (Hammami *et al.* 2013). Another mechanism of defense by the commensal microbiota is the production of SCFAs via fermentation of polysaccharides (Rios-Covian *et al.* 2016). SCFAs are aliphatic organic acids including acetate, propionate, and butyrate (Iacob *et al.* 2018). These metabolites, principally acetate and butyrate, contribute to maintaining ecological homeostasis in the GIT by decreasing the luminal pH, stimulating mucin production, enhancing tight junction integrity, and induction of T-regulatory cell differentiation (Rios-Covian *et al.* 2016; Jung *et al.* 2015). Constituents of the commensal microbiota can utilize the Type VI secretion system to deliver effector proteins that directly attack pathogens that compete for the same niche (Jana *et al.* 2019). In conclusion, a variety of direct mechanisms of CR have been implicated in regulating pathogen colonization of the GIT.

Autochthonous microorganisms can also compete with pathogens indirectly. For example, microorganisms can enhance the mucus barrier, and modulate other innate as well as adaptive immune responses. It has been proposed that the normal development of the mucosa layer depends on the commensal microbiota. For example, studies conducted in germ-free mice demonstrated that these animals possess a thinner mucus layer as compared to mice possessing a normal GIT flora (Petersson *et al.* 2011). However, when these animals were treated with lipopolysaccharides or peptidoglycan, the thickness of the mucus layer was restored, which highlights the importance of the microbiota in mucus development (Petersson *et al.* 2011). Another mechanism of indirect competition with pathogens is the enhancement of additional aspects of the innate immune response. For example, a study examining disease incited by *Clostridium difficile* showed that during the infection, commensals were translocated through the large intestine wall resulting in the stimulation of cytokine Il1β production and recruitment of neutrophils to the site of infection, thereby facilitating clearance of *C. difficile* (Hasegawa *et al.* 2012). Another indirect mechanism by which commensals enhance the innate immune response is by stimulating secretion of antimicrobial peptides from Paneth cells (Sansonetti *et al.* 2004). In this regard,

the recognition of pathogen associated molecular patterns of commensals by toll-like receptors can induce secretion of C-type lectins such as RegIIIγ which contributes to the maintenance/restoration of intestinal homeostasis (Vaishnava *et al.* 2011). Moreover, commensal microorganisms can stimulate secretion of α and β-defensins to mitigate pathogen colonization (Salzman *et al.* 2003; Schlee *et al.* 2007). The commensal microbiota can also modulate adaptive immunity by modulating the differentiation of T cells (Ivanov *et al.* 2009). Although the protective role that the microbiota confers to the host is broadly accepted, further studies are needed to fully elucidate the mechanisms by which enteric bacteria directly and indirectly protect the host from enteropathogens including *S*. Typhimurium.

### 1.5.1 The commensal microbiota and *Salmonella* Typhimurium

Some enteropathogens have evolved the ability to overcome CR, and thus colonize the intestine and cause disease. One hypothesis suggests that pathogens incite inflammation to allow the infection to develop (Lupp *et al.* 2007). A proposed mechanism detailing how pathogens may benefit from inflammation is known as the "differential killing hypothesis" (Figure 1.2) (Stecher *et al.* 2008). This hypothesis predicts that inflammation is accompanied by the release of antimicrobial substances (e.g. host-defense peptides, SIgA, nitric oxide) that target specific commensal bacteria responsible for CR, while the pathogen has developed strategies for resisting the harmful substances (Stecher *et al.* 2008). It is possible that *S.* Typhimurium utilizes this strategy to overcome the microbiota and incite disease in pigs and mice (Stecher *et al.* 2007; Drumo *et al.* 2015; Chirullo *et al.* 2015). The major changes observed in the composition of microbiota in animals with *Salmonella* enterocolitis have been attributed to the release of oxygen (e.g. nitric oxide; NO) as a result of the inflammatory response mounted by the host (Drumo *et al.* 2015). Since several members of the microbiota are extremely sensitive to oxygen, the increase in oxygen concentration can alter the structure of commensal bacterial communities (Rigottier-Gois *et al.* 2013). An inflammatory response is normally characterized by an oxidative burst triggered by neutrophils. These immune cells release reactive oxygen species (ROS) such as NO that will target the pathogen leading to its elimination (Winterbourn *et al.* 2016). While certain species can be benefited by this condition (e.g. lactobacilli), others microorganisms are negatively affected (Drumo *et al.* 2015). One of the main groups of bacteria that become less abundant in piglets with *Salmonella* enterocolitis are the SCFA-producing bacteria (Bearson *et al.* 2013). For example, the relative abundance of *Ruminococacceae* and *Prevotellaceae* is lower in infected animals (Drumo *et al.* 2015; Arguello *et al.* 2018). A hallmark of alterations to the enteric microbiota associated with inflamed tissues is a conspicuous increase in the abundance of *Proteobacteria* relative to other phyla (Litvak *et al.* 2017). Since this phylum contains several pathogenic bacteria, including *S. enterica*, the increased relative

abundance of these bacteria in animals infected with *Salmonella* is expected (Lupp *et al.* 2007). However, other *Enterobacteriaceae* taxa (e.g. *E. coli*) associated with inflamed tissue, also increase in abundance independent of the cause of inflammation (e.g. enteropathogens, chemically induce colitis, or host immune deficiencies) (Zeng *et al.* 2017). During inflammation, the higher concentration of ROS and high levels of antimicrobial peptides that occur can have a collateral effect on the commensals, while bacteria within the family *Enterobacteriaceae* have evolved different strategies to subvert these host defense mechanisms (Zeng *et al.* 2017). In this regard, *S. enterica* expresses PhoP-PhoQ inducing acylation of lipid A increasing its resistance to antimicrobial peptides (Broz *et al.* 2012). Another example of how *S. enterica* can benefit from inflammation is the used of tetrathionate as terminal electron acceptor (Winter *et al.* 2010). Colonic bacteria produce high quantities of toxic hydrogen sulphide. To avoid damage by this metabolite, the cecal mucosa converts this component into thiosulfate. When inflammation occurs, thiosulfate in digesta within the intestinal lumen is oxidized to tetrathionate, which *S. enterica* is able to use as an electron acceptor for respiration allowing it to out complete commensal bacteria (Winter *et al.* 2010). Ethanolamine, a nutrient derived from phosphatidylethanolamine and fructose-asparagine is one of the carbon sources that *S. enterica* can consume in the presence of tetrathionate (Thiennimitr *et al.* 2011; Ali *et al.* 2014). Although the commensal microbiota is normally able to outcompete *S. enterica,* the pathogen has evolved several strategies to overcome CR, including exploiting the host inflammatory response to its advantage.

That normal flora mice infected with *S.* Typhimurium infection do not develop enterocolitis has raised several questions related to the role that microbiota plays in this protection (Roy *et al.* 2002). The requirement to disrupt the microbiota (e.g. with the broad-spectrum antibiotic, streptomycin) to produce *Salmonella* enterocolitis illustrates the importance of the microbiota in this pathology (Barthel *et al.* 2003). A study conducted in mice pretreated with streptomycin showed that *S.* Typhimurium interfered with the restoration of the enteric microbiota after 4 days of infection (Stecher *et al.* 2007). As indicated previously, bacteria in the phylum, *Protebacteria* become dominant in the large intestine of mice infected with *S*. Typhimurium (Stecher *et al.* 2007). Early efforts to establish CR against *S.* Typhimurium using defined microbiota mice were unsuccessful, indicating that the presence of a complex microbiota is essential to impair colonization by this pathogen (Stecher *et al.* 2010). However, a recent study showed that colonizing mice with a mix of 12 different strains from the phyla, *Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia,* and *Actinobacteria* was able to establish CR against *S.* Typhimurim (Brugiroux *et al.* 2016). Thus, the presence of a complex microbiota is essential to fully

elucidate mechanisms of *Salmonella* enterocolitis (i.e. pertaining to the host-pathogen-microbiota interaction) in mice and other animals.

**1.6 Host defense peptides and infection**

Host-defense peptides also known as antimicrobial peptides are an essential component of the innate immune system of mammals. They may also be important regulators of CR (Figure 1.2B). These peptides comprise α- and β-defensins, C-type lectins, and cathelicidins (Brogden *et al.* 2005). Their amphipathic characteristics allow these molecules to attach to the bacterial wall inducing cellular lysis (Gallo *et al.* 2012). Their antimicrobial activity is essential to regulate densities of bacteria, including opportunistic and obligate pathogens in proximity of mucosal surfaces (Sansonetti *et al* 2004). Alpha-defensins are continuously secreted by specialized epithelial cells located at the bottom of the intestinal crypt, known as Paneth cells in the small intestine (Keshav *et al* 2006). In contrast, β-defensins are expressed along the entire GIT including the cecum and colon (Veldhuizen *et al.* 2007). Both types of defensins have a broad antimicrobial spectrum, and are active against Gram-positive bacteria, Gram-negative bacteria, viruses, fungi, and protozoa (Gallo *et al.* 2012). In piglets, the production of β-defensin 2 has been shown to impair growth of *S.* Typhimurium (Veldhuizen *et al.* 2008). C-type lectins include the regenerating islet-derived protein (REG) family of peptides. The RegIII family of host defense peptides are excreted in the small intestine of mammals, but can also be produced in the large intestine in response to infection (Ogawa *et al.* 2003). Their capability to target peptidoglycans is responsible for their bactericidal activity against Gram-positive bacteria (Gallo *et al.* 2012). Notably, elevated expression of RegIIIγ has been observed in cecal tissue after *S.* Typhimurium infection (Godinez *et al.* 2009); however, the direct impact of RegIIIγ on the pathogen is currently unknown.

Cathelicidins are a group of antimicrobial peptides that have been identified in invertebrates, vertebrates, and various mammals including human beings (Gudmundsson *et al.* 1996), monkeys (Bals *et al.* 2001), pigs (Holani *et al.* 2016), cows (Zanetti *et al.* 1993), rats (Travis *et al.* 2000), and mice (Gallo *et al.* 1997), among others. These peptides are encoded by a single gene that contains four exons. Exons 1 to 3 encode an N-terminal conserved signal peptide, and a cathelin-like pro-peptide while exon 4 encodes a hypervariable C-terminal domain that must be cleaved for the peptide to effect the antimicrobial activity (Figure 1.3A) (Iimura *et al.* 2005; Neeloffer Mookherjee *et al.* 2013). The hypervariable C-terminal domain has determined the different types of cathelicidins observed in different species. Thus, while in swine thirteen different cathelicidins have been described (Table 1.1) in mice the murine cathelicidin-related antimicrobial peptide (mCRAMP) is the only cathelicidin described at the moment (Neeloffer Mookherjee *et al.* 2013). Cathelicidins are widely distributed in different

tissues including, skin, intestine, lungs, mucosa, among others, and they are mainly found in their unprocessed form in epithelial cells, neutrophils, mast cells, keratinocytes and lymphocytes (Bals *et al.* 2003). These proteins are divided into three different groups according to their biochemical characteristics (Gallo *et al.* 2012). Human cathelicidin (LL-37) and murine cathelicidin (mCRAMP) are part of the same group presenting an amphipathic α-helix structure. The second group of cathelicidins is characterized by its high content of proline and arginine; PR-39 produced by pigs is a primary cathelicidin of this type. The last group of cathelicidins comprises the protegrins, which have a β-sheet structure (Gallo *et al.* 2012) (Figure 1.3B). Similarly to defensins, cathelicidins possess a broad antimicrobial spectrum of activity against Gram-positive bacteria, Gram-negative bacteria, fungi, viruses, and protozoa (Bals *et al.* 2003). Their mechanism of action is produced by an initial electrostatic interaction between their positive charged and the negative charged molecules on the surface of their target cells (Zaiou *et al.* 2002). After the first contact has occurred, these peptides are able to form pores in the membrane of the target cell altering their permeability leading to cellular death (Bals *et al.* 2003). Additionally, PR-39 can inhibit protein and RNA synthesis (Veldhuizen *et al.* 2014). The importance of these proteins in immune defense extends beyond their antimicrobial activity and includes immunomodulation (Veldhuizen *et al.* 2014), enhance wound healing (Gallo *et al.* 1994), and stimulate angiogenesis (Koczulla *et al.* 2003). The immunomodulatory action of these peptides involves several mechanisms (Figure 1.4). For example, cathelicidins can stimulate direct and indirect chemoattraction of leukocytes by direct activation of formyl peptide receptors like (FPRL) or by upregulation of chemokine release such as CCL2, CCL10, IL8 among others (Kurosaka *et al.* 2005). Cathelicidins have also been demonstrated to modulate phagocytes function by enhancing degranulation of neutrophils and mast cells, stimulating NO production by macrophages, activating phagocytosis by acting as opsonins or by upregulation of TLR expression in phagocytes and inducing bacterial DNA/RNA uptake (van Harten *et al.* 2018). These host defense peptides can also modulate the immune response by upregulation of pro-inflammatory cytokines and receptors (e.g. TNFα, IL1β, IL6, IL36, IL1R, IFNγR) or controlling an exacerbated immune reaction by inducing anti-inflammatory cytokines (e.g. IL10) or by neutralizing endotoxins (van Harten *et al.* 2018). Additionally, cathelicidins have been described to modulate B cell proliferation and dendritic cells differentiation (van Harten *et al.* 2018). The activity of these peptides against a variety of different enteropathogens has been investigated *in vitro*. In this regard, the antimicrobial activity of PR-39 has been demonstrated against *E. coli* (Fan *et al.* 2010), *Mycobacterium tuberculosis* (Linde *et al.* 2001), and *S.* Typhimurium (Mukherjee *et al.* 2015). mCRAMP has also been shown to impair skin infection by Group A *Streptococcus* (Nizet *et al.* 2001), mitigate urinary infections

by *E. coli* (Chromek *et al.* 2006), and ameliorate infection of the GIT by *Citrobacter rodentium* (Iimura *et al.* 2005). To my knowledge, the impact of mCRAMP on *S.* Typhimurium has been restricted to *in vitro* evaluations (Gallo *et al.* 1997). Additionally, intracellular expression of mCRAMP in macrophages has been shown to impair *S.* Typhimurium cell division (Rosenberger CM *et al.* 2004 Feb 24). The *in vivo* impacts of cathelicidins on salmonellosis are currently unknown. However, the secretion of mCRAMP has been shown to modulate the composition of the microbiota (Yoshimura *et al.* 2018), and it is thought to be important in maintaining homeostasis within the colon. Elucidating the degree and how cathelicidins influence infection by *S*. Typhimurium and ensuing disease (e.g. enterocolitis) may provide valuable information to facilitate the development of novel mitigation strategies.

**1.7 A knockout mouse model**

Knockout mice models have been used extensively to elucidate mechanisms of intestinal disease. The use of mice lacking a particular gene allows evaluation of a specific function(s) associated with that gene, and the effects that its absence imparts on the host (i.e. relative to the wild type genotype). The development of a cathelicidin-knock out mice has facilitated the understanding of the role of cathelicidin in mucosal immune responses. In this regard, mCRAMP null mice show increased susceptibility to infections of skin (Nizet *et al.* 2001), urinary tract (Chromek *et al.* 2006), and GIT (Chromek *et al.* 2006), which have been attributed to the antimicrobial and immunomodulatory role of this cathelicidin. Additionally, a comparative examination of wild type and mCRAMP null mice has shown the important role that cathelicidin plays in homeostasis of the colonic microbiota (Yoshimura *et al.* 2018). Despite the salient advantages of using knockout models to elucidate mechanisms, it is important to consider potential confounding effects that may exist when using these model organisms. For example, the removal of the mCRAMP gene may result in qualitative differences in the commensal bacterial flora within the intestine of the knockout relative to wild type mice, thereby confounding the ability to identify direct effects of cathelicidin on the immune modulation. Taking into account the limitations that this model can have, it is still a valuable tool available to elucidate the mechanisms by which cathelicidins can modify the host response to *S.* Typhimurium infection, and also to characterize the key aspects of the pathogen-host-microbiota interactions *in vivo.*

**1.8 Knowledge gaps**

Although *S.* Typhimurium infection has been studied extensively, there are many aspects of the host-pathogen interaction that remain unknown. Importantly, effective and non-antibiotic methods to control porcine infection with the pathogen and contamination of the food chain are yet to be developed. Moreover, the capacity of the bacterium to persists in piglets being these asymptomatic

carriers is a primary concern of the swine sector. Importantly, the use of antibiotics has resulted in the selection of *S.* Typhimurium strains that are resistant, which adversely impacts both the swine industry and human health. Comprehensive studies that characterize salmonellosis in pigs with emphasis on the elucidation of temporal and spatial host responses in concert with modifications to the microbiota are lacking. The structure of the microbiota varies from the small intestine to the large intestine, and from the mucosa to the lumen (Donaldson *et al.* 2016). Therefore, assessing the impact that *S.* Typhimurium has in the different locations of the intestinal tract and at the different stages of the disease is expected to provide valuable information to establish which bacterial species are more affected by the pathogen, and may be key regulators of CR. Therefore, I propose to evaluate modifications in the structure of the microbiota in relation to temporal and spatial immune response in piglets experimentally infected with a virulent serotype of *S.* Typhimurium DT104. Given the emergence of antimicrobial resistance in *S.* Typhimurium and increasing restrictions on antibiotic use in swine production the development of new and non-antibiotic strategies to treat these infections is imperative. Elucidation of function of host defense peptides, such as cathelicidin PR-39, at the different stages of salmonellosis could provide valuable information toward the use of this peptide as a new strategy to mitigate this important zoonotic pathogen.

Mouse models have been used to study various human pathologies for years. In the particular case of the murine model of *Salmonella* enterocolitis, a dysbiosis must be generated to mimic the disease that occurs in human beings and pigs. To mimic the complex interplay between the microbiota and the host during *Salmonella* enterocolitis, the structure of the microbiota needs to be conserved when studying enteric diseases. As indicated previously, mCRAMP null mice are more susceptible to infection, but to my knowledge, the use of mCRAMP$^{-/-}$ mice to ascertain the function that mCRAMP has on *S.* Typhimurium infection and ensuring disease has not been done. Thus, I propose to temporally compare infection and disease in wild type and mCRAMP knockout C57BL/6J mice challenged with *S.* Typhimurium to elucidate the role that this cathelicidin plays in salmonellosis. Additionally, the use of this model without the administration of streptomycin may allow me to ascertain the mechanisms of CR in relation to disease incited by this important pathogen.

### 1.9 Objectives and hypotheses

#### 1.9.1 Porcine *Salmonella* inflammation study

The first study presented in this thesis aimed to achieve the following objectives: (i) establish a porcine model of acute, subacute, and chronic inflammation cause by *S.* Typhimurium; (ii) temporally and spatially characterize the enteric microbiota with or without *S.* Typhimurium; (iii) examine temporal

host responses to colonization and disease by *S.* Typhimurium; and (iv) examine the potential role that host defense peptides play in *Salmonella* enterocolitis. The hypotheses tested were: (a) infection by *Salmonella* Typhimurium will progress temporally, from acute to chronic disease, which will be indicated by the host response to infection; (b) *Salmonella* Typhimurium will induce a pro-inflammatory response that will correspond to temporal and spatially modification of the enteric microbiota; and (c) the cathelicidin, PR-39 will be temporally and spatially upregulated in piglets with *Salmonella* enterocolitis.

### 1.9.2 mCRAMP null mice and *Salmonella* enterocolitis

The second study presented in this thesis included the following objectives: (i) establish and use an mCRAMP knockout murine model to ascertain the pathogenicity/virulence of *Salmonella* Typhimurium; (ii) examine temporal and spatial colonization of the intestine by *S.* Typhimurium; (iii) elucidate the role that cathelicidin plays in *Salmonella* Typhimurium infection; (iv) determine how cathelicidins affect host immune responses; and (v) elucidate the role that cathelicidin plays in homeostasis of the microbiota of animals infected with S. Typhimurium. The hypotheses tested were: (a) mCRAMP regulates local and systemic infection by *S.* Typhimurium; (b) mCRAMP modulates the enteric microbiota and CR increasing susceptibility to *S.* Typhimurium; and (c) mCRAMP plays an important role in regulating the host innate immune response to *Salmonella* Typhimurium.

### 1.10 Tables and figures

Table 1.1 Cathelicidins in swine

| Peptide | Structure | Sequence |
|---|---|---|
| PMAP23 | α-Helical | RIIDLLWRVRRPQKPKFVTVWVR |
| PMAP36 | α-Helical | GRFRRLRKKTRKRLKKIGKVLKWIPPIVGSIPLGC |
| PMAP37 | α-Helical | GLLSRLRDFLSDRGRRLGEKIERIGQKIKDLSEFFQS |
| PR39 | Extended | RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRFPPRFP |
| Prophenin-1 | Extended | AFPPPNVPGPRFPPPNFPGPRFPPPNFPGPRFPPPNFPGPRFPPPNFPGPPFP PPIFPGPWFPPPPPFRPPPFGPPRFP |
| Prophenin-2 | Extended | AFPPPNVPGPRFPPPNVPGPRFPPPNFPGPRFPPPNFPGPRFPPPNFPGPPFP PPIFPGPWFPPPPPFRPPPFGPPRFP |
| Protegrin-1 | β-Hairpin | RGGRLCYCRRRFCVCVGR |
| Protegrin-2 | β-Hairpin | RGGRLCYCRRRFCICV |
| Protegrin-3 | β-Hairpin | RGGGLCYCRRRFCVCVGR |
| Protegrin-4 | β-Hairpin | RGGRLCYCRGWICFCVGR |
| Protegrin-5 | β-Hairpin | RGGRLCYCRPRFCVCVGR |

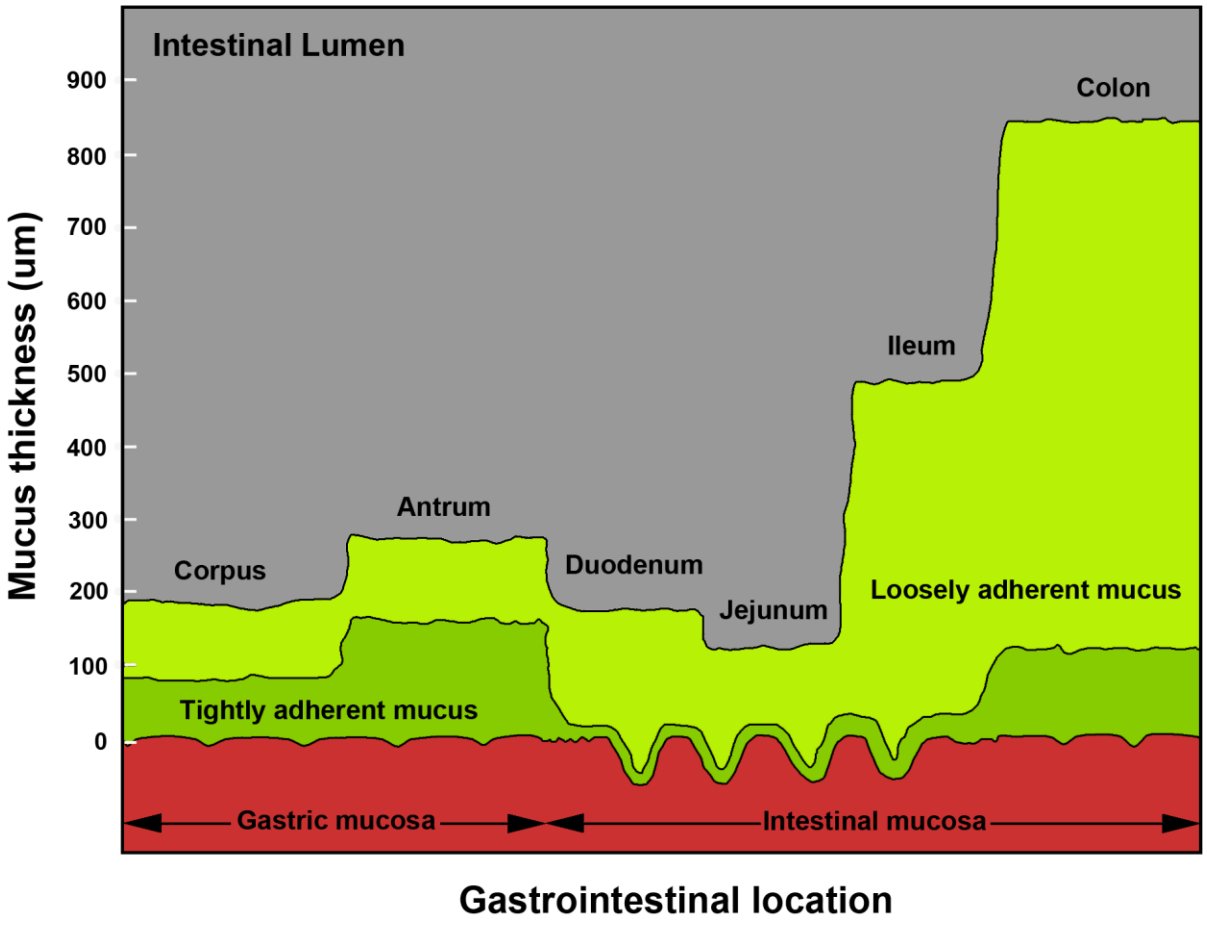


Figure 1.1 Schematic representation of the thickness of the tightly adherent and loosely adherent mucus layer along the gastrointestinal tract. Recreated from (Inglis *et al.* 2012).

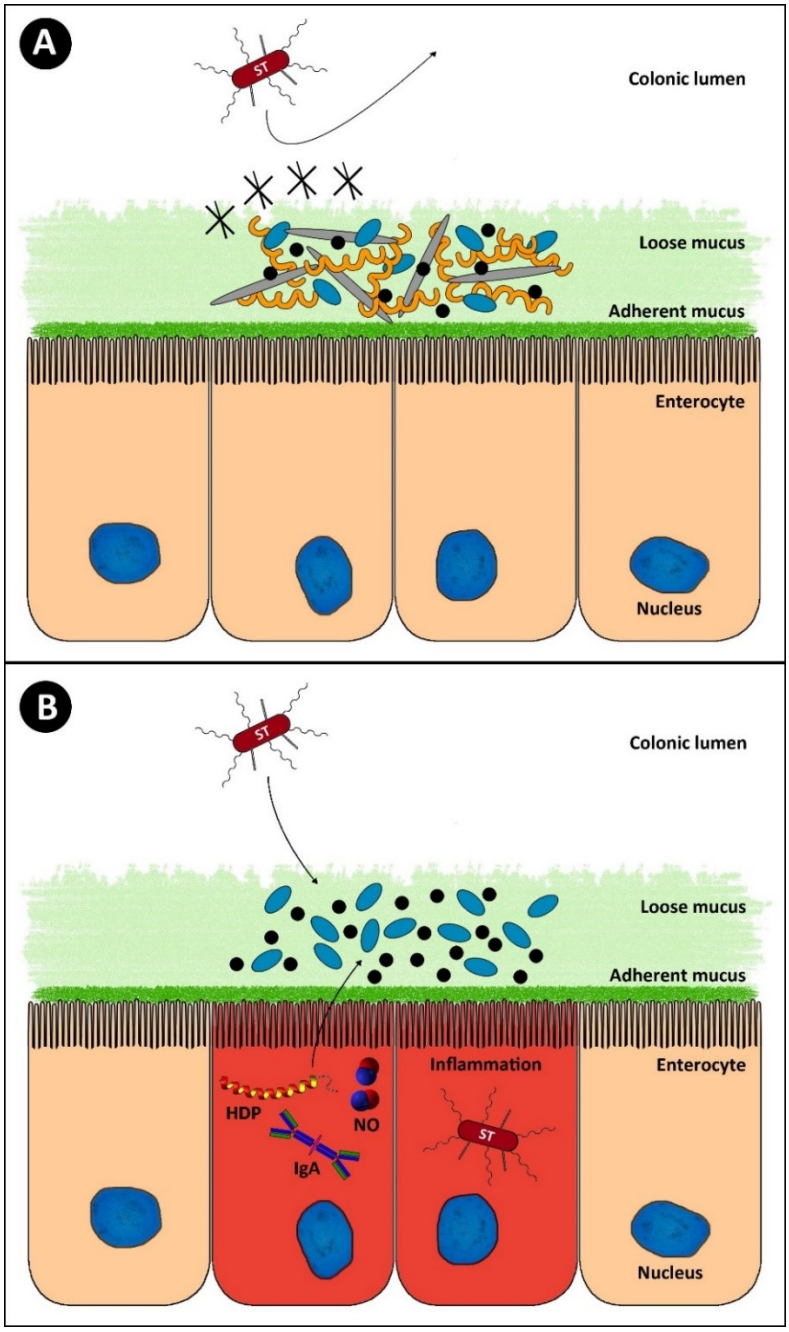


Figure 1.2 Schematic figure provided by Nathal Puhl and G. Douglas Inglis representing the differential killing hypothesis. (A) Direct and indirect mechanisms of CR performed by a eubiotic microbiota are in place avoiding in this way colonization by the pathogen. (B) *Salmonella enterica* remotely induces a host response (e.g. secretion of HDPs, production of sIgA, and/or neutrophil infiltration with increase of NO) that differently affects the autochthonous bacterial community resulting in a dysbiosis within the loosely adherent mucus layer, which reduces CR allowing the pathogen to access the epithelium and incite an inflammatory response.

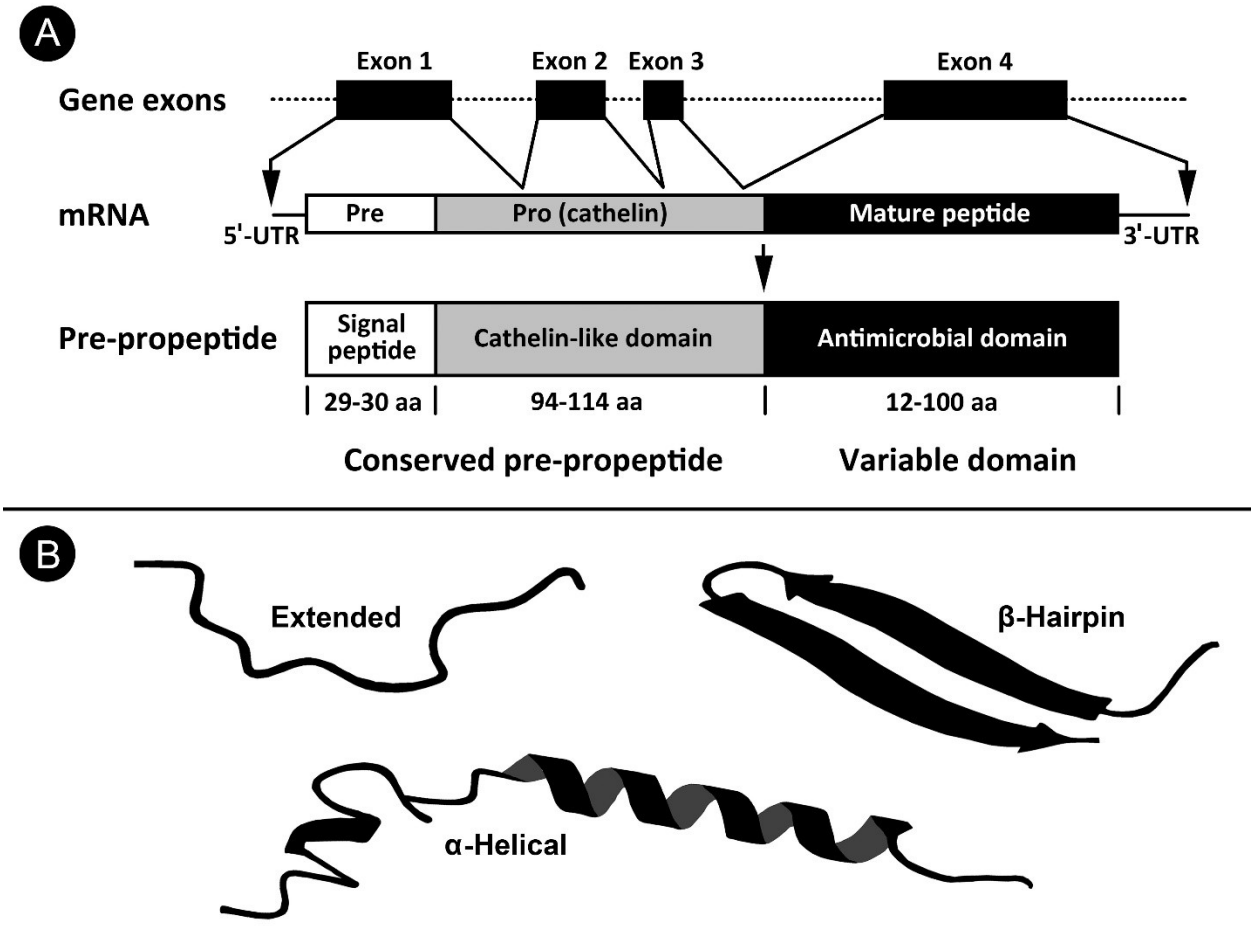


Figure 1.3 Schematic representation of cathelicidins. (A) Gene structure and processing. Exon 1-3 encode the conserved signal peptide and the cathelin-like domain, while exon 4 encodes the hypervariable region for the antimicrobial domain; (B) Ribbon structures of cathelicidin mature peptides extended (PR-39), β-hairpin, and α-helical (mCRAMP) structures. Recreated from Neeloffer Mookherjee *et al.* (2013).

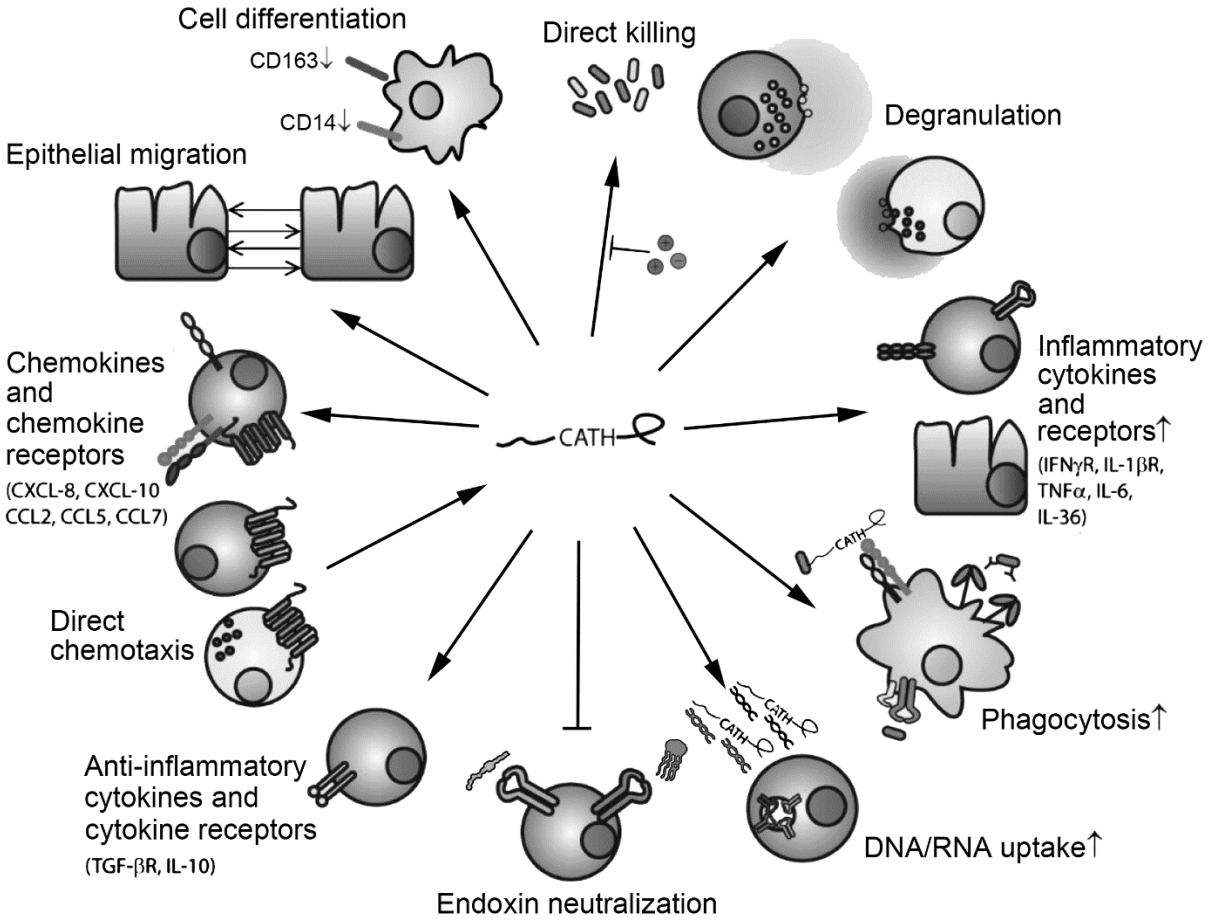


Figure 1.4 Summary of cathelicidin biological functions, figure adapted from van Harten *et al.* (2018). Additionally to the direct killing of Gram-positive, Gram-negative bacteria, viruses, fungi and parasites, cathelicidin can enhance degranulation of granulocytes, induce inflammatory cytokines and their receptors, stimulate phagocytosis opsonizing bacteria, increasing pattern recognition receptors and increasing DNA/RNA uptake. They can avoid activation of TLR2 and TLR4 by neutralizing endotoxins. Cathelicidins can also upregulate anti-inflammatory cytokines and their receptors. Furthermore, they can directly and indirectly induce chemotaxis, wound healing. In addition, cathelicidins can modulate cell differentiation by polarizing macrophages to inflammatory phenotypes (M1).

### 1.11 References


Agbor, T. A., and B. A. McCormick. 2011. '*Salmonella* effectors: important players modulating host cell function during infection', *Cell Microbiol*, 13: 1858-69.

Ali, M. M., D. L. Newsom, J. F. Gonzalez, A. Sabag-Daigle, C. Stahl, B. Steidley, J. Dubena, J. L. Dyszel, J. N. Smith, Y. Dieye, R. Arsenescu, P. N. Boyaka, S. Krakowka, T. Romeo, E. J. Behrman, P. White, and B. M. Ahmer. 2014. 'Fructose-asparagine is a primary nutrient during growth of *Salmonella* in the inflamed intestine', *PLoS Pathog*, 10: e1004209.

Althouse, C. , S. Patterson, P. Fedorka-Cray, and R. E. Isaacson. 2003. 'Type 1 fimbriae of *Salmonella enterica* serovar Typhimurium bind to enterocytes and contribute to colonization of swine in vivo', *Infect Immun*, 71: 6446-52.

Arguello, H., J. Estelle, S. Zaldivar-Lopez, A. Jimenez-Marin, A. Carvajal, M. A. Lopez-Bascon, F. Crispie, O. O'Sullivan, P. D. Cotter, F. Priego-Capote, L. Morera, and J. J. Garrido. 2018. 'Early *Salmonella* Typhimurium infection in pigs disrupts microbiome composition and functionality principally at the ileum mucosa', *Sci Rep*, 8: 7788.

Baggesen, D. L., G. Sorensen, E. M. Nielsen, and H. C. Wegener. 2010. 'Phage typing of *Salmonella* Typhimurium - is it still a useful tool for surveillance and outbreak investigation?', *Euro Surveill*, 15: 19471.

Bals, R., C. Lang, D. J. Weiner, C. Vogelmeier, U. Welsch, and J. M. Wilson. 2001. 'Rhesus monkey (Macaca mulatta) mucosal antimicrobial peptides are close homologues of human molecules', *Clin Diagn Lab Immunol*, 8: 370-5.

Bals, R., and J. M. Wilson. 2003. 'Cathelicidins--a family of multifunctional antimicrobial peptides', *Cell Mol Life Sci*, 60: 711-20.

Barthel, M. , S. Hapfelmeier, L. Quintanilla-Martinez, M. Kremer, M. Rohde, M. Hogardt, K. Pfeffer, H. Russmann, and W. D. Hardt. 2003. 'Pretreatment of Mice with Streptomycin Provides a *Salmonella enterica* Serovar Typhimurium Colitis Model That Allows Analysis of Both Pathogen and Host', *Infect Immun*, 71: 2839-58.

Bearson, S. M., H. K. Allen, B. L. Bearson, T. Looft, B. W. Brunelle, J. D. Kich, C. K. Tuggle, D. O. Bayles, D. Alt, U. Y. Levine, and T. B. Stanton. 2013. 'Profiling the gastrointestinal microbiota in response to *Salmonella*: low versus high *Salmonella* shedding in the natural porcine host', *Infect Genet Evol*, 16: 330-40.

Berends, B. R. , H. A. Urlings, J. M. Snijders, and F. Van Knapen. 1996. 'Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs', *Int J Food Microbiol*, 30: 37-53.

Bohnhoff, M. , B. L. Drake, and C. P. Miller. 1954. 'Effect of streptomycin on susceptibility of intestinal tract to experimental *Salmonella* infection', *Proc Soc Exp Biol Med*, 86: 132-7.

Boyen, F. , F. Pasmans, F. Van Immerseel, E. Morgan, C. Adriaensen, J. P. Hernalsteens, A. Decostere, R. Ducatelle, and F. Haesebrouck. 2006. '*Salmonella* Typhimurium SPI-1 genes promote intestinal but not tonsillar colonization in pigs', *Microbes Infect*, 8: 2899-907.

Boyen, F. Haesebrouck, F. Maes, D. Van Immerseel, F. Ducatelle, and F. R. Pasmans. 2008. 'Non-typhoidal *Salmonella* infections in pigs: a closer look at epidemiology, pathogenesis and control', *Vet Microbiol*, 130: 1-19.

Boyle, E. C. , N. F. Brown, and B. B. Finlay. 2006. '*Salmonella enterica* serovar Typhimurium effectors SopB, SopE, SopE2 and SipA disrupt tight junction structure and function', *Cell Microbiol*, 8: 1946-57.

Brisson, Yan. 2014. 'The changing face of the Canadian hog industry. Available at: https://www.statcan.gc.ca/'.

Brogden, K. A. 2005. 'Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?', *Nat Rev Microbiol*, 3: 238-50.

Brown, K., R. R. E. Uwiera, M. L. Kalmokoff, S. P. J. Brooks, and G. D. Inglis. 2017. 'Antimicrobial growth promoter use in livestock: a requirement to understand their modes of action to develop effective alternatives', *Int J Antimicrob Agents*, 49: 12-24.

Broz, P., M. B. Ohlson, and D. M. Monack. 2012. 'Innate immune response to *Salmonella* Typhimurium, a model enteric pathogen', *Gut Microbes*, 3: 62-70.

Brugiroux, S., M. Beutler, C. Pfann, D. Garzetti, H. J. Ruscheweyh, D. Ring, M. Diehl, S. Herp, Y. Lotscher, S. Hussain, B. Bunk, R. Pukall, D. H. Huson, P. C. Munch, A. C. McHardy, K. D. McCoy, A. J. Macpherson, A. Loy, T. Clavel, D. Berry, and B. Stecher. 2016. 'Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium', *Nat Microbiol*, 2: 16215.

Canibe, N. , O. Hojberg, S. Hojsgaard, and B. B. Jensen. 2005. 'Feed physical form and formic acid addition to the feed affect the gastrointestinal ecology and growth performance of growing pigs', *J Anim Sci*, 83: 1287-302.

Casey, P. G. , G. E. Gardiner, G. Casey, B. Bradshaw, P. G. Lawlor, P. B. Lynch, F. C. Leonard, C. Stanton, R. P. Ross, G. F. Fitzgerald, and C. Hill. 2007. 'A five-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with *Salmonella enterica* Serovar Typhimurium', *Appl Environ Microbiol*, 73: 1858-63.

Chirullo, B. , M. Pesciaroli, R. Drumo, J. Ruggeri, E. Razzuoli, C. Pistoia, P. Petrucci, N. Martinelli, L. Cucco, L. Moscati, M. Amadori, C. F. Magistrali, G. L. Alborali, and P. Pasquali. 2015. '*Salmonella* Typhimurium exploits inflammation to its own advantage in piglets', *Front Microbiol*, 6: 985.

Chromek, M., Z. Slamova, P. Bergman, L. Kovacs, L. Podracka, I. Ehren, T. Hokfelt, G. H. Gudmundsson, R. L. Gallo, B. Agerberth, and A. Brauner. 2006. 'The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection', *Nat Med*, 12: 636-41.

Clark, M. A., M. A. Jepson, N. L. Simmons, and B. H. Hirst. 1994. 'Preferential interaction of *Salmonella* Typhimurium with mouse Peyer's patch M cells', *Res Microbiol*, 145: 543-52.

Coburn, B. , G. A. Grassl, and B. B. Finlay. 2007. '*Salmonella*, the host and disease: a brief review', *Immunol Cell Biol*, 85: 112-8.

Collado-Romero, M. , C. Arce, M. Ramirez-Boo, A. Carvajal, and J. J. Garrido. 2010. 'Quantitative analysis of the immune response upon *Salmonella* Typhimurium infection along the porcine intestinal gut', *Vet Res*, 41: 23.

Creus, E. , J. F. Perez, B. Peralta, F. Baucells, and E. Mateu. 2007. 'Effect of acidified feed on the prevalence of *Salmonella* in market-age pigs', *Zoonoses Public Health*, 54: 314-9.

De Freitas Neto, O. C., Penha Filho, R. A. C., P. Barrow, and A. & Berchieri Junior. 2010. 'Sources of human non-typhoid salmonellosis: a review', *Rev Bras Cien Avic*, 12(1): 01-11.

Donaldson, G. P., S. M. Lee, and S. K. Mazmanian. 2016. 'Gut biogeography of the bacterial microbiota', *Nat Rev Microbiol*, 14: 20-32.

Drumo, R., M. Pesciaroli, J. Ruggeri, M. Tarantino, B. Chirullo, C. Pistoia, P. Petrucci, N. Martinelli, L. Moscati, E. Manuali, S. Pavone, M. Picciolini, S. Ammendola, G. Gabai, A. Battistoni, G. Pezzotti, G. L. Alborali, V. Napolioni, P. Pasquali, and C. F. Magistrali. 2015. '*Salmonella enterica* serovar typhimurium exploits inflammation to modify swine intestinal microbiota', *Front Cell Infect Microbiol*, 5: 106.

Everest, P., J. Ketley, S. Hardy, G. Douce, S. Khan, J. Shea, D. Holden, D. Maskell, and G. Dougan. 1999. 'Evaluation of *Salmonella* Typhimurium mutants in a model of experimental gastroenteritis', *Infect Immun*, 67: 2815-21.

Fan, F., Y. Wu, and J. Liu. 2010. 'Expression and purification of two different antimicrobial peptides, PR-39 and Protegrin-1 in *Escherichia coli*', *Protein Expr Purif*, 73: 147-51.

Fedorka-Cray, P. J. , L. C. Kelley, T. J. Stabel, J. T. Gray, and J. A. Laufer. 1995. 'Alternate routes of invasion may affect pathogenesis of *Salmonella* Typhimurium in swine', *Infect Immun*, 63: 2658-64.

'FoodNet Canada annual report 2017'. 2018. *Available at: https://www.canada.ca/*.
Fu, Y., and J. E. A & Galán. 1999. '*Salmonella* protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion.', *Nature,*, 401(6750): 293-97.
Furter, M., M. E. Sellin, G. C. Hansson, and W. D. Hardt. 2019. 'Mucus Architecture and Near-Surface Swimming Affect Distinct *Salmonella* Typhimurium Infection Patterns along the Murine Intestinal Tract', *Cell Rep*, 27: 2665-78 e3.
Gallo, R. L., and L. V. Hooper. 2012. 'Epithelial antimicrobial defence of the skin and intestine', *Nat Rev Immunol*, 12: 503-16.
Gallo, R. L., K. J. Kim, M. Bernfield, C. A. Kozak, M. Zanetti, L. Merluzzi, and R. Gennaro. 1997. 'Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse', *J Biol Chem*, 272: 13088-93.
Gallo, R. L., M. Ono, T. Povsic, C. Page, E. Eriksson, M. Klagsbrun, and M. Bernfield. 1994. 'Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds', *Proc Natl Acad Sci U S A*, 91: 11035-9.
Gewirtz, A. T. , T. A. Navas, S. Lyons, P. J. Godowski, and J. L. Madara. 2001. 'Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression', *J Immunol*, 167: 1882-5.
Gewirtz, A. T. , P. O. Simon, C. K. Jr. Schmitt, L. J. Taylor, C. H. Hagedorn, A. D. O'Brien, A. S. Neish, and J. L. Madara. 2001. '*Salmonella* Typhimurium translocates flagellin across intestinal epithelia, inducing a proinflammatory response', *J Clin Invest*, 107: 99-109.
Giannella, R. A., S. B. Formal, G. J. Dammin, and H. & Collins. 1973. 'Pathogenesis of salmonellosis Studies of fluid secretion, mucosal invasion, and morphologic reaction in the rabbit ileum.', *J Clin Invest*, 52(2): 441.
Glynn, J. R. , and S. R. Palmer. 1992. 'Incubation period, severity of disease, and infecting dose: evidence from a *Salmonella* outbreak', *Am J Epidemiol*, 136: 1369-77.
Godinez, I., M. Raffatellu, H. Chu, T. A. Paixao, T. Haneda, R. L. Santos, C. L. Bevins, R. M. Tsolis, and A. J. Baumler. 2009. 'Interleukin-23 orchestrates mucosal responses to *Salmonella enterica* serotype Typhimurium in the intestine', *Infect Immun*, 77: 387-98.
Gudmundsson, G. H., B. Agerberth, J. Odeberg, T. Bergman, B. Olsson, and R. Salcedo. 1996. 'The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes', *Eur J Biochem*, 238: 325-32.
Gutzmann, F. , H. Layton, K. Simkins, and H. Jarolmen. 1976. 'Influence of antibiotic-supplemented feed on occurrence and persistence of *Salmonella* typhimurium in experimentally infected swine', *Am J Vet Res*, 37: 649-55.
Haley, C. A. , D. A. Dargatz, E. J. Bush, M. M. Erdman, and P. J. Fedorka-Cray. 2012. '*Salmonella* prevalence and antimicrobial susceptibility from the National Animal Health Monitoring System Swine 2000 and 2006 studies', *J Food Prot*, 75: 428-36.
Hammami, R., B. Fernandez, C. Lacroix, and I. Fliss. 2013. 'Anti-infective properties of bacteriocins: an update', *Cell Mol Life Sci*, 70: 2947-67.
Hapfelmeier, S. , and W. D. Hardt. 2005. 'A mouse model for *S.* typhimurium-induced enterocolitis', *Trends Microbiol*, 13: 497-503.
Hardt, W. D., L. M. Chen, K. E. Schuebel, X. R. Bustelo, and J. E & Galán. 1998. '*S.* typhimurium encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells.', *Cell Host Microbe*, 93(5): 815-26.
Hasegawa, M., N. Kamada, Y. Jiao, M. Z. Liu, G. Nunez, and N. Inohara. 2012. 'Protective role of commensals against *Clostridium difficile* infection via an IL-1beta-mediated positive-feedback loop', *J Immunol*, 189: 3085-91.

Helms, M., S. Ethelberg, K. Molbak, and D. T. Study Group. 2005. 'International *Salmonella* Typhimurium DT104 infections, 1992-2001', *Emerg Infect Dis*, 11: 859-67.
Hensel, M. 2000. '*Salmonella* pathogenicity island 2', *Mol Microbiol*, 36: 1015-23.
Henzler, D. J. , and H. M. Opitz. 1992. 'The role of mice in the epizootiology of *Salmonella* Enteritidis infection on chicken layer farms', *Avian Dis*, 36: 625-31.
Hobbie, S., L. M. Chen, R. J. Davis, and J. E. & Galan. 1997. 'Involvement of mitogen-activated protein kinase pathways in the nuclear responses and cytokine production induced by *Salmonella* Typhimurium in cultured intestinal epithelial cells.', *J Immunol*, 159(11): 5550-59.
Holani, R., C. Shah, Q. Haji, G. D. Inglis, R. R. E. Uwiera, and E. R. Cobo. 2016. 'Proline-arginine rich (PR-39) cathelicidin: structure, expression and functional implication in intestinal health', *Comp Immunol Microbiol Infect Dis*, 49: 95-101.
Iacob, S., D. G. Iacob, and L. M. Luminos. 2018. 'Intestinal Microbiota as a Host Defense Mechanism to Infectious Threats', *Front Microbiol*, 9: 3328.
Iimura, M., R. L. Gallo, K. Hase, Y. Miyamoto, L. Eckmann, and M. F. Kagnoff. 2005. 'Cathelicidin Mediates Innate Intestinal Defense against Colonization with Epithelial Adherent Bacterial Pathogens', *J Immunol*, 174: 4901-07.
Inglis, G. D., M. C. Thomas, D. K. Thomas, M. L. Kalmokoff, S. P. Brooks, and L. B. Selinger. 2012. 'Molecular methods to measure intestinal bacteria: a review', *J AOAC Int,* 95: 5-23.
Ivanov, II, K. Atarashi, N. Manel, E. L. Brodie, T. Shima, U. Karaoz, D. Wei, K. C. Goldfarb, C. A. Santee, S. V. Lynch, T. Tanoue, A. Imaoka, K. Itoh, K. Takeda, Y. Umesaki, K. Honda, and D. R. Littman. 2009. 'Induction of intestinal Th17 cells by segmented filamentous bacteria', *Cell*, 139: 485-98.
Jana, B., and D. Salomon. 2019. 'Type VI secretion system: a modular toolkit for bacterial dominance', *Future Microbiol*, 14: 1451-63.
Jones, B. D., N. Ghori, and S & Falkow. 1994. '*Salmonella* typhimurium initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches.', *J Exp Med*, 180(1): 15-23.
Jung, T. H., J. H. Park, W. M. Jeon, and K. S. Han. 2015. 'Butyrate modulates bacterial adherence on LS174T human colorectal cells by stimulating mucin secretion and MAPK signaling pathway', *Nutr Res Pract*, 9: 343-9.
Kaiser, P., M. Diard, B. Stecher, and W. D. & Hardt. (2012). 'The streptomycin mouse model for *Salmonella* diarrhea: functional analysis of the microbiota, the pathogen's virulence factors, and the host's mucosal immune response', *Immunol Rev*, 245: 56-83.
Keshav, S. 2006. 'Paneth cells: leukocyte-like mediators of innate immunity in the intestine', *J Leukoc Biol*, 80: 500-8.
Kim, C. H. , D. Kim, Y. Ha, K. D. Cho, B. H. Lee, I. W. Seo, S. H. Kim, and C. Chae. 2009. 'Expression of mucins and trefoil factor family protein-1 in the colon of pigs naturally infected with *Salmonella* Typhimurium', *J Comp Pathol*, 140: 38-42.
Koczulla, R., G. von Degenfeld, C. Kupatt, F. Krotz, S. Zahler, T. Gloe, K. Issbrucker, P. Unterberger, M. Zaiou, C. Lebherz, A. Karl, P. Raake, A. Pfosser, P. Boekstegers, U. Welsch, P. S. Hiemstra, C. Vogelmeier, R. L. Gallo, M. Clauss, and R. Bals. 2003. 'An angiogenic role for the human peptide antibiotic LL-37/hCAP-18', *J Clin Invest*, 111: 1665-72.
Kurosaka, K., Q. Chen, F. Yarovinsky, J. J. Oppenheim, and D. Yang. 2005. 'Mouse cathelin-related antimicrobial peptide chemoattracts leukocytes using formyl peptide receptor-like 1/mouse formyl peptide receptor-like 2 as the receptor and acts as an immune adjuvant', *J Immunol*, 174: 6257-65.
Kyrova, K. , H. Stepanova, I. Rychlik, O. Polansky, L. Leva, Z. Sekelova, M. Faldyna, and J. Volf. 2014. 'The response of porcine monocyte derived macrophages and dendritic cells to *Salmonella* Typhimurium and lipopolysaccharide', *BMC Vet Res*, 10: 244.
Lawley, T. D., and A. W. Walker. 2013. 'Intestinal colonization resistance', *Immunology*, 138: 1-11.

Letellier, A. Messier, S. Pare, J. Menard, J. Quessy, S. 1999. 'Distribution of *Salmonella* in swine herds in Quebec', *Vet Microbiol*, 67: 299-306.
Lhocine, N. , E. T. Arena, P. Bomme, F. Ubelmann, M. C. Prevost, S. Robine, and P. J. Sansonetti. 2015. 'Apical invasion of intestinal epithelial cells by *Salmonella* Typhimurium requires villin to remodel the brush border actin cytoskeleton', *Cell Host Microbe*, 17: 164-77.
Li, H., J. P. Limenitakis, T. Fuhrer, M. B. Geuking, M. A. Lawson, M. Wyss, S. Brugiroux, I. Keller, J. A. Macpherson, S. Rupp, B. Stolp, J. V. Stein, B. Stecher, U. Sauer, K. D. McCoy, and A. J. Macpherson. 2015. 'The outer mucus layer hosts a distinct intestinal microbial niche', *Nat Commun*, 6: 8292.
Linde, C. M., S. E. Hoffner, E. Refai, and M. Andersson. 2001. 'In vitro activity of PR-39, a proline-arginine-rich peptide, against susceptible and multi-drug-resistant *Mycobacterium tuberculosis*', *J Antimicrob Chemother*, 47: 575-80.
Litvak, Y., M. X. Byndloss, R. M. Tsolis, and A. J. Baumler. 2017. 'Dysbiotic *Proteobacteria* expansion: a microbial signature of epithelial dysfunction', *Curr Opin Microbiol*, 39: 1-6.
Liu, J. Z. , S. Jellbauer, A. J. Poe, V. Ton, M. Pesciaroli, T. E. Kehl-Fie, N. A. Restrepo, M. P. Hosking, R. A. Edwards, A. Battistoni, P. Pasquali, T. E. Lane, W. J. Chazin, T. Vogl, J. Roth, E. P. Skaar, and M. Raffatellu. 2012. 'Zinc sequestration by the neutrophil protein calprotectin enhances -*Salmonella* growth in the inflamed gut', *Cell Host Microbe*, 11: 227-39.
Lostroh, C. P. , and C. A. Lee. 2001. 'The *Salmonella* pathogenicity island-1 type III secretion system', *Microbes Infect*, 3: 1281-91.
Lupp, C., M. L. Robertson, M. E. Wickham, I. Sekirov, O. L. Champion, E. C. Gaynor, and B. B. Finlay. 2007. 'Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of *Enterobacteriaceae*', *Cell Host Microbe*, 2: 119-29.
Ly, K. T. , and J. E. Casanova. 2007. 'Mechanisms of *Salmonella* entry into host cells', *Cell Microbiol*, 9: 2103-11.
Mathias, A., and B. Corthesy. 2011. 'N-Glycans on secretory component: mediators of the interaction between secretory IgA and gram-positive commensals sustaining intestinal homeostasis', *Gut Microbes*, 2: 287-93.
Meurens, F. , M. Berri, G. Auray, S. Melo, B. Levast, I. Virlogeux-Payant, C. Chevaleyre, V. Gerdts, and H. Salmon. 2009. 'Early immune response following *Salmonella enterica* subspecies *enterica* serovar Typhimurium infection in porcine jejunal gut loops', *Vet Res*, 40: 5.
Meurens, F. Berri, G. M. Auray, S. Melo, B. Levast, I. Virlogeux-Payant, C. Chevaleyre, V. Gerdts, and H Salmon. 2009. 'Early immune response following *Salmonella enterica* subspecies *enterica* serovar Typhimurium infection in porcine jejunal gut loops', *Vet Res*, 40: 5.
Meyerholz, D. K. , T. J. Stabel, M. R. Ackermann, S. A. Carlson, B. D. Jones, and J. Pohlenz. 2002. 'Early epithelial invasion by *Salmonella enterica* serovar Typhimurium DT104 in the swine ileum', *Vet Pathol*, 39: 712-20.
Misselwitz, B. , S. K. Kreibich, S. Rout, B. Stecher, B. Periaswamy, and W. D. Hardt. 2011. '*Salmonella enterica* serovar Typhimurium binds to HeLa cells via Fim-mediated reversible adhesion and irreversible type three secretion system 1-mediated docking', *Infect Immun*, 79: 330-41.
Mukherjee, S., and L. V. Hooper. 2015. 'Antimicrobial defense of the intestine', *Immunity*, 42: 28-39.
Neeloffer Mookherjee, Kelly L. Brown and Robert E.W. Hancock. 2013. 'Cathelicidins.' in, Handbook of biologically active peptides (Academic press).
Nizet, V., T. Ohtake, X. Lauth, J. Trowbridge, J. Rudisill, R. A. Dorschner, V. Pestonjamasp, J. Piraino, K. Huttner, and R. L. Gallo. 2001. 'Innate antimicrobial peptide protects the skin from invasive bacterial infection', *Nature*, 414: 454-7.
Ogawa, H., K. Fukushima, H. Naito, Y. Funayama, M. Unno, K. Takahashi, T. Kitayama, S. Matsuno, H. Ohtani, S. Takasawa, H. Okamoto, and I. Sasaki. 2003. 'Increased expression of HIP/PAP and

regenerating gene III in human inflammatory bowel disease and a murine bacterial reconstitution model', *Inflamm Bowel Dis*, 9: 162-70.
Parmley, E. J. , K. Pintar, S. Majowicz, B. Avery, A. Cook, C. Jokinen, V. Gannon, D. R. Lapen, E. Topp, T. A. Edge, M. Gilmour, F. Pollari, R. Reid-Smith, and R. Irwin. 2013. 'A Canadian application of one health: integration of *Salmonella* data from various Canadian surveillance programs (2005-2010)', *Foodborne Pathog Dis*, 10: 747-56.
Patel, J. C., and J. E. Galan. 2006. 'Differential activation and function of Rho GTPases during *Salmonella*-host cell interactions', *J Cell Biol*, 175: 453-63.
Petersson, J., O. Schreiber, G. C. Hansson, S. J. Gendler, A. Velcich, J. O. Lundberg, S. Roos, L. Holm, and M. Phillipson. 2011. 'Importance and regulation of the colonic mucus barrier in a mouse model of colitis', *Am J Physiol Gastrointest Liver Physiol*, 300: G327-33.
Que, J. U., and D. J. & Hentges. 1985. 'Effect of streptomycin administration on colonization resistance to *Salmonella* Typhimurium in mice.', *Infect Immun*, 48(1): 169-74.
Rabsch, W. 2007. '*Salmonella* typhimurium phage typing for pathogens', *Methods Mol Biol*, 394: 177-211.
Raupach, B. , S. K. Peuschel, D. M. Monack, and A. Zychlinsky. 2006. 'Caspase-1-mediated activation of interleukin-1beta (IL-1beta) and IL-18 contributes to innate immune defenses against *Salmonella enterica* serovar Typhimurium infection', *Infect Immun*, 74: 4922-6.
Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J. P. Kraehenbuhl, and P. Ricciardi-Castagnoli. 2001. 'Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria', *Nat Immunol*, 2: 361-7.
'Responsible use of Medically Important Antimicrobials in Animals', 2018. Available at: https://www.canada.ca/en/public-health/services/antibiotic-antimicrobial-resistance/animals/actions/responsible-use-antimicrobials.html.
Rigottier-Gois, L. 2013. 'Dysbiosis in inflammatory bowel diseases: the oxygen hypothesis', *ISME J*, 7: 1256-61.
Rios-Covian, D., P. Ruas-Madiedo, A. Margolles, M. Gueimonde, C. G. de Los Reyes-Gavilan, and N. Salazar. 2016. 'Intestinal short chain fatty acids and their link with diet and human health', *Front Microbiol*, 7: 185.
Rosenberger CM, Gallo RL, and Finlay BB. 2004 Feb 24. 'Interplay between antibacterial effectors: a macrophage antimicrobial peptide impairs intracellular *Salmonella* replication.', *Proc Natl Acad Sci U S A*, 101(8):: 2422-7.
Roy, M. F., and D. Malo. 2002. 'Genetic regulation of host responses to *Salmonella* infection in mice', *Genes Immun*, 3: 381-93.
Salzman, N. H., D. Ghosh, K. M. Huttner, Y. Paterson, and C. L. Bevins. 2003. 'Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin', *Nature*, 422: 522-6.
Sansonetti, P. J. 2004. 'War and peace at mucosal surfaces', *Nat Rev Immunol*, 4: 953-64.
Santos RL, Zhang S, Tsolis RM, Kingsley RA, Adams LG, and Bäumler AJ. 2001. 'Animal models of *Salmonella* infections: enteritis versus typhoid fever.', *Microb Infect*, 3(14): 1335-44.
Sassone-Corsi, M., and M. Raffatellu. 2015. 'No vacancy: how beneficial microbes cooperate with immunity to provide colonization resistance to pathogens', *J Immunol*, 194: 4081-7.
Scherer, K., I. Szabo, U. Rosler, B. Appel, A. Hensel, and K. Nockler. 2008. 'Time course of infection with *Salmonella* Typhimurium and its influence on fecal shedding, distribution in inner organs, and antibody response in fattening pigs', *J Food Prot*, 71: 699-705.
Schlee, M., J. Wehkamp, A. Altenhoefer, T. A. Oelschlaeger, E. F. Stange, and K. Fellermann. 2007. 'Induction of human beta-defensin 2 by the probiotic *Escherichia coli* Nissle 1917 is mediated through flagellin', *Infect Immun*, 75: 2399-407.

Sicard, J. F., G. Le Bihan, P. Vogeleer, M. Jacques, and J. Harel. 2017. 'Interactions of Intestinal Bacteria with Components of the Intestinal Mucus', *Front Cell Infect Microbiol*, 7: 387.

Spees, A. M. , D. D. Kingsbury, T. Wangdi, M. N. Xavier, R. M. Tsolis, and A. J. Baumler. 2014. 'Neutrophils are a source of gamma interferon during acute *Salmonella enterica* serovar Typhimurium colitis', *Infect Immun*, 82: 1692-7.

Srikanth, C. V. , R. Mercado-Lubo, K. Hallstrom, and B. A. McCormick. 2011. '*Salmonella* effector proteins and host-cell responses', *Cell Mol Life Sci*, 68: 3687-97.

Stecher, B. , R. Robbiani, A. W. Walker, A. M. Westendorf, M. Barthel, M. Kremer, S. Chaffron, A. J. Macpherson, J. Buer, J. Parkhill, G. Dougan, C. von Mering, and W. D. Hardt. 2007. '*Salmonella enterica* serovar Typhimurium exploits inflammation to compete with the intestinal microbiota', *PLoS Biol*, 5: 2177-89.

Stecher, B., S. Chaffron, R. Kappeli, S. Hapfelmeier, S. Freedrich, T. C. Weber, J. Kirundi, M. Suar, K. D. McCoy, C. von Mering, A. J. Macpherson, and W. D. Hardt. 2010. 'Like will to like: abundances of closely related species can predict susceptibility to intestinal colonization by pathogenic and commensal bacteria', *PLoS Pathog*, 6: e1000711.

Stecher, B., and W. D. Hardt. 2008. 'The role of microbiota in infectious disease', *Trends Microbiol*, 16: 107-14.

Steele-Mortimer, O., S. Méresse, J. P. Gorvel, B. H. Toh, and B. B. & Finlay. 1999. 'Biogenesis of *Salmonella* typhimurium-containing vacuoles in epithelial cells involves interactions with the early endocytic pathway', *Cell Microbiol*, 1(1): 33-49.

Thiennimitr, Parameth, Sebastian E. Winter, Maria G. Winter, Mariana N. Xavier, Vladimir Tolstikov, Douglas L. Huseby, Torsten Sterzenbach, Renée M. Tsolis, John R. Roth, and and Andreas J. Bäumler. 2011. 'Intestinal inflammation allows *Salmonella* to use ethanolamine to compete with the microbiota.', *Proc Natl Acad Sci U S A*, 108: 17480-85.

Toms, C. , and F. Powrie. 2001. 'Control of intestinal inflammation by regulatory T cells', *Microbes Infect*, 3: 929-35.

Travis, S. M., N. N. Anderson, W. R. Forsyth, C. Espiritu, B. D. Conway, E. P. Greenberg, P. B. McCray, Jr., R. I. Lehrer, M. J. Welsh, and B. F. Tack. 2000. 'Bactericidal activity of mammalian cathelicidin-derived peptides', *Infect Immun*, 68: 2748-55.

Tsolis, R. M., R. A. Kingsley, S. M. Townsend, T. A. Ficht, L. G. Adams, and A. J. & Bäumler. (1999). Of mice, calves, and men. *In Mechanisms in the Pathogenesis of Enteric Diseases 2* (Springer, Boston, MA.).

Uzzau, Sergio, Derek J. Brown, T. Wallis, Salvatore Rubino, Guido Leori, Serge Bernard, Josep Casadesús, David J. Platt, and and John Elmerdahl Olsen. 2000. 'Host adapted serotypes of *Salmonella enterica*', *Epidemiol Infect*, 125: 229-55.

Vaishnava, S., M. Yamamoto, K. M. Severson, K. A. Ruhn, X. Yu, O. Koren, R. Ley, E. K. Wakeland, and L. V. Hooper. 2011. 'The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine', *Science*, 334: 255-8.

Van der Wolf, P. J. , F. W. Van Schie, A. R. Elbers, B. Engel, H. M. Van der Heijden, W. A. Hunneman, and M. J. Tielen. 2001. 'Administration of acidified drinking water to finishing pigs in order to prevent *Salmonella* infections', *Vet Q*, 23: 121-5.

van Harten, R. M., E. van Woudenbergh, A. van Dijk, and H. P. Haagsman. 2018. 'Cathelicidins: Immunomodulatory Antimicrobials', *Vaccines (Basel)*, 6.

Vazquez-Torres, A., J. Jones-Carson, A. J. Baumler, S. Falkow, R. Valdivia, W. Brown, M. Le, R. Berggren, W. T. Parks, and F. C. Fang. 1999. 'Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes', *Nature*, 401: 804-8.

Vazquez-Torres, A., J. Jones-Carson, A. J. Bäumler, S. Falkow, R. Valdivia, W. Brown, Mysan Le, W. Ruth Berggren, Tony Parks, and and Ferric C. Fang. 1999. 'Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes', *Nat Rev Immunol*, 401: 804.

Veldhuizen, E. J., M. Rijnders, E. A. Claassen, A. van Dijk, and H. P. Haagsman. 2008. 'Porcine beta-defensin 2 displays broad antimicrobial activity against pathogenic intestinal bacteria', *Mol Immunol*, 45: 386-94.

Veldhuizen, E. J., V. A. Schneider, H. Agustiandari, A. van Dijk, J. L. Tjeerdsma-van Bokhoven, F. J. Bikker, and H. P. Haagsman. 2014. 'Antimicrobial and immunomodulatory activities of PR-39 derived peptides', *PLoS One*, 9: e95939.

Veldhuizen, E. J., A. van Dijk, M. H. Tersteeg, S. I. Kalkhove, J. van der Meulen, T. A. Niewold, and H. P. Haagsman. 2007. 'Expression of beta-defensins pBD-1 and pBD-2 along the small intestinal tract of the pig: lack of upregulation in vivo upon *Salmonella* Typhimurium infection', *Mol Immunol*, 44: 276-83.

Verbrugghe, E. , A. Van Parys, B. Leyman, F. Boyen, F. Haesebrouck, and F. Pasmans. 2015. 'HtpG contributes to *Salmonella* Typhimurium intestinal persistence in pigs', *Vet Res*, 46: 118.

Wall, D. M. , W. J. Nadeau, M. A. Pazos, H. N. Shi, E. E. Galyov, and B. A. McCormick. 2007. 'Identification of the *Salmonella enterica* serotype typhimurium SipA domain responsible for inducing neutrophil recruitment across the intestinal epithelium', *Cell Microbiol*, 9: 2299-313.

Wilcock, B. P., C. H. Armstrong, and H. J. & Olander. 1976. 'The significance of the serotype in the clinical and pathological features of naturally occurring porcine salmonellosis.', *Can J Comp Med*, 40: 80.

Wilkins, W. , A. Rajic, C. Waldner, M. McFall, E. Chow, A. Muckle, and L. Rosengren. 2010. 'Distribution of *Salmonella* serovars in breeding, nursery, and grow-to-finish pigs, and risk factors for shedding in ten farrow-to-finish swine farms in Alberta and Saskatchewan', *Can J Vet Res*, 74: 81-90.

Winter, S. E., P. Thiennimitr, M. G. Winter, B. P. Butler, D. L. Huseby, R. W. Crawford, J. M. Russell, C. L. Bevins, L. G. Adams, R. M. Tsolis, J. R. Roth, and A. J. Baumler. 2010. 'Gut inflammation provides a respiratory electron acceptor for *Salmonella*', *Nature*, 467: 426-9.

Winterbourn, C. C., A. J. Kettle, and M. B. Hampton. 2016. 'Reactive oxygen species and neutrophil function', *Annu Rev Biochem*, 85: 765-92.

Wood, R. L. , A. Pospischil, and R. Rose. 1989. 'Distribution of persistent *Salmonella* typhimurium infection in internal organs of swine', *Am J Vet Res*, 50: 1015-21.

Xu, J., and J. I. Gordon. 2003. 'Honor thy symbionts', *Proc Natl Acad Sci U S A*, 100: 10452-9.

Xu, Jian, Magnus K. Bjursell, Jason Himrod, Su Deng, Lynn K. Carmichael, Herbert C. Chiang, Lora V. Hooper, and and Jeffrey I. Gordon. (2003). 'A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis', *Science,*, 299: 2074-76.

Yoshimura, T., M. H. McLean, A. K. Dzutsev, X. Yao, K. Chen, J. Huang, W. Gong, J. Zhou, Y. Xiang, H. Badger J, C. O'HUigin, V. Thovarai, L. Tessarollo, S. K. Durum, G. Trinchieri, X. W. Bian, and J. M. Wang. 2018. 'The Antimicrobial Peptide CRAMP Is Essential for Colon Homeostasis by Maintaining Microbiota Balance', *J Immunol*, 200: 2174-85.

Zaiou, M., and R. L. Gallo. 2002. 'Cathelicidins, essential gene-encoded mammalian antibiotics', *J Mol Med (Berl)*, 80: 549-61.

Zanetti, M., G. Del Sal, P. Storici, C. Schneider, and D. Romeo. 1993. 'The cDNA of the neutrophil antibiotic Bac5 predicts a pro-sequence homologous to a cysteine proteinase inhibitor that is common to other neutrophil antibiotics', *J Biol Chem*, 268: 522-6.

Zeng, M. Y., N. Inohara, and G. Nunez. 2017. 'Mechanisms of inflammation-driven bacterial dysbiosis in the gut', *Mucosal Immunol*, 10: 18-26.

Zhang, L., W. Wu, Y. K. Lee, J. Xie, and H. Zhang. 2018. 'Spatial heterogeneity and co-occurrence of mucosal and luminal microbiome across swine intestinal tract', *Front Microbiol*, 9: 48.

Zhang, S. , R. A. Kingsley, R. L. Santos, H. Andrews-Polymenis, M. Raffatellu, J. Figueiredo, J. Nunes, R. M. Tsolis, L. G. Adams, and A. J. Baumler. 2003. 'Molecular Pathogenesis of *Salmonella enterica* Serotype Typhimurium-Induced Diarrhea', *Infect. Immun*, 71: 1-12.

## Chapter 2: *Salmonella enterica* serovar Typhimurium temporally modulates the enteric microbiota and host responses to overcome colonization resistance in swine

### 2.1 Introduction

The gastrointestinal tract (GIT) of mammals is inhabited by commensal and mutualistic microorganisms; referred to as the normal microbiota. Bacterial densities in the GIT system vary within sites ranging from $10^3$ cells per milliliter in the stomach to $10^{11}$ cells per gram in the colon (Xu *et al.* 2003). These bacterial communities have been shown to play an essential role in nutrient acquisition, development of gut-associated lymphoid tissue (GALT), defense against pathogens, and maturation of the intestine (Sansonetti *et al.* 2004; Hooper *et al.* 2002). Changes in numbers and diversity of bacteria that comprise the normal microbiota can be triggered by diverse factors potentially leading to health consequences for the host. Dramatic changes in the intestinal microbiota of swine have been observed after enteropathogenic infections (Drumo *et al.* 2015), as a result of nutritional and dietary additives (Liu *et al.* 2012), and due to the administration of antibiotics (Allen *et al.* 2011). Furthermore, the presence of an inflammatory response triggered by infections by enteric pathogens such as *Salmonella enterica*, *Citrobacter rodentium*, and *Campylobacter jejuni* has been associated with alterations to the composition of the intestinal microbiota (Lupp *et al.* 2007; Drumo *et al.* 2015).

*Salmonella enterica* serovar Typhimurium is an important zoonotic pathogen that successfully infects the intestinal tract of swine. A number of studies to elucidate mechanisms of pathogenesis (Boyen, F. Maes, *et al.* 2008; Agbor *et al.* 2011), host immune responses (Collado-Romero *et al.* 2010; Wang *et al.* 2007), and the epidemiology and control of salmonellosis have been conducted in pigs (Boyen, F. Maes, *et al.* 2008). High throughput sequencing methods have been used to profile the enteric microbiota of swine under the influence of different feed efficiencies (Quan *et al.* 2018) and different levels of fatness (Yang *et al.* 2016). A similar culture-independent approach was employed to investigate changes in the ileal microbiota of swine subjected to an early *S.* Typhimurium infection (Arguello *et al.* 2018). However, the majority of studies conducted to date have focused on the evaluation of differences in the fecal microbiota of pigs such as, evaluation of fecal microbiota during weaning transition (Alain *et al.* 2014), after antibiotic administration (Looft *et al.* 2012), and a comparison between high or low *Salmonella* shedders (Bearson *et al.* 2013). Importantly, evidence indicates that the fecal microbiota is not representative of the intestinal microbiota (e.g. mucosa-associated communities) (Zhao *et al.* 2015). Differences in protocols among laboratories, specifically in DNA extraction, variation of sequencing coverage to detect minority populations (Lynch *et al.* 2015), differences within methods for bioinformatics analyses, the incapacity to differentiate live from dead

bacteria, and relatively poor taxonomic resolution are some of the salient limitations of the culture-independent strategies. Moreover, bacteria are not recovered for additional analysis (e.g. for functional determinations). Thus, comprehensive analyses that include culturomics together with sequence-based microbiome analysis approaches are necessary to properly characterize the intestinal microbiota.

Arguello *et al.* (2018) and Bearson *et al.* (2013) examined changes in the composition of the enteric microbiota of pigs infected by *S.* Typhimurium. However, they did not characterize the composition and structure of the microbiota as a function of inflammation intensity; in pigs, salmonellosis follows a temporal progression developing from acute infection characterized by severe diarrhea, vomiting and fever to subclinical infection represented by intermittent shedding of the pathogen (Scherer *et al.* 2008). Additionally, they exclusively used high throughput sequencing techniques to characterize the microbiota, and they did not characterize communities associated with the mucosa. It has been observed that the composition of bacterial communities in association with mucosa can vary with those in digesta within the intestinal lumen (Zhang *et al.* 2018). Thus, a primary objective of the current study was to comprehensively characterize the microbiota in the ileum, cecum, and colon of pigs at different stages of salmonellosis experimentally incited by *S.* Typhimurium DT104 (i.e. acute, subacute, and recovery phases) using both culture-based and culture-independent methods. Moreover, I collated temporal changes to the microbiota with a variety of host metrics to glean information on how the pathogen overcomes colonization resistance. This study provides a more comprehensive and extensive understanding of temporal interactions between the host and the intestinal microbiota, and the impact that enteric inflammation incited by *S.* Typhimurium has in this interplay.

## 2.2 Materials and methods

### 2.2.1 Ethics

The project was approved by the LeRDC Animal Care Committee (Animal Use Protocol Review 1512), and LeRDC Biosafety and Biosecurity Committee before commencement. As infection by *S.* Typhimurium in pigs is reportable in Alberta, approval to conduct *Salmonella* inoculations in piglets was also obtained from the Head Provincial Veterinarian, Dr. Gerald Hauer, Alberta Agriculture and Forestry, Edmonton, Alberta.

### 2.2.2 Experimental design

The experiment was arranged as a completely randomized design with three levels of sample time (2, 6, and 10 dpi) and two levels of immunological stress (± *S.* Typhimurium) (Figure 2.1). The goal was to obtain samples that corresponded temporally to acute, subacute, and recovery phases of salmonellosis in swine. Each replicate included six piglets. To ensure humane animal care, piglets were housed in pairs

within individual animal rooms. The limited number of animal rooms available in the Livestock Containment Unit [LCU] at LeRDC necessitated that the experiment was conducted on separate occasions (i.e. two replicates at time one, and two replicates at time two); the separate times were treated as a random effect in the statistical model.

**2.2.3 Animal maintenance**

Castrated male piglets at 6-weeks of age were used in the experiment. Piglets were vaccinated for circovirus, ileitis, and erysipelothrix. Neither the sow (during pregnancy or post-partum) nor piglets were administered antibiotics. Piglets were group housed for 3 days in the LCU under a 14:10 h dark:light cycle. After the adaptation period, arbitrarily-selected animals were assigned to individual pens, with two animals per room. Piglets were provided a mini-pellet ration diet that was free of antibiotics (Proform Pig Starter 2; Hi-Pro Feeds, Okotoks, AB). Feed was provided daily, and piglets were permitted to eat and drink *ad libitum*. Straw was used for bedding, and toys were provided for environmental enrichment. Animals were monitored daily for activity level, and behavioral signs of pain and stress (i.e. diarrhea) were recorded. Bedding, residual food and water were replaced daily. Initial body weights were recorded upon assignment of animals to individual cages, and every other day thereafter. Food consumption was determined daily.

**2.2.4 Inoculation**

Piglets were orally inoculated with *S. enterica* Typhimurium DT104 (strain SA970934) (Yin *et al.* 2014). The bacterium was grown aerobically on MacConkey's agar (MA) (Difco BD, Mississauga, ON) at 37°C for 16-24 h. Biomass was removed from the surface of the agar and transferred into Columbia Broth (CB) (Difco BD, Mississauga, ON). Cultures were maintained for 180 to 210 min at 37°C, shaking at 150 rpm, until an optical density (600 nm) of greater than 1.2 was obtained. Cultures were centrifuged at 4,000 x g for 15 min, supernatants were removed to a volume of 20 ml, and the density adjusted to a target of $3.0 \times 10^9$ cells $ml^{-1}$. To confirm densities of viable cells, inoculum was diluted in a 10-fold dilution series, 100 µl of each dilution was spread in duplicate onto MA, cultures were incubated at 37°C, and the number of *S*. Typhimurium colonies were counted at the dilution yielding 30 to 300 CFU after 24 h. Individual piglets were gavaged on 2 consecutive days with *S.* Typhimurium cells in CB (1.0 ml, SA+) or with CB alone (1.0 ml; SA-). Each piglet was administered the two treatments in 1 ml aliquots using sterile 3.0 ml syringes. The animals were individually restrained, the syringe was placed in the rear of the mouth and the liquid slowly dispensed taking care to avoid aspiration.

### 2.2.5 Body temperature, feed consumption, weight gain, and feces collection

Rectal temperature and food consumption were measured daily. In addition, body weights were measured at 2-day intervals using a Model 75 scale (Reliable Scale Corporation, Calgary, AB). Fresh feces were collected from the pen floor immediately before inoculation and at 2-day intervals thereafter, including just before humane euthanization. Fecal samples were transported to the laboratory within 30 min of collection for analysis. Samples of fresh feces were processed for the presence of *S.* Typhimurium via dilution plating. In addition, aliquots of the feces were weighed and placed at -80°C for quantitative PCR (qPCR).

### 2.2.6 Intestinal tissue collection

At 2, 6, and 10 dpi, randomly designated animals were anaesthetized for sample collection from live animals. Piglets were premedicated intramuscularly with a cocktail of ketamine (Ketaset, Pfizer, Kirkland, QC) and xylazine (Xylamax, Bimeda, Cambridge, ON) at a dose of 22 mg $kg^{-1}$ and 2.2 mg $kg^{-1}$ respectively. Animals were placed in dorsal recumbency on a v-trough surgical table, intubated, and general anesthesia established with 2-3% isoflurane 500 -1500 ml/min $O_2$ (Abbott Laboratories, Chicago, IL). The abdomen was scrubbed with chlohexadine (Omega Laboratories Ltd., Montreal, QC) 70% ethanol, and propyliodine (West Penetone Inc., Ville D'Anjou, QC).

Harvested from animals under general anesthesia, segments of intestine (≈10 cm-long) were collected from the duodenum, proximal jejunum, mid-jejunum, distal jejunum, ileum, cecum (two segments located at the free end and adjacent to the ileal-cecal junction), ascending colon, spiral colon (two segments located at the central flexure and ≈100 cm distal from the flexure), and descending colon. To ensure the integrity of the intestinal segment and to minimize release of ingesta, double ligatures were established at the two ends of the segment, and the segment was excised from the intestine by cutting between the two ligatures. Care was taken to ligate mesentery blood vessels immediately prior to intestinal segment removal, and to ensure the maintenance of blood flow to adjacent intestinal tissue. All intestinal samples were processed within ca. 5-10 min of their removal from live animals. To prevent introduction of air, additional ligatures were established on segments from which anaerobic bacteria were to be isolated (i.e. ileum, cecum, and spiral colon). Ligated sub-segments for culturomics were removed and immediately placed in an anaerobic jar; the ambient atmosphere was removed by vacuum, replaced with $N_2$, and the segments transported to the laboratory. With the exception of intestinal segments used to isolate anaerobic bacteria, segments were longitudinally incised in ambient atmosphere, and luminal contents (i.e. digesta) were aseptically collected and weighed for DNA extraction for quantitation of *S.* Typhimurium and select commensal

bacterial taxa, and characterization of bacterial communities. Sections of the intestine were collected for RNA extraction for gene expression, and DNA extraction for quantitation of mucosa-associated *S.* Typhimurium and select commensal bacterial taxa, histology, and characterization of bacterial communities. Samples for characterization of mucosa-associated *S.* Typhimurium and microbial communities were gently flooded with sterile phosphate buffered saline (≈3 ml) to remove residual ingesta with minimal disruption to the adhered mucus. Samples for RNA extraction (three subsamples per segment) were placed in RNAprotect® (Qiagen Inc., Toronto, ON) and maintained at -80°C until processed. For histopathological scoring, intestinal segments were placed in 10% neutral buffered formalin (Surgipath Canada, Inc., Winnipeg, MB) for a minimum of 24 h.

**2.2.7 Blood collection and animal euthanization**

Blood (≈20 ml) was collected from the portal vein (draining the intestine) and from the heart (systemic blood) using an 18 gauge needle. Blood was collected in BD Vacutainer® with $K_2$EDTA (BD, Franklin Lake, NJ) for complete blood count (CBC) analysis and detection of *S.* Typhimurium. Immediately after blood removal, the animal was euthanized under general anesthesia.

**2.2.8 Accessory tissue collection**

Within 5-10 min of euthanasia, ileal-cecal lymph nodes, jejunal lymph nodes (one to two per animal), the liver, and spleen were removed, and samples excised from these tissues were placed in RNAprotect® (Qiagen Inc.) and maintained at -80°C until processed. In addition, samples from the above tissues, and from the frontal cortex, midbrain, and brain stem were collected for isolation of *Salmonella*.

**2.2.9 Histopathology**

Tissues for hematoxylin and eosin (H&E) were prepared with the standard procedure from the lab (Jiminez *et al.* 2017). Tissues from duodenum, proximal jejunum, medium jejunum, distal jejunum, ileum, cecum, spiral colon, ascending colon and distal colon in formalin were dehydrated using a tissue processor (Leica TP 1020, Leica Biosystems), and then embedded in paraffin (Fisherfinest™ Histoplast PE, Thermo Fisher Scientific, Edmonton, AB) using a Shandon Histocentre 3 (Thermo Fisher Scientific). Five µm sections were transferred to positively charged slides (Fisherbrand Superfrost™ Plus Gold; Thermo Fisher Scientific) and allowed to dry prior to being deparaffinized with xylene. Slides were then rehydrated in ethanol and stained with H&E using a standard protocol. Histopathologic changes were assessed by a veterinary pathologist, (RREU), blinded to the treatment protocol. The scoring system used was a modification of previous scoring protocols developed by Boyer *et al.* (2015) and Garner *et al.* (2009). The tissues were scored for villus blunting, villus fusion, lymphoid depletion, neutrophil

infiltration, epithelial injury, and fibrosis (Table 2.1). The total histopathologic score was sum of all individual tissue measurements (maximum score of 21).

**2.2.10 Blood analysis**

Complete blood counts were performed on a Hematrue blood analyser (Heska, Des Moines, IA) within 45 min of collection. The Heska Hematrue system employs an electronic impedance method for cell counting and sizing. An electrical current is constantly applied to the sample. When a cell is drawn into this constant current, the electrical conductivity of the environment changes, generating an equivalent voltage pulse. The number of pulses corresponds to the number of cells detected, and the amplitude of each pulse is also directly proportional to the volume of the cell. No separation between different granulocytes is allowed with this technology.

Blood chemistry was analyzed on a Vettest blood analyzer (Idexx Laboratories, Westbrook, ME) using a pre-anesthetic blood panel. The pre-anesthetic blood panel included tests for alanine aminotransferase, alkaline phosphatase, creatinine, glucose, total protein, and blood urea nitrogen.

**2.2.11 Isolation and genotyping of *Salmonella***

*Salmonella* was isolated and identified from feces, systemic blood, ileal-cecal lymph nodes, jejunal lymph nodes, liver, spleen, frontal cortex, midbrain, and brain stem as previously described (Molla *et al.* 2010). Briefly, for feces, ≈1 g of fecal matter, was weighed, diluted 1:10 in buffered peptone water (Oxoid Inc., Nepean, ON), and suspended in the liquid by vortexing. For blood, 1 ml of systemic blood was added to 9 ml of buffered peptone water, and vortexed. For tissues, any bacteria on the outside of the samples were killed by immersion into boiling water for 10 s; this method does not kill *Salmonella* internalized within the tissues. As with feces, ≈1 g of tissue was weighed and diluted 1:10 in buffered peptone water. Samples were homogenized using a Tissue Tearor (Model 985370; Biospec Products Inc., Bartlesville, OK). All suspensions were incubated at 37°C for 16-24 h, and 50 µl of the suspension was transferred to 5 ml of Rappaport-Vassiliadis enrichment broth (Oxoid Inc.), and incubated at 42°C for 16-24 h. A 10 µl inoculation loop was used to transfer a subsample of the Rappaport-Vassiliadis enrichment broth to both Brilliant Green Agar (BGA; BD Difco, Mississauga, ON) and Modified Lysine Iron Agar (MLIA; Oxoid Inc.). The BGA and MLIA cultures were incubated for 48 h at 37°C to allow time for $H_2S$ production to manifest. Representative red colonies on BGA were transferred to Triple Sugar Iron agar (TSIA; BD Difco) slants. Two representative black colonies from MLIA were transferred to TSIA slants. The TSIA slant cultures were incubated at 37°C for 16-24 h. Representative colonies from *Salmonella* positive TSIA slants were transferred to MacConkey Agar (BD Difco) and incubated at 37°C for 16-24 h.

Colorless colonies on MacConkey Agar are considered *Salmonella* positive and were stored in 30% glycerol in broth at -80°C.

To identify presumptive *Salmonella* positive colonies, genomic DNA was extracted using an automated DNA extraction robot (Model Autogen 740; Autogen, Inc., Holliston, MA) according to the manufacturer's recommendations. Taxon-specific PCR was conducted using the primers F-(Sal) and R-(Sal), which target the *invA* gene of *S. enterica* (Kumar *et al.* 2010). The reactions were run on Eppendorf Mastercycler Pro S thermocycler (VWR international, Mississauga, ON) at 95°C for 5 min, followed by 35 cycles of 94°C for 1 min, 64°C for 1 min, 72°C for 1 min, and one cycle at 72°C for 10 min. DNA obtained from *S.* Typhimurium SA970934 was used as a positive control, and Optima water (Fisher Scientific, Edmonton, AB) was used as a template negative control. To genotype *Salmonella* isolates, the pulsed field gel electrophoresis (PFGE) protocol used by PulseNet (PNL05) was applied to representative *Salmonella* isolates recovered from piglets relative to *S.* Typhimurium SA970934.

**2.2.12 Meat characteristics**

Exsanguinated animals were hung in a 4°C cooler, and the pH of the right *longissimus dorsi* muscle (≈2-3 cm posterior to the last rib) was determined at 45 min and 24 h post-mortem using a portable meat pH meter (HI99163; Hanna Instruments, Laval, QC). At 24 h post-mortem, the right and left longissimus dorsi muscles were removed, and moisture drip loss test was conducted. Briefly, the *longissimus dorsi* muscle from each side was treated as an observation (n=2). The individual muscles were trimmed to equal dimensions, cut in two, weighed, placed in a porous nylon bag, suspended within a liquid impervious bag for 48 h at 4°C, and re-weighed.

**2.2.13 RNA extraction**

To quantify mRNA of targets of interest, RNA was extracted from samples (≈0.5 x 0.5 cm) from the intestines (duodenum, jejunum, ileum, cecum, ascending colon, spiral colon, and descending colon) and spleen stored in RNAprotect® (Qiagen Inc.) using an RNeasy Mini kit (Qiagen Inc.) with a DNase step added to eliminate residual genomic DNA (Qiagen Inc.). RNA quantity and quality was determined using a Bioanalyzer 2100 (Agilent Technologies Canada Inc., Mississauga, ON), and 1000 ng of RNA was transcribed to cDNA following the manufacturer's protocol (Qiagen Inc). Reactions were run on a 384-well plate, and each reaction contained 5.0 µl QuantiTect SYBR Green Master Mix (Qiagen Inc.), 0.5 µl of each primer (10 µM) (Table 2.2), 3.0 µl of RNase-free water, and 1.0 µl of cDNA. Quantitative PCR was performed using ABI7900HT thermocycler (Applied Biosystems, Carlsbad, CA) with the following cycle conditions: 95°C for 15 min; 40 cycles of 95°C for 15 sec, at the primer annealing temperature (Table 2.2) for 30 sec, and 72°C for 30 sec; and melt curve analysis from 55-95°C. Some primer sequences specific to

gene targets were generated using NCBI Primer-BLAST to generate an amplicon between 75 and 200 base pairs. Reactions were run in triplicate and average Ct values were used to calculate expression relative to Peptidylprolyl isomerase A (*PPIA*), hypoxanthine-guanine phosphoribosyltransferase (*HPRT*), and beta-glucuronidase (*GUSβ*) reference genes. These genes were selected using the geNorm algorithm in qbase+ (Biogazelle, Zwijnaarde, Belgium), which identifies stability of expression among samples (Vandesompele *et al.* 2002).

**2.2.14 Bacterial genomic DNA extraction from digesta and tissue samples**

For quantification of *S.* Typhimurium and select commensal bacteria associated with mucosa by qPCR, DNA was extracted from the ileal, cecal, and spiral colonic samples using the Qiagen Blood and Tissue kit (Qiagen Inc.) gram positive protocol. For characterization of mucosa-associated bacteria by next-generation sequencing, DNA extracted from intestinal tissue of the spiral colon was enriched using the NEBNext® Microbiome DNA Enrichment Kit (New England Biolabs, Ipswich, MA). For quantification of *S.* Typhimurium and select commensal bacterial taxa, and characterization of bacterial communities in feces and digesta from the ileum, cecum, and spiral colon, DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen Inc.). A bead homogenization step using 5.0 mm-diam stainless steel beads following the Qiagen protocol for isolation of bacterial DNA using a TissueLyser LT (Qiagen Inc.) at 30 Hz, three times for 30 s, was included to ensure comprehensive extraction of genomic DNA.

**2.2.15 Quantification of *Salmonella***

To enumerate *S.* Typhimurium by qPCR, duplicate reactions (20 µl) were prepared as follows: 10 µl of QuantiTect SYBR® Green Mastermix (Qiagen Inc.), 0.5 µM of each primer (IDT, San Diego, CA), 2 µl BSA (0.1µg $\mu l^{-1}$) (Promega, Madison, WI), 2 µl DNA, and 4 µl DNase free water (Qiagen Inc.). The primers used were F-(Sal) and R-(Sal) (Kumar *et al.* 2010). Data was collected using an Mx3005p Real Time PCR instrument (Agilent Technologies Canada Inc. Mississauga, ON). Cycle conditions used were 95°C for 5 min, followed by 40 cycles of 94°C for 15 s, 64°C for 30 s, and 72°C for 30 s. Serial dilutions of genomic DNA containing 1.5 x $10^6$ copies $\mu l^{-1}$ were used to prepare a standard curve, concentrations of the *Salmonella* DNA in the sample were determined based on standard curve Ct values, and copies $cm^{-2}$ were calculated. A dissociation curve was included with each run to verify amplicon specificity.

**2.2.16 Characterization of bacterial communities by culturomics**

**(i) Bacteriological media.** The basic protocol described in Moote *et al.* (Moote *et al.* 2020) was used. All media (Table 2.3-2.4) were reduced before use. Media were prepared without the addition of cysteine and autoclaved for 5 min. Once autoclaved, warmed media was immediately transferred into chamber containing a nitrogen ($N_2$)-predominant atmosphere consisting of 85:10:5% $N_2$:$CO_2$:$H_2$ or a

carbon dioxide ($CO_2$)-predominant atmosphere consisting of 90:10% $CO_2$:$H_2$, and vigorously agitated to displace oxygen from the media. For media used in the $CO_2$ atmosphere, 40 ml $l^{-1}$ of 8% sodium carbonate (Sigma-Aldrich, Ottawa, ON) was added to prevent acidification. When cooled, media were decanted into separate bottles containing the desired L-cysteine monohydrate content to remove any residual oxygen, the bottles were sealed with a screw cap containing a rubber stopper, removed from the chamber, and autoclaved for 30 min at 121°C and 15 kPa. The pH of media was tested using pH paper (Micro Essential Laboratory, Brooklyn, NY). Agar (1.5% agar; BD Difco) and resazurin as an oxygen indicator (25 µg $ml^{-1}$; Temecula, CA) were added to media before autoclaving for 30 min. Media was dispensed into Petri dishes, and maintained in the $N_2$ and $CO_2$ atmosphere chambers for 24 h before use. For enrichments, resazurin sodium salt (25 µg $ml^{-1}$) was added, media (10 ml) were dispensed into 15-ml glass Hungate tubes (Kimble-Chase, Vineland, NJ), tubes were sealed with a screw cap fitted with a black butyl rubber stopper (Bellco Glass Inc., Vineland, NJ), autoclaved for 30 min, and then transferred to the $N_2$ and $CO_2$ atmosphere chambers.

**(ii) Liberation of bacteria from digesta and the mucosal surface.** Ligated intestinal samples were transferred into an anaerobic chamber (Forma Scientific, Inc., Marietta, OH) containing the $N_2$ atmosphere. The anoxic status of anaerobic chambers was routinely monitored using a resazurin anaerobic indicator (Oxoid Inc.). Once in the chamber, ligations were aseptically removed, and the intestinal segment was incised to expose the mucosal surface and digesta. A 1 $cm^2$ sample of the intestinal wall with digesta was removed, and transferred into 5 ml of reduced CB (HiMedia Laboratories LLC, West Chester, PA) in a 50-ml Falcon tube, where it was gently mixed by rocking side to side for 30 s. To isolate mucosa-associated bacteria, individual washed tissue segments were transferred to a new tube containing 5 ml of CB. The remaining suspension in the initial tube was used to isolate digesta-associated bacteria. Tubes were then vortexed vigorously (high setting for 0.5 min), and the resultant suspensions were used to isolate bacteria. Half of the tubes were retained within the $N_2$ atmosphere chamber, and half of the tubes were transferred to a $CO_2$ atmosphere chamber.

**(iii) Direct plating.** Bacteria suspended in CB (10 µl) were streaked onto each agar medium within the $N_2$ and $CO_2$ atmosphere chambers, and cultures were maintained for 7 days at 37°C. Where possible, cells from a minimum of five morphologically distinct colonies per culture were transferred to fresh reduced Columbia agar supplemented with 10% whole sheep blood (CBA), and streaked for purity as warranted.

**(iv) Enrichment.** Bacteria suspended in CB (10 µl) were added to enrichment media, and cultures were maintained at 37°C for 12 weeks in chambers with $N_2$ and $CO_2$ atmospheres. After the incubation period, bacteria were isolated by streaking the culture onto CBA as described above for direct plating.

**(v) Ichip.** A modified version of the original Ichip method (Berdy *et al.* 2017) was used. The primary modification was the use of a pipette tip holder as the Ichip apparatus, to which a membrane containing 0.2 µm pores (Sterlitech Corporation, Kent, WA) was attached to the Ichip bottom using Silicone II sealant (General Electric Company, Fairfield, CT). The assembled Ichip was sterilized by autoclaving and maintained in the $N_2$ and $CO_2$ atmosphere chambers for 24 h before use. Warm (37°C) reduced phosphate buffered saline (PBS; 0.1 M, pH 7.2) containing 0.5% agarose was used to dilute bacteria liberated from digesta and mucosal surfaces in CB. Bacterial cells in suspension (20 µl) were stained with Trypan Blue (20 µl) (Sigma-Aldrich), and enumerated using a Petroff-Hausser chamber at 100X magnification. Bacterial suspensions were diluted in the reduced PBS-agarose medium to a target density of one cell in 200 µl, and 200 µl aliquots were dispensed into individual cells within the Ichip apparatus. The top of the Ichip was sealed with a non-porous adhesive membrane (BioRad Laboratories Inc., Hercules, CA). Another salient modification from previous reported applications of the Ichip was the continuous submergence of the apparatus in freshly collected rumen fluid, which provided limiting nutrients including vitamins and co-factors; rumen fluid was collected from fistulated beef cattle (LeRDC Animal Use Protocol Review 1614). Ichips were maintained for 12 weeks, and rumen fluid was replaced with fresh fluid at 2 week intervals. After the incubation period, the Ichips were removed, and left on sterile paper towel within anaerobic chambers to dry. The Ichip top seal was then carefully removed taking care to prevent cross contamination among wells, and a 10 µl aliquot was removed from each well, and streaked onto reduced CBA as above.

**(vi) Endospore germination.** Two strategies were applied to kill vegetative bacteria, and to facilitate the isolation of endospore-forming taxa. These were the basic ethanol-killing method described by Browne et. al (Browne *et al.* 2016), and Tyndallization. For the former, an equal volume of ethanol (70%) was added to 750 µl of the bacterial cells suspended in CB in 2 ml sample vials, rigorously vortexed, and maintained at 37°C for 4 h. Subsamples of the CB-ethanol mixture (10 µl) were streaked onto CBA containing 0.1% taurocholic acid (Millipore, Burlington, MA). In addition, 10 µl of the suspensions were transferred into 10 ml Hungate tubes containing reduced Dehority's medium amended with 0.5% xylan (Sigma-Aldrich) or 0.5% porcine mucin (Sigma-Aldrich), as well as CB with 5% sheep blood for enrichment. Enrichments were maintained for 12 weeks at 37°C, and 10 µl aliquots were streaked onto CBA as above. For Tyndallization, ≈500 µl of the bacterial suspension was transferred to a sterile 1.5 ml

glass tube, and then sealed within the anaerobic chambers. The samples were transferred to an oven, and maintained at 100°C for 30 min to kill vegetative cells and to stimulate the germination of endospores. After the heat treatment, the tubes were returned to the anaerobic chambers, and processed as for the ethanol-killing method.

**(vii) Recovery and storage of bacteria.** Based on colony morphology, five representative colonies were selected per culture (1523 isolates in total), and cells from the colonies were streaked for purity on CBA. After ≥7 days, bacterial biomass was suspended in 1.5 ml of CB containing 40% glycerol, the tubes were sealed, snap frozen on dry ice, and transferred to -80°C for medium-term storage.

**(viii) Bacterial identification.** Bacteria were rejuvenated from glycerol stocks on CBA at 37°C in the atmospheres from which they were originally isolated until sufficient biomass was produced. Biomass was scraped from the agar surface, placed in CB, and sedimented by centrifugation (13,200 x *g* for 10 min). Genomic DNA was extracted using an automated DNA extraction robot (Autogen, Inc.) according to the manufacturer's recommendations. Amplicons of the 16S rRNA gene were generated using the primers 27F and 1492R (Costa *et al.* 2009). Amplicons were purified using a QIAquick PCR Purification kit (Qiagen N.V., Hilden, Germany) and sequenced by Eurofins genomics (Toronto, ON) using the 27F primer. Sequence chromograms were visualized, assessed for quality, and trimmed using Geneious (Biomatters, Inc., San Diego, CA).

**(ix) Analysis of isolated bacteria.** Bacterial taxonomy was assigned using the Seqmatch tool of the Ribosomal Database Project (RDP) with the following settings selected: strain = both (Type and Non-Type); source = Isolates; size = both (>1200 and <1200; quality = good; taxonomy = nomenclature; and KNN matches = 1 (Cole *et al.* 2014). Diversity metrics were made using functions of the vegan package in R (Version 3.4.3) and phylogenetic relationships were analyzed using the LefSE tool on the Galaxy Instance of the Huttenhower lab (http://huttenhower.sph.harvard.edu/galaxy/). Phylogenetic trees were generated and evolutionary analyses were conducted from the 16S rRNA gene sequences using the MEGA-X software package (Kumar *et al.* 2018). Briefly, a multiple sequence alignment was made using MUSCLE software and a tree was generated using the unweighted pair group method with arithmetic mean (UPGMA) method (Edgar *et al.* 2004). A bootstrap test for phylogeny was used with the number of replications set to 1000. A tree figure was generated with FigTree version 1.4.4 using the resulting Newick file (Rambaut *et al.* 2012.; Pohlert *et al.* 2018.). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.* 2004) and are in the units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option); there were a total of 1,258 positions in the final dataset (Kumar *et al.* 2018).

### 2.2.17 Bacterial community characterization by next-generation sequencing

DNA extracted from digesta and mucosa was processed with an Illumina protocol for creating 16S rRNA sequencing libraries. Extracted genomic DNA was amplified with Illumina indexed adaptor primers (V4 Schloss primers (James Kozich 2013. Access at https://github.com/SchlossLab/MiSeq_WetLab_SOP/blob/master/MiSeq_WetLab_SOP.md). The PCR reaction contained 5 µl of PCR buffer, 1 µl of 10 mM dNTPs, 1 µl of 25 mM of $MgCl_2$, 2.5 of µl of each primer, 0.25 µl of Hot Start Taq (Qiagen Inc.), 32.8 µl of molecular grade water, and 5 µl of bacterial community DNA. Amplicons were purified with QIAquick PCR Purification kit (Qiagen Inc.) following the manufacturer's recommendations. The effectiveness of the clean-up was checked by agarose gel electrophoresis, followed by quantification with a Qubit (Fisher Scientific, Ottawa, ON). Indexed DNA libraries were normalized to 1.5 ng $\mu l^{-1}$ and pooled together. A PhiX control (10%) was run with the normalized DNA library, and both were denatured and diluted to 8 pM prior to loading into the MiSeq Reagent Kit v2 500-cycle (Illumina, San Diego, CA). QIIME2 (Bolyen *et al.* 2019) was used to classify bacterial reads for digesta and mucosa-associated communities. This analysis was done using the tourmaline Snakemake reproducible workflow to automate QIIME2 (version 2019.7) analyses (https://github.com/ropolomx/tourmaline). Briefly, raw reads were denoised with DADA 2 (Callahan *et al.* 2016), and representative sequences, amplicon sequence variants (ASV) were generated. A phylogenetic tree of ASV sequences was generated, and the taxonomy of each ASV was identified by using a machine learning classifier pre-trained with the reference SILVA 132 database (silva-132-99-515-806-nb-classifier.qza). Alpha diversity metrics including number of taxa observed, the Shannon's index of diversity, and the Inverse Simpson index were calculated. The phyloseq package (version 1.28.0) of R version 3.6.1 was used to evaluate beta-diversity with a Principal Coordinate Analysis (PCoA) of the calculated Unweighted Unifrac distances, generating an ordination plot. Detection of differential abundance between tissues was done with the analysis of composition of microbiomes (ANCOM) in QIIME2 (Mandal *et al.* 2015).

### 2.2.18 Quantification of commensal bacterial taxa by quantitative PCR

To enumerate commensal taxa of interest (e.g. *B. uniformis* and *S. gallolyticus*) by qPCR, duplicate reactions (20 µl) consisting of 10 µl of QuantiTect SYBR® Green Mastermix (Qiagen Inc.), 0.5 µM of each primer (Table 2.5), 2 µl of community DNA, and 6 µl of DNase free water (Qiagen Inc.) were prepared. Data was collected using an Mx3005p Real Time PCR instrument (Agilent Technologies Canada Inc.). Cycle conditions were 95°C for 15 min, followed by 40 cycles at 94°C for 15 s, at the individual annealing temperatures (Table 2.5) for 30 s, and at 72°C for 30 s. Serial dilutions of genomic DNA containing 1.0 x

$10^6$ copies $\mu l^{-1}$ were used to prepare a standard curve. Concentrations of the bacterial DNA in the sample were determined based on standard curve Ct values, and copies $mg^{-1}$ or $g^{-1}$ were calculated. A dissociation curve was included with each run to verify amplicon specificity.

#### 2.2.19 Statistical analyses

The experiment was arranged as a two (*Salmonella* ±) by three (2, 6, and 10 dpi) factorial experiment with four replicates. These two factors and the interaction between them were treated as fixed effects. Given the experiment was run on two separate occasions (i.e. runs), run was treated as a random effect in the statistical model. Analyses for gene expression, histopathologic measurements, *Salmonella* quantification, bacteria quantification, physiopathology changes, and meat pH were performed using Statistical Analysis Software (SAS Institute Inc. Cary, NC). Normality was checked and analyzed in continuous data using the MIXED procedure of SAS. In the event of a main treatment event effect (P≤0.050), the least squares means test was used to compare treatments within factors. Histopathologic measurement data was analyzed using the pairwise Fisher's exact test in SAS. Quantification data for commensal bacteria was not normally distributed, and the data was analysed using the Kruskal-Wallis test in SAS. Data are represented by mean ± standard error of the mean (SEM).

### 2.3 Results

#### 2.3.1 Infection by *Salmonella* Typhimurium induced temporal changes in health status

All piglets inoculated with *S.* Typhimurium (SA+) showed clinical evidence of disease in a temporal manner. Inoculated piglets rapidly developed transient watery diarrhea (some with mucus discharge). This was particularly conspicuous within 2-3 days post-inoculation (dpi). Moreover, all inoculated piglets were modesty depressed and lacked appetite. By 6 dpi, diarrhea had largely abated, and by 10 dpi all piglets showed distinct evidence of recovery (e.g. semi-solid stools) and restoration of normal food consumption.

#### 2.3.2 Infection by *Salmonella* Typhimurium affected body temperature, feed consumption, and weight gain

SA+ animals exhibited an increase (P=0.028) in body temperature over time (P=0.007) (Figure 2.2A); the body temperature of inoculated piglets was elevated (P≤0.050) 1, 2, and 3 dpi. The consumption of feed was suppressed (P≤0.001) in infected animals 2 to 5 dpi (Figure 2.2B). SA+ piglets lost weight for 4 days, and weighed less (P=0.003) than control piglets (SA-) at all experimental end points (Figure 2.2C).

#### 2.3.3 Infection by *Salmonella* Typhimurium induced gross pathologic changes

At 2 and 6 dpi, all SA+ piglets showed gross evidence of enteritis in the cecum, ascending colon, and spiral colon, and to a lesser extent in the ileum. This included evidence of hyperemia and excessive gas

and liquefied digesta accumulation. Fibrinous mucosal necrosis with casts were frequently observed in the spiral and ascending colon of SA+ piglets. All SA+ piglets displayed enlarged lymph nodes, and some inoculated animals exhibited splenomegaly. In no instance were conspicuous gross pathologic changes observed in the duodenum or jejunum.

### 2.3.4 Infection by *Salmonella* Typhimurium induced temporal histopathologic alterations in the distal small intestine and large intestine

Substantive histopathologic changes to the intestine were observed in SA+ relative to SA- piglets. No differences were observed in histologic changes within infected animals between the two cecal locations (P=0.408) or the two spiral colon locations (P=1.000) examined; therefore, information from the two locations within the cecum and spiral colon were grouped. Higher (P≤0.041) total histopathologic scores (i.e. epithelial injury, neutrophil infiltration, fibrosis, villus fusion) were observed in SA+ piglets in the distal small intestine (distal jejunum and ileum), and throughout the large intestine at 2, 6, and 10 dpi (Figure 2.3). The degree of neutrophil infiltration (mucosa to submucosa) between SA+ and SA- piglets in the distal small intestine and large intestine was the highest (P≤0.001) at 2 dpi, and progressively subsided by 10 dpi (Figure 2.4). Changes were particularly evident in these intestinal sites, as there is marked transmural neutrophilic inflammation with multifocal to coalescing areas of epithelial erosion (Figure 2.5). In contrast, fibrosis in the large intestine of SA+ pigs was more prominent (P≤0.001) at 10 dpi (Figure 2.6).

### 2.3.5 Infection by *Salmonella* Typhimurium affected total white cells densities in blood

There was no difference (P=0.627) in the numbers or types of immune cells collected from the portal vein or systemic venous blood. Densities of total white blood cells, and the percentage of granulocytes in blood of SA+ piglets were elevated (P≤0.001) only at 10 dpi (Figure 2.7A,D). Moreover, the percentage of lymphocytes circulating in the blood of SA+ piglets was lower (P≤0.005) at 10 dpi (Figure 2.7B). The percentage of monocytes in blood were lower (P≤0.020) at 10 dpi (Figure 2.7C). Inoculation of pigs with *S*. Typhimurium had no effect (P>0.500) on blood chemistry, including alanine aminotransferase, alkaline phosphatase, creatinine, glucose, total protein, and blood urea nitrogen (*data not presented*).

### 2.3.6 Higher densities of *Salmonella* Typhimurium were observed in infected piglets at 2 days post-inoculation

*Salmonella* Typhimurium was not isolated from the feces of any of the piglets upon arrival at the Lethbridge Research and Development Centre (LeRDC), nor was bacterium isolated from SA- treatment animals during the experimental period. In contrast, *S.* Typhimurium was isolated from the feces of SA+

at high densities throughout the study period; densities of the bacterium shed in feces peaked at day 2 and 4 dpi (Figure 2.8). High densities of *S.* Typhimurium DNA were also observed in digesta and associated with mucosa throughout the intestinal tract (Figure 2.9). Densities of the pathogen associated with mucosa, and to a lesser extent in digesta, were highest (P≤0.039) in the distal small intestine and large intestine, and decreased (P≤0.017) over time. *Salmonella* Typhimurium was frequently isolated from the jejunal and ileal-cecal lymph nodes, and from the liver and spleen. The bacterium was isolated from the brain (frontal cortex and mid-brain) of one piglet 2 dpi, and from systemic blood of an additional animal 10 dpi. Genotyping of arbitrarily selected strains of *S.* Typhimurium isolated from piglets showed that recovered isolates possessed the same PFGE fingerprint as the *S.* Typhimurium SA970934 (*data not presented*).

### 2.3.7 The pH of meat was affected in piglets infected with *Salmonella* Typhimurium

The pH of *longissimus dorsi* muscle from SA+ piglets was lower (P<0.001) than SA- piglets (Figure 2.10). Moreover, the pH of *longissimus dorsi* muscle was lower (P<0.001) in pigs at 6 and 10 dpi regardless of the *Salmonella* treatment. There was no effect (P=0.824) of *S.* Typhimurium infection on drip moisture loss from the *longissimus dorsi* muscle harvested from piglets (*data not presented*).

### 2.3.8 Infection by *Salmonella* Typhimurium temporally modulated immune responses

Expression of genes encoding immune response proteins were evaluated to temporally characterize responses incited by *S.* Typhimurium throughout the intestinal tract. Differential expression of mRNA for immune genes was not observed in the duodenum or jejunum (*data not presented*). In contrast, the differential regulation of a variety of immune genes was observed in the ileum, cecum, and spiral colon (Figure 2.11). In the ileum, *βD2* (P=0.011), *MUC4* (P=0.038), and *REGIIIγ* (P=0.011) were upregulated at 6 dpi and/or at 2 dpi. In the spiral colon, *βD2* (P=0.029) and *MUC1* (P=0.024) were downregulated in SA+ at 2 dpi and/or 6 dpi. At 2 dpi, *TNFα* (P<0.001), *IFNγ* (P=0.006), *IL17* (P=0.014), *IL10* (P=0.006), *PR39* (P=0.002), *IL1β* (P<0.001), *IL8* (P=0.016), *iNOS* (P=0.006), *TLR4* (P=0.033), and *TGFβ* (P=0.030) were upregulated in the cecum of SA+ piglets as compared with SA- animals (Figure 2.12). At 6 dpi, *TNFα* (P=0.043), *IL8* (P=0.008), *IL17* (P=0.016), and *IL1β* (P=0.045) were upregulated in cecum of SA+ piglets compared with SA- animals. Additionally, at 6 dpi, *MUC1* (P=0.028) was downregulated in the cecum of SA+ piglets compared with SA- animals (Figure 2.11).

### 2.3.9 Bacterial communities characterized by next-generation sequencing differed in the small and large intestine

The digesta microbiota within the ileum mainly contained bacteria within the *Firmicutes* (75.0 ± 18.6%) and *Proteobacteria* (24.9 ± 18.8%) (Figure 2.13). In the cecum and spiral colon, the digesta

microbiota was primarily dominated by bacteria within the *Firmicutes* (39.0 ± 13.6% and 33.9 ± 21.9%, respectively) and *Bacteroidetes* (58.7 ± 13.9% and 64.0 ± 21.3%, respectively). At a family level of resolution, greater numbers of bacteria within *Clostridiaceae*-1, *Peptostreptococcaceae*, and *Pasteurellaceae* were observed in the ileum than in the large intestine. The large intestine was mainly dominated by bacteria within *Prevotellaceae, Lachnospiraceae*, *Ruminococcaceae,* and *Veillonellaceae*. At a genus level of resolution, an increase in the relative abundance of *Clostridium sensu stricto, Streptococcus*, and *Romboutsia* was observed in the ileum, and *Prevotellaceae*-9 abundance increased in the cecum and spiral colon.

Number of amplicon sequence variants (ASVs), Shannon index, Pielou's evenness, and Faith's phylogenetic diversity were evaluated to characterize the alpha diversity of luminal samples of each intestinal site. In all instances, bacterial communities in the ileum differed from the cecum and spiral colon, which were similar to each other. For example, ASV counts were lower in ileum as compared with cecum ($P<0.001$) and spiral colon ($P<0.001$). Moreover, Shannon's index indicated lower diversity in the ileum as compared to the cecum ($P<0.001$) and spiral colon ($P<0.001$), and no difference ($P=0.589$) was observed between the cecum and spiral colon. There was no difference ($P=0.290$) in species evenness between the cecum and spiral colon, but evenness was reduced ($P<0.001$) in the ileum relative to the other two sites. A clear separation in beta diversity of bacterial communities (using unweighted principal coordinate analysis) was observed between digesta in the ileum relative to the cecum and spiral colon (Figure 2.14). There was no difference in beta diversity of bacteria between the cecum and spiral colon.

To determine if bacterial communities observed in digesta corresponded with those associated with mucosa, I examined the composition of the microbiota associated with the mucosa of the spiral colon. Bacterial communities associated with mucosa were similar in composition to those within digesta, and no significant differences were observed at the phyla, family, or genus levels (Figure 2.15).

### 2.3.10 Next-generation sequence analysis showed taxon- and location-specific changes in bacterial communities in piglets infected with *Salmonella* Typhimurium

Amplicon Sequencing Variant counts were lower ($P=0.008$) in SA+ animals. Piglets infected with *S*. Typhimurium showed an increase in the relative abundance of *Prevotellaceae* in digesta within the cecum at 2 and 6 dpi (71-79% and 50-56%) (Figure 2.13). A higher abundance of *Enterobacteriaceae* was observed in infected animals at day 6, and to a lesser extent at day 2 in the ileum. Although some genera, such as *Ruminococcaceae* and *Veillonellaceae*, tended to decrease in the cecum and spiral colon of SA+ piglets, no significant differences in the relative abundance were observed in SA+ piglets.

A lower (P=0.048) alpha diversity of bacteria was observed in the ceca of SA+ relative to SA- piglets at 2 dpi, but not at 6 and 10 dpi (Figure 2.16). In contrast, alpha diversity was equivalent within the ileum and spiral colon of SA+ and SA- piglets (Figure 2.16).

**2.3.11 The culturable bacteria differed in piglets infected with *Salmonella* Typhimurium**

To increase the taxonomic resolution and ascertain information on function, comprehensive isolation methods were applied to recover bacteria from the ileum, cecum, and spiral colon of SA+ and SA- piglets. A total of 1,526 bacterial isolates were isolated and identified; of these, 419, 331 and 484 were collected from the ileum, cecum and spiral colon, respectively. The composition of the culturable and culture-independent bacteria communities differed (Figure 2.17). For example, no *Bacteroides* or *Parabacteroides* were detected by next-generation sequence (NGS) analysis, whereas, twelve species of these two genera were isolated (*B. caccae*, *B. denticanum*, *B. eggerthii*, *B. fragilis*, *B. pyogenes*, *B. stercoris*, *B. uniformis*, *B. vulgatus*, *B. xylanisolvens*, *B. heparinolyticus*, *P. distasosis*, and *P. merdae*) (Figure 2.18). Moreover, *Streptococcus* species were not detected in cecum and spiral colon by NGS, whereas *Streptococcus gallolyticus* was commonly isolated from the ileum, cecum, and spiral colon (Figure 2.19). Analysis of taxa revealed that the abundance of taxa isolated differed between infected and control pigs (Figure 2.20). For example, *S. gallolyticus* was isolated only from the intestines of pigs infected with SA+ but not from SA- piglets. Additionally, higher abundances of *Gammaproteobacteria* (*Escherichia/Shigella, Proteus*, and *Salmonella*) were observed in SA+ piglets at 6 dpi (Figure 2.20). Bacteria that were more common in SA- animals included unclassified members of the *Ruminococcaceae* family (Figure 2.19). These bacteria included putative members of the *Intestinimonas* spp., as well as some bacteria that are most closely related to *Soleaferrea massiliensis*, among other previously undescribed bacteria. At 10 dpi, the composition of the culturable microbiota from SA+ animals did not differ from control piglets with the exception of *Gammaproteobacteria* whose abundance remained higher in SA+ animals (Figure 2.20). In the ileum, members of the *Actinobacteria* order (e.g. *Bifidobacterium pseudolongum*) were more commonly isolated from SA- piglets (Figure 2.19). In contrast, *Gammaproteobacteria* (*Escherichia/Shigella* and *S. enterica*) and *Fusobacterium* (*F. varium* and *F. gastrosuis*) were commonly isolated from ileum of SA+ animals. In the cecum and spiral colon of SA+ piglets, members of *Bacteroidia* (*B. uniformis*, *B. fragilis* and *B. heparinolyticus*) were more abundant. Bacteria within the *Firmicutes* phylum (i.e. *Bacilli*, *Clostridia*, *Erysiphelotrichia*, and *Negativicutes*) were recovered from all locations, and from both SA+ and SA- piglets. However, members of the *Bacilli* (i.e. *Lactobacillales and Bacilliales*) were more prevalent in SA- piglets. Although *Clostridia*

bacteria were more commonly associated with samples collected from the spiral colon of SA+ piglets, a higher abundance of the *Clostridiaceae* family was observed in cecum of SA- piglets.

### 2.3.12 Quantitative PCR confirmed that densities of bacterial genera differed by intestinal location and in piglets infected by *Salmonella* Typhimurium

Lower quantities of DNA for *B. uniformis*, *S. gallolyticus, Intestinimonas* spp., *Prevotella* spp., and *Ruminococcus* spp. were observed in the ileum compared to the cecum ($P<0.001$) and spiral colon ($P<0.001$) (Figure 2.21-2.22). Although the concentration of *Clostridium* cluster I spp. DNA was higher in ileum in comparison to the other genera examined, concentrations were still lower ($P<0.007$) in the ileum relative to the cecum (Figure 2.22C). *Bacteroides uniformis* and *S. gallolyticus* DNA was detected in the digesta and in association with mucosa (*data not presented*) of the ileum, cecum, and spiral colon, albeit at low densities. Consistent with the frequency of isolation, densities of *B. uniformis* were higher ($P\leq0.058$) in the digesta of infected animals in cecum at day 10 and spiral colon at 6 dpi (Figure 2.21). Similarly, a trend for higher ($P\leq0.124$) densities of *S. gallolyticus* in the digesta of infected animals was observed in the cecum and spiral colon at both 6 and 10 dpi (Figure 2.21).

## 2.4 Discussion

The composition of the intestinal microbiota of piglets has been studied by a number of research groups (Alain *et al.* 2014; Dou *et al.* 2017; Kelly *et al.* 2017; Liu *et al.* 2012; Looft *et al.* 2012). However, the impact of infection and ensuing inflammation on the enteric microbiota is poorly understood at present. In this study, I temporally (2, 6 and 10 dpi) and spatially (small to the large intestine) characterized host responses due to *S.* Typhimurium infection in piglets. In this regard, I concomitantly measured immune responses, histopathologic alterations, and changes to the structure of the enteric microbiota at the acute, subacute, and chronic stages of salmonellosis. The cecum was the most affected location. To my knowledge, the current study is the first to characterize the microbiota of piglets infected with *S.* Typhimurium using culture-dependent and culture-independent methods. NGS analysis allowed me to conclude that the primary changes to the microbiota happened in the cecum at the earliest stages of disease (i.e. 2 dpi), which correlated with the highest level of inflammation. A substantially lower Shannon diversity, an increase in the relative abundance of *Proteobacteria,* and a decrease in the relative abundance of *Clostridiaceae* and *Lachnospiraceae* were conspicuous alterations to the microbiota observe in the cecum of infected animals. Additionally, culture-based methods allowed us to examine bacteria at a higher resolution than NGS analysis, and demonstrated higher association of some species with inflamed tissues (e.g. *S. gallolyticus* and *B. uniformis*). This study

reports on the coordinated influence that *S.* Typhimurium has on the enteric microbiota and host in infected piglets.

Salmonellosis is a concern to the swine sector for two primary reasons. Firstly, the impact of the disease causes significant economic losses, and secondly, *S*. Typhimurium is an important foodborne pathogen, responsible for significant morbidity of people (Scherer *et al.* 2008), as well as additional losses to the swine sector (e.g. meat recalls). Swine infected with *S.* Typhimurium exhibit a disease progression, but the temporal characteristics of acute salmonellosis in pigs are not well defined. I incited salmonellosis by inoculating piglets with a highly virulent strain of *S.* Typhiumurium DT104 (SA970934) (Yin *et al.* 2014), and I temporally measured a variety of metrics in infected and uninfected animals. Similarly to Scherer *et al.* (Scherer *et al.* 2008) I observed evidence of enteritis (Balaji *et al.* 2000; Johnson 2005) in piglets 1 to 2 dpi, including elevated body temperature, inappetence, body weight loss and diarrhea. To temporally characterize the impacts of salmonellosis on the host, piglets were humanely euthanized, and intestinal (i.e. duodenum to the descending colon) and extra-intestinal (e.g. portal vein and systemic blood, mesenteric lymph nodes, spleen, liver, brain, muscle) samples were recovered and analyzed at 2, 6, and 10 dpi. Substantive histopathologic changes were observed throughout the intestine in infected piglets at all three time points. In this regard, pathogen densities in digesta and mucosa samples, and total histopathologic scores, including epithelial injury and neutrophil infiltration, were higher at 2 dpi in piglets infected with *S*. Typhimurium. I observed that densities of *S.* Typhimurium within digesta and associated with mucosa decreased over time. There was also an attenuation of intestinal injury, which had returned near normal levels by 10 dpi. However, levels of fibrosis were elevated at 10 dpi in the cecum, ascending colon, spiral colon and descending colon of piglets infected with *S*. Typhimurium; an expected observation of tissue repair following injury. Spatially, histopathologic changes were most pronounced in the distal small intestine, and within the cecum and spiral colon, which is consistent with previous studies (Chirullo *et al.* 2015; Wilcock *et al.* 1976). It is suggested that the location of tissue injury may be attributed to the lower concentration of bile salts and beta-defensins present at these sites (Prouty *et al.* 2000). My findings based on histopathologic changes, indicated that the three sample times at which piglets were evaluated in the current study (i.e. 2, 6, and 10 dpi), represented, acute, subacute, and recovery stages of salmonellosis, respectively.

Examination of immune marker modulation is essential to fully understand the progression of salmonellosis in swine. Immune responses triggered after infection with *S.* Typhimurium in swine have been previously described (Collado-Romero *et al.* 2010; Meurens *et al.* 2009; Uthe *et al.* 2007). However, evaluation of the immune response within the entire GIT has not been examined. In the

current study I measured different T helper cell responses that occurred after *Salmonella* infection, and the temporal progression of these responses. More specifically, I evaluated Th1 (*IFNγ*, *TNFα*), Th2 (*IL4*), Th17 (*IL17*) and T regulatory (*IL10* and *TGFβ*) responses. I also examined innate mechanisms of defense such as, host defense peptides, including defensins (*βD1* and *βD2*), C-type lectins (*REGIIIγ*), and cathelicins (*PR39*). As well, I evaluated expression of pattern recognition receptors (*TLR4*). Importantly, these metrics were comparatively investigated both temporally and spatially along the intestinal tract. I observed that *S.* Typhimurium did not affect the expression of the target genes in the duodenum, jejunum, and proximal ileum. In contrast, targeted genes were upregulated in *S*. Typhimurium infected piglets in the distal ileum, cecum, and colon, which corresponded to the histopathologic changes observed. In these locations, differential regulation of gene expression primarily occurred at 2 dpi (acute stage), with modest changes at 6 dpi, and no difference between infected and non-infected piglets at 10 dpi (recovery). The cecum was the most affected intestinal site. In the cecum, I observed elevated expression of *IL17* and *IFNγ*, which corresponds to the findings of other studies (O'Donnell *et al.* 2014; Godinez *et al.* 2008). Secretion of IL17 and contact between the pathogen and enterocytes have been shown to enhance the secretion of IL8 attracting neutrophils from the microvasculature to the intestinal mucosa (McCormick *et al.* 1995). In the current study, a greater neutrophilic infiltration was observed in cecum at 2 dpi, which corresponded with the elevated expression of *IL8* that was observed at this time point. I observed elevated expression of inducible nitric oxide synthase (*iNOS*) in the cecum at 2 dpi. Since its secretion has been associated with elimination of *S*. Typhimurium and also with epithelial injury (Cherayil *et al.* 2001), this is consistent with the mucosal damage that I observed in the cecum at 2 dpi. Induction of a pro-inflammatory response by pathogens is often accompanied by an anti-inflammatory response triggered by the host (Toms *et al.* 2001). In the current study, I observed that *S*. Typhimurium infected piglets which exhibited a higher pro-inflammatory response in the cecum at 2 dpi, concurrently presented a higher expression of *IL10* and *TGFβ*. Both of these anti-inflammatory cytokines have previously been shown to incite a regulatory response to attenuate inflammation within the intestine (Toms *et al.* 2001); therefore, the elevated expression of these cytokines is likely directed to control the inflammatory response triggered by *S.* Typhimurium. One of the main mechanisms of innate defense that the host utilizes to eliminate pathogens, and also to shape the microbiota, is the secretion of antimicrobial and host defense peptides (HDPs) (Salzman *et al.* 2007). In the current study, I examined the expression of β-defensins (e.g. *βD2*), C-type lectins (e.g. *REGIIIγ*), and cathelicidins (e.g. *PR39*). I observed that these three HDPs were upregulated after *Salmonella* infection. The secretion of β-defensins is induced after detection of pathogens due to their bactericidal activity (Wang *et al.* 2014). In

piglets, the secretion of βD2 has previously been shown to inhibit the growth of *S.* Typhimurium (Veldhuizen *et al.* 2008). Elevated expression of REGIIIγ has also been observed following infection with enterotoxigenic *E. coli* in pigs, which was implicated with its bactericidal action by pore-forming activity (Soler *et al.* 2015). A primary group of HDPs are the cathelicidins (Holani *et al.* 2016). In pigs, *PR39*, a homologue of *LL37* and *mCRAMP* in human and mice, respectively, has been thoroughly studied due to its broad antimicrobial spectrum, but not extensively in pigs with salmonellosis (Holani *et al.* 2016; Veldhuizen *et al.* 2014). The release of cathelicidins by neutrophils and their expression in the mucosa surfaces have been associated with local host defense. Their antimicrobial activity is not only limited to the potent, rapid and broad spectrum bactericidal function, but also to their capacity to bind and neutralize endotoxins (Mookherjee *et al.* 2007). PR39 has been observed to inhibit bacterial DNA and protein synthesis (Veldhuizen *et al.* 2014). Its immunomodulatory effects include leukocyte chemotaxis, modulation of cytokine production, as well as stimulation of wound healing (Gallo *et al.* 1994) and angiogenesis (Li *et al.* 2000). In the current study, HDPs were the only genes upregulated not only in the cecum of *S*. Typhimurium infected piglets, but also in the ileum of infected animals. In this regard, two- and three-fold increases in expression of *βD2* and *REGIIIγ*, respectively, were observed in the distal ileum of infected piglets at 2 dpi, while *PR39* showed a three-fold increase in the cecum of infected animals at 2 dpi. The differential regulation of *βD2* and *REGIIIγ* in the ileum compared with the cecum was expected, since the higher expression of both of these immune markers in the ileum of infected pigs has previously been reported (Soler *et al.* 2015; Veldhuizen *et al.* 2008). Since *PR39* is mainly secreted by neutrophils, the higher expression in the cecum, that I observed in the current study, could be likely related to the level of neutrophil infiltration observed at this intestinal site. Although the roles that cathelicidins perform in modulating the enteric microbiota and salmonellosis are enigmatic at present, the changes in expression that I observed could be intimately related to their bactericidal activity directed against *S.* Typhimurium (Mukherjee *et al.* 2015).

A primary goal of the current study was to describe how the commensal microbiota is temporally modified during the induction and progression of intestinal disease. We comparatively characterized bacterial communities in the ileum, cecum, and spiral colon of *S*. Typhimurium infected and control piglets using a combination of culture-independent and culture-dependent methods. Results from next-generation sequencing showed a clear separation between the ileal and large intestinal communities. In the small intestine, and primarily in the ileum, the microbiota was predominated by facultative anaerobic bacteria that are adapted to acidic pH, high concentrations of bile acids, antimicrobial peptides, and higher levels of oxygen, which allows them to outcompete many obligate anaerobic

bacteria for simple carbohydrates (e.g. oligosaccharides) (Zhang *et al.* 2018). It has been proposed that the microbiota of the ileum originates from the cecum, a hypothesis that is primarily based on the presence of similar taxa at both sites (Brooks SPJ *et al.* 2011). In the ileum, we observed a higher abundance of *Enterobacteriaceae* in the luminal digesta relative to the large intestine. This may be attributed to the higher concentration of oxygen found in the small intestine that would differentially benefit facultative anaerobic taxa in this family (Donaldson *et al.* 2016). In contrast, the neutral pH, lower concentration of antimicrobials, slower transit time, and greater access to complex carbohydrates in the large intestine facilitate the colonization and proliferation of a substantially higher diversity of autochthonous bacteria, with cell densities in the range of $10^{11}$ to $10^{12}$ cells $g^{-1}$ in the large intestine compared with $10^{4}$-$10^{8}$ cells $g^{-1}$ in the small intestine (Rastall *et al.* 2004; Donaldson *et al.* 2016).

In the cecal and spiral colonic digesta, we observed that bacteria within the *Bacteroidetes* and *Firmicutes* were most prevalent, which has been observed previously (Zhang *et al.* 2018). Overall, we observed taxon-specific changes in the composition of bacterial communities within digesta between *S*. Typhimurium infected and control piglets. The most pronounced changes between the two treatment groups occurred at 2 dpi, which corresponded with the highest degree of intestinal damage (i.e. acute salmonellosis). Communities in cecal digesta were more affected by inflammation than in the spiral colon. In this regard, a decrease in alpha diversity was observed in the cecal digesta of *S*. Typhimurium infected piglets at 2 dpi, which corresponds to a similar observation in chickens infected with *S.* Typhimurium (Mon *et al.* 2015). A higher abundance of *Proteobacteria* (e.g. *Enterobacteriaceae*) was observed in both the cecum and spiral colon of *S*. Typhimurium infected piglets, and this was most pronounced in the cecum at 2 dpi as has been observed previously (Litvak *et al.* 2017). An increase in bacterial proportion within this phylum is a hallmark of dysbiosis and epithelial injury (Litvak *et al.* 2017; Mon *et al.* 2015), and since the *Proteobacteria* phylum contains many human and porcine pathogens, including *Salmonella*, the increase in abundance of pathogenic organisms within inflamed tissues is expected. Correspondingly, obligate anaerobes such as *Clostridiaceae*-1 and *Lachnospiraceae* decreased in abundance within the cecum and spiral colon of *S*. Typhimurium infected animals at 2 dpi, but recovered to normal levels by 10 dpi. Changes in the relative abundance of *Enterobacteriaceae*, *Clostridiaceae*, and *Lachnospiraceae* are influenced by levels of oxygen in the lumen of infected piglets (Litvak *et al.* 2017; Rigottier-Gois *et al.* 2013). When inflammation is triggered within the GIT, higher levels of oxygen are released into the lumen (Rigottier-Gois *et al.* 2013). The increase in luminal oxygen could be due to higher irrigation of the GIT in chronic inflammation with an extravasation of hemoglobin carrying oxygen into the lumen (Rigottier-Gois *et al.* 2013). A change in oxygen level could also be the

result of inflammation itself, as an oxidative burst triggered by neutrophils releases reactive oxygen species into the lumen benefiting facultative anaerobes (Winter *et al.* 2010). Moreover, a negative correlation between *Enterobacteriaceae* and *Prevotellaceae*, *Ruminococcaceae,* and *Lachnospiraceae* has previously been described in other hosts infected with *Salmonella* (Mon *et al.* 2015). The production of short-chain fatty acids (SCFAs), specially butyric acid, by obligate anaerobic bacteria within *Prevotellaceae*, *Ruminococcaceae,* and *Lachnospiraceae*, has been shown to inhibit *Salmonella* in an acidic environment (Bearson *et al.* 2013), reduce *Salmonella* invasion (Gantois *et al.* 2006), and decrease the pro-inflammatory response triggered by this pathogen in enterocyte cells (Malago *et al.* 2005). This could explain the relatively low abundance of *Enterobacteriaceae*, and the high abundance of *Prevotellaceae*, *Ruminococcaceae*, and *Lachnospiraceae* that we observed in digesta from the cecum and spiral colon of *S*. Typhimurium infected piglets in the current study. In addition to spatial differences along the GIT, the composition of the microbiota varies in the cross-sectional axis of the intestine. Unique niches for enteric bacteria (Kelly *et al.* 2017) are provided by higher concentrations of oxygen, the proximity to the mucosa, and the abundance of mucin glycoproteins and other nutrient sources. I observed higher densities of *S.* Typhimurium in association with the mucosa as compared with the luminal digesta in the cecum and spiral colon. This is consistent with the lowered densities of *Prevotellaceae*, *Lachnospiraceae,* and *Ruminococcaceae* previously observed in association with cecal mucosa (Kelly *et al.* 2017). In the current study, communities associated with the mucosa were not significantly different in their composition than those in the digesta of the spiral colon (i.e. via Illumina sequencing of the community). Although I observed a trend for a higher abundance of bacteria within the *Enterobacteriaceae* and *Campylobactereaceae* in association with mucosa (i.e. families that contain species known to associate with mucosa) these differences were not statistically different. Studying mucosa-associated bacteria is challenging. For example, removal of residual digesta from mucosa surfaces without disrupting the integrity of the loosely adherent mucus is exceptionally difficult. Furthermore, the predominance of host DNA can lead to preferential amplification of host DNA; to circumvent this possibility I used a DNA enrichment method that allowed capture and elimination of methylated host DNA while conserving and enriching bacterial DNA. Although some studies have reported a unique bacterial community associated with mucosa (Zhang *et al.* 2018), conclusions regarding differential composition of mucosa-associated and luminal communities without supporting evidence (e.g. fluorescent *in situ* microscopy) should be interpreted carefully.

Despite the current emphasis on NGS technologies to characterize the intestinal microbiota of mammals, there are inherent limitations of sequence-based methods (e.g. poor taxonomic resolution,

taxa overrepresentation, poor sensitivity, and incapacity to differentiate live from dead bacteria) (Inglis *et al.* 2012). Thus, we also used culture-based methods to characterize enteric communities in the intestines of *S*. Typhimurium infected and control piglets. To isolate bacteria, we applied a variety of media and strict anaerobic methods (e.g. differential killing of vegetative cells, induction of endospore germination, direct plating, long-term enrichments, and an Ichip method modified to isolate enteric bacteria) (Lagier *et al.* 2012; Browne *et al.* 2016; Berdy *et al.* 2017). We observed that no single isolation method was comprehensive, and that a combination of methods was required. In particular, the use of long-term enrichments and Ichip recovered the highest diversity of bacteria. Similarly to Lagier *et al.* (2016) we observed that Columbia agar supplemented with sheep's blood provided good recovery of bacteria by direct plating. Of the isolation methods utilized in the current study, the use of heat treatment was found to noticeably change the diversity of isolated bacteria, which is in agreement with Fenske *et al.* (2020). However the diversity of the bacteria isolated via Tyndallization as well as via ethanol exposure was reduced relative to the long-term enrichment and the Ichip methods. This decrease in diversity is in disagreement with that of Browne *et al.*(2016), who suggested spore germination methods such as the ethanol exposure would increase the diversity and isolation of novel taxa. It is worth noting that we relied solely on identification of bacteria by sequencing the 16S rRNA gene, whereas Browne *et al.* (2016) use a MALDI-TOF to initially screen bacteria. It is noteworthy that Russell *et al.*(1979) isolated and characterized bacteria from the intestines of health pigs in the late 1970's. They relied on a single method, and recovered 46 presumptive taxa that were identified using physiological characters. In contrast, we recovered a minimum of 173 different bacterial species, representing seven phyla using a combination of methods concert. Our findings of prominent taxa broadly correspond with that of the Russell *et al.* (1979) study in that *Eubacterium*, *Lactobacillus*, and Gram positive cocci were frequently recovered. That we targeted bacteria associated with inflamed tissues coupled with the use of a combination of culturomic methods likely contributed to the higher diversity of bacteria that we recovered from piglets.

Our culturomic and NGS analysis of enteric bacteria in piglets with salmonellosis both showed an elevated abundance of *Gammaproteobacteria* (*Escherichia/Shigella, Proteus* and *Salmonella*), which has been previously described (Zeng *et al.* 2017). However, in other instances the composition of the bacterial communities as determined by culturomics and NGS analysis differed conspicuously. In this regard, a number of isolated taxa were observed to be differentially associated with inflamed tissues. For instance, *S. gallolyticus* was solely isolated from *S*. Typhimurium infected piglets, and *Bacteroides fragilis*, *Bacteroides heparinolyticus, B. uniformis*, and *Acidominococcus fermentants* were more

commonly isolated from pigs with salmonellosis; these three genera were not detected using NGS analysis. Fenske *et al.* (2020) recovered *S. gallolyticus* from healthy adult pigs maintained in a commercial production setting, whereas the isolates that we recovered were isolated from piglets infected with S. Typhimurium in a controlled setting. That *S. gallolyticus* was isolated at a conspicuously higher frequency from inflamed tissues suggests that this bacterium is favored by this condition. An association between *S. gallolyticus* and colon cancer tissues in human beings has been recently reported (Boleij *et al.* 2013). Therefore, the presence of this bacterium in *S*. Typhimurium infected piglets may be a result of a propensity of this bacterium to colonize inflamed tissue sites (Al-Jashamy *et al.* 2010; Fyderek *et al.* 2009). A characteristic of *S*. *gallolyticus* that may favor the colonization of inflamed tissues is its ability to form biofilms when alterations in the physical barrier allows exposure of collagen type I and IV (Boleij *et al.* 2013). Another possibility is that *S. gallolyticus* is able to evade the immune response mounted by the host (e.g. host defense peptides) as has been observed for other taxa effective at overcoming colonization resistance in the intestine. Of the ten species of *Bacteroides* that we isolated, only *B. fragilis*, *B. uniformis*, and *B. heparinolyticus* were more commonly isolated from piglets with salmonellosis. An association of enterotoxigenic *B. fragilis* with inflamed tissues in piglets and human beings has been described previously (Duimstra *et al.* 1991; Sears *et al.* 2008). This bacterium is known to colonize crypts (Lee *et al.* 2013). Moreover, the formation of capsules provides resistance to the complement system, and to phagocytic uptake and killing (Wexler *et al.* 2007). The utilization of a comprehensive isolation strategy revealed a number of bacteria that are differentially associated with infected piglets, and demonstrated the value of using culture-dependent methods in concert with NGS analysis of communities to characterize bacteria potentially associated with salmonellosis. Ancillary examination of cell densities by taxon-specific qPCR showed a tendency of *S. gallolyticus* and *B. uniformis* to increase in cecum and spiral colon of *S*. Typhimurium infected piglets. However, the low level of detection of these taxa associated with mucosa and the limitation of real-time qPCR to differentiate between nucleic acids from live and dead cells emphasizes the requirement of conducting *in vivo* analyses to confirm the propensity of these taxa to colonize inflamed tissue.

A number of complementary strategies can be applied to ascertain bacterial function. The application of transcriptomics and shotgun metagenomics are two approaches that can be useful to elucidate function *in vivo*. In pigs, a shotgun metagenomic approach to characterize metabolic profiles of bacteria in feces was recently reported (Wang *et al.* 2019). This is a major advantage over traditional NGS analysis as it provides researchers with not only the information of the bacterial taxa present within a sample, but also their entire genomic repertoire including important genes such as antimicrobial

resistance determinants. However, a major disadvantage of metagenomics is the inability to experimentally elucidate the function of individual taxa or assemblages of taxa. Moreover, bacteria are not recovered from the samples for subsequent empirical testing. In the current study, we used culturomics in concert with NGS characterization of bacteria communities to identify bacterial species associated with inflamed tissues, and to acquire the bacteria for subsequent experimentation. For instance, we plan to confirm the propensity of isolated bacteria to colonize inflamed intestine as autochthonous taxa (e.g. in gnotobiotic models of salmonellosis). Furthermore, we will elucidate mechanisms of competitive colonization and impacts on colonization resistance (He *et al.* 2019). It is noteworthy that >90 undescribed bacteria were recovered in the current study, and the ecological role that these bacteria have in the intestines of pigs and other animals is uncertain.

The 'commensal' microbiota participates in host nutrition, development of the gastrointestinal immune system, maturation of the intestine, and defense of the GIT (Sansonetti *et al.* 2004; Hooper *et al.* 2002). Crucially, commensal microorganisms impede pathogens from effectively colonizing the GIT via a variety of mechanisms, thereby attenuating or preventing disease (Sassone-Corsi *et al.* 2015). The production of SCFAs by the large intestinal microbiota can influence colonization resistance. Although the addition of non-starch polysaccharides in the diet and a shift to fermentation-based nutrition is not desirable in production pigs, the production of SCFAs via fermentation of polysaccharides can provide beneficial impacts for intestinal health (Rios-Covian *et al.* 2016). Therefore, SCFA can enhance intestinal health by reduction of pH, stimulation of mucin secretion, improvement of tight junction integrity and induction of T-regulatory cells differentiation to impair pathogen colonization (Rios-Covian *et al.* 2016). Although we did not measure SCFA production, we observed a negative correlation between SCFA-producing taxa (e.g. *Ruminococcacea, Prevotellaceae*, and *Lachnospiraceae*) and bacteria within the *Enterobacteriaceae*, which could be intimately related to their excretion of SCFA and thus their mechanisms of defense (Boyen, Haesebrouck, *et al.* 2008). Additionally, the upregulation of *MUC4* that I observed in *S*. Typhimurium infected animals at 2 dpi could be associated with microbial-stimulated enhancement of barrier function, which has been reported after secretion of butyric acid (Jung *et al.* 2015). Stimulation of host immune defenses by the commensal flora, mainly the release of HDPs, has been described as an indirect mechanism of colonization resistance (Sassone-Corsi *et al.* 2015). In the current study, the elevated expression of HDPs (e.g. *PR39*, *REGIIIγ*, and *βD2*) that was observed in *S*. Typhimurium infected piglets at 2 dpi may be a key step in restoring homeostasis. However, pathogens have developed different strategies to compete with the microbiota for nutrients and binding sites to facilitate their ability to colonize the GIT. In this regard, *S.* Typhimurium has been shown to induce

inflammation in an attempt to outcompete the microbiota (Chirullo *et al.* 2015; Stecher *et al.* 2007; Thiennimitr *et al.* 2011). Therefore, the higher degree of inflammation, the higher abundance of *S.* Typhimurium, and the lower diversity of commensals observed in the cecum of animals with salmonellosis at 2 dpi could be also a clear indication of the importance of the normal microbiota in maintaining an equilibrium in the GIT.

In conclusion, I evaluated the progression of salmonellosis in pigs by characterizing histopathologic changes in the GIT, host immune responses, and alterations to the enteric microbiota. To characterize the microbiota, we applied a combination of culture-dependent and culture-independent methods, and found that a reliance on NGS analysis was insufficient to detect taxon-specific changes associated with inflammation. Furthermore, we recovered a number of taxa that were differentially abundant in pigs with salmonellosis (e.g. *S. gallolyticus*), and the acquisition of these bacteria will facilitate functional assessments. A detailed evaluation of the host immune responses pointed to upregulation of host defense peptides (HDPs) as an important mechanism modulating disease. It is currently believed that these peptides execute their bactericidal function in a prescribed manner; however, the mechanisms by which HDPs trigger an immune response and also modify the microbiota are not well understood. The utilization of knock-out (KO) mice lacking antimicrobial peptides genes could be used to elucidate the role that these peptides play in salmonellosis. Moreover, delivery of HDPs to sites of enteric inflammation may represent a novel and non-antibiotic strategy to mitigate enteritis. These are areas of current research by our team.

### 2.5 Tables and figures

Table 2.1 Histopathologic scoring system.

| Score | Villus blunting (i.e. crypt to villus ratio) | Villus fusion | Lymphoid depletion (reduction in lymphoid cells) | Neutrophil infiltration | Epithelial injury | Fibrosis (desmoplasia) |
|---|---|---|---|---|---|---|
| 0 | normal | normal | none | none | none | normal |
| 1 | <25% reduction | mild (small increases in numbers of fused villi) | mild (small reduction) | rarely observed in tissue | rare (< 10 surface epithelial cells shedding) | rare with small foci of collections of reactive fibroblast or small areas with increased amounts of mature collagens |
| 2 | 26-50% reduction | moderate (prominent increases in numbers of fused villi) | moderate (prominent reduction) | few scattered neutrophils within the tissue | mild (focal epithelial erosions; 11-50 surface epithelial cells shedding) | small focal to multifocal areas of collections of reactive fibroblast or small focal to multifocal areas with increased amounts of mature collagens |
| 3 | 51-75% reduction | severe (substantive increases in numbers of fused villi) | severe (marked reduction) | many foci with collections of few numbers of neutrophils | moderate (multi focal surface epithelium erosions; <50 of surface epithelial cells shedding) | large areas of reactive fibroblast or large areas of increased amounts of mature collagens |
| 4 | complete villus loss | – | – | large numbers of neutrophils present within the tissue | severe (multi focal to coalescing area of surface epithelium erosions; >50 of surface epithelial cells shedding) | – |

Table 2.2 Sequences and annealing temperatures for primers used for gene expression.

| Target gene | Primer | Sequence (5' to 3') | Annealing Temperature (°C) | Source |
|---|---|---|---|---|
| *Ppia* | Ppia-F | GACAGCAGAAAACTTCCGTG | 58 | NCBI Blast |
| | Ppia-R | ACCACCCTGGCACATAAATC | | |
| *HPRT* | pHPRT-F | AGGCTATGCCCTTGACTACA | 58 | NCBI Blast |
| | pHPRT-R | GGCTTTGTATTTTGCCTTTCCA | | |
| *Gusβ* | pGusB-F | CATGAGGCCTACCAGAAACC | 58 | NCBI Blast |
| | pGusB-R | GAGGTGGATCCTCGTGAAAC | | |
| *βD2* | pBD2-F | AGCTGGCTGCAGGTATTAAC | 58 | NCBI Blast |
| | pBD2-R | TCAATCCTGTTGAAGAGCGG | | |
| *Muc4* | pMuc4-R | GTCCCCTGGGTGTTTCTGAG | 58 | NCBI Blast |
| | pMuc4-R | CATAGTGTTTCCACCCAGGAC | | |
| *RegIIIγ* | REG3g-F | AGCCTGTCAAGAAACACAGGATA | 58 | NCBI Blast |
| | REG3g-R | TCCAATCTCATCTAGCCCTTG | | |
| *Muc1* | pMuc1-F | ACCCCTATGAGCAGGTTTCT | 58 | NCBI Blast |
| | pMuc1-R | CCCCTACAAGTTGGCAGAAG | | |
| *TNFα* | pTnf-a-F | CCACGTTGTAGCCAATGTCA | 58 | NCBI Blast |
| | pTnf-a-R | GTTGTCTTTCAGCTTCACGC | | |
| *IFNγ* | pIFN-g-F | AGAATTGGAAAGAGGAGAGTGAC | 58 | NCBI Blast |
| | pIFN-g-R | TCAGTTTCCCAGAGCTACCA | | |
| *IL17* | IL17a-F | CCAGACGGCCCTCAGATTAC | 65 | (Meurens, Berri, *et al.* 2009) |
| | IL17a-R | CACTTGGCCTCCCAGATCAC | | |
| *IL10* | pIL-10-F | CTGGAAGACGTAATGCCGAA | 58 | NCBI Blast |
| | pIL-10-R | CAGAAATTGATGACAGCGCC | | |
| *PR39* | PR39-F | TAATCTCTACCGCCTCCTGG | 62 | (Meurens, Berri, *et al.* 2009) |
| | PR39-R | CCCGTTCTCCTTGAAGTCAC | | |
| *IL1β* | pIL1b-F | CCCATCATCCTTGAAACGTG | 58 | NCBI Blast |
| | pIL1b-R | CTCATGCAGAACACCACTTC | | |
| *IL8* | IL8-F | TCCTGCTTTCTGCAGCTCTC | 62 | (Meurens, Berri, *et al.* 2009) |
| | IL8-R | GGGTGGAAAGGTGTGGAATG | | |
| *INOS* | iNOS-F | GAGAGGCAGAGGCTTGAGAC | 62 | NCBI Blast |
| | iNOS-R | TGGAGGAGCTGATGGAGTAG | | |
| *TLR4* | pTLR4-F | CAGCCATGGCCTTTCTCTC | 58 | NCBI Blast |
| | pTLR4-R | ATGTTAGGAACCACCTGCAC | | |
| *TGFβ* | pTGF-B1-F | CCGGAACCTGTATTGCTCTC | 58 | NCBI Blast |
| | pTGF-B1-R | TGACATCAAAGGACAGCCAC | | |

Table 2.3 Dehority's medium.

| **Ingredient** | **Concentration** |
|---|---|
| | g $L^{-1}$ |
| Carbohydrate | 5 |
| Tripticase | 4.5 |
| Yeast Extract | 0.5 |
| | ml $L^{-1}$ |
| Mineral 1[a] | 40 |
| Mineral 2[b] | 40 |
| Hemin (0.01%)[c] | 10 |
| VFA solution[d] | 10 |
| Resazurin solution (25 mg $ml^{-1}$) | 1 |
| L-Cysteine HCL solution | 10 |
| Clarified rumen fluid[e] | 50 |

[a]Mineral 1 = 6 g $L^{-1}$ $K_2HPO_4$.

[b]Mineral 2 = 6g L $KH_2PO_4$, 6 g $L^{-1}$ $((NH_4)_2)SO_4$, 12 g $L^{-1}$ NaCl, 2.45 g $L^{-1}$ $MgSO_4$, 1.69 g $L^{-1}$ $CaCl_2 \bullet 2H_2O$.

[c]Hemin was dissolved in 1 M NaOH and then diluted to appropriate concentration.

[d] VFA solultion = 6.85 mL $L^{-1}$ acetic acid, 3 mL $L^{-1}$ propionic acid, 1.85 mL $L^{-1}$ butyric acid, 0.5 mL $L^{-1}$ isobutyric acid, 0.55 mL $L^{-1}$ 2-methyl butyric acid, 0.55 mL $L^{-1}$ N-valeric acid, and 0.55 mL $L^{-1}$ isovaleric acid.

[e]Clarified rumen fluid was prepared by centrifuging to remove debris and gently decanting in a fresh vial for storage at -20°C.

Table 2.4 Columbia blood agar.

| **Ingredient** | **Concentration** $g\ L^{-1}$ |
|---|---|
| HiMedia Columbia broth | 35 g |
| Cysteine | 1 g |
| Agar | 15 g |
| Autoclave | 30 min |
| Sheep's Blood[a] | 100 ml |

[a] Added to cooled media (≈50°C). The anticoagulant CPDA-1 is added to blood bags in advance of bleeding to prevent clotting. CPDA-1 is composed of 26.30 g $L^{-1}$ trisodium citrate, 3.27 g $L^{-1}$ citric acid, 3.22 g $L^{-1}$ sodium dihydrogen phosphate, 3.18 g $L^{-1}$ dextrose, and 0.275 g $L^{-1}$ adenine dissolved in water and adjusted to a pH of 5.6 to 5.8.

Table 2.5 Sequences and annealing temperatures for primers used to quantify bacteria.

| Target organism | Primer | Sequence (5' to 3') | Annealing Tmp (°C) | Reference |
|---|---|---|---|---|
| | PrevF | CACCAAGGCGACGATCA | 58 | (Larsen *et al.* |
| *Prevotella spp.* | PrevR | GGATAACGCCYGGACCT | | 2010) |
| *Ruminococcus* | Rflbr730F | GGCGGCYTRCTGGGCTTT | 58 | (Ramirez-Farias |
| | Clep866mR | ACCTTCCTCCGTTTTGTCAAC | | *et al.* 2009) |
| *Clostridium* | CI-F1 | TACCHRAGGAGGAAGCCAC | 55 | (Song *et al.* |
| cluster I | CI-R2 | GTTCTTCCTAATCTCTACGCAT | | 2004) |
| *Intestinimonas* | PFF590F | AAAACTATGGGCTCAACCCA | 58 | (Bui *et al.* |
| AF211 | PFF702R | GTCAGTTAATGTCCAGCAGG | | 2015) |
| Total bacteria | F-tot | GCAGGCCTAACACATGCAAGTC | 56 | (Castillo *et al.* |
| | R-tot | TGCTGCCTCCCGTAGGAGT | | 2006) |
| *Bacteroides* | BaUNI-F | TACCCGATGGCATAGTTCTT | 55 | (Rashidan *et al.* |
| *uniformis* | BaUNI-R | GGACCGTGTCTCAGTTCCAA | | 2018) |
| *Streptococcus* | Sg-F | TGACGTACGATTGATATCATCAAC | 60 | (Kumar *et al.* |
| *gallolyticus* | Sg-R | CGCTTAACACATTTTTAGCTAATACG | | 2017) |

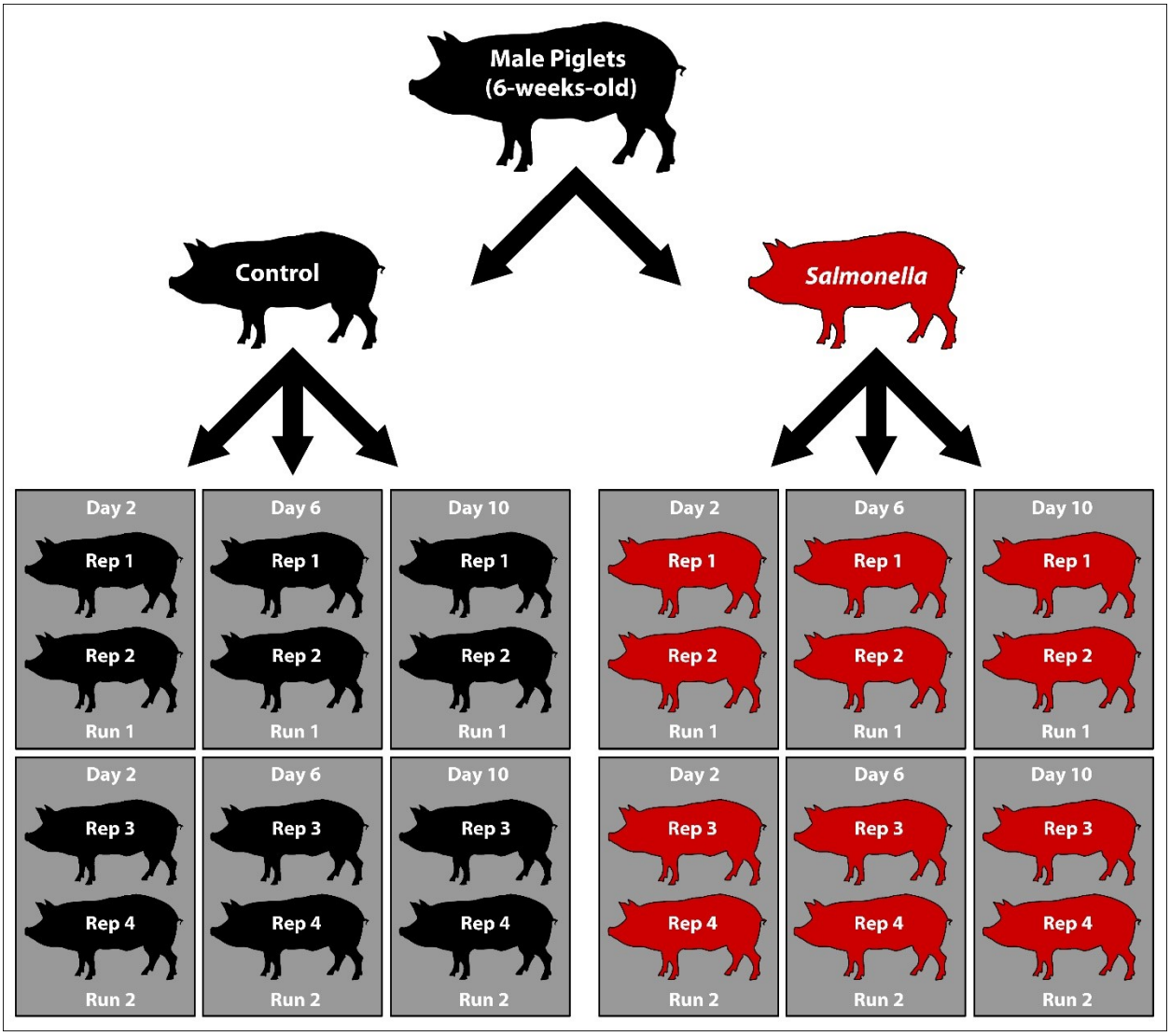


Figure 2.1 Schematic representation of the experimental design.

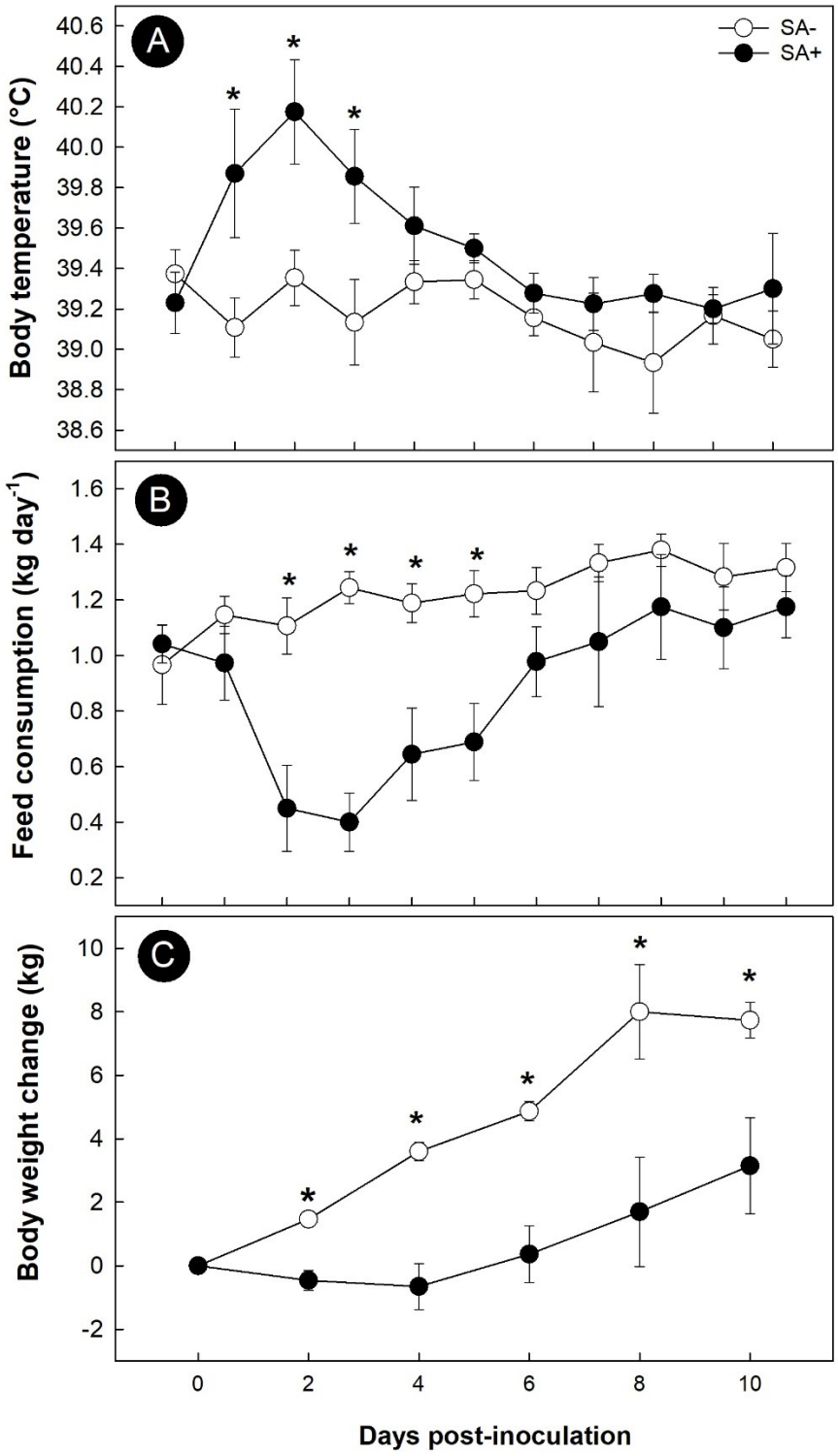


Figure 2.2 Change in host parameters in piglets orally inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). (A) Body weight change in kg; (B) rectal temperature; (C) daily feed consumption (kg day$^{-1}$). Vertical lines associated with markers are standard errors of the mean. Markers with an asterisk indicate a difference ($P<0.050$) between treatments at the corresponding time point.

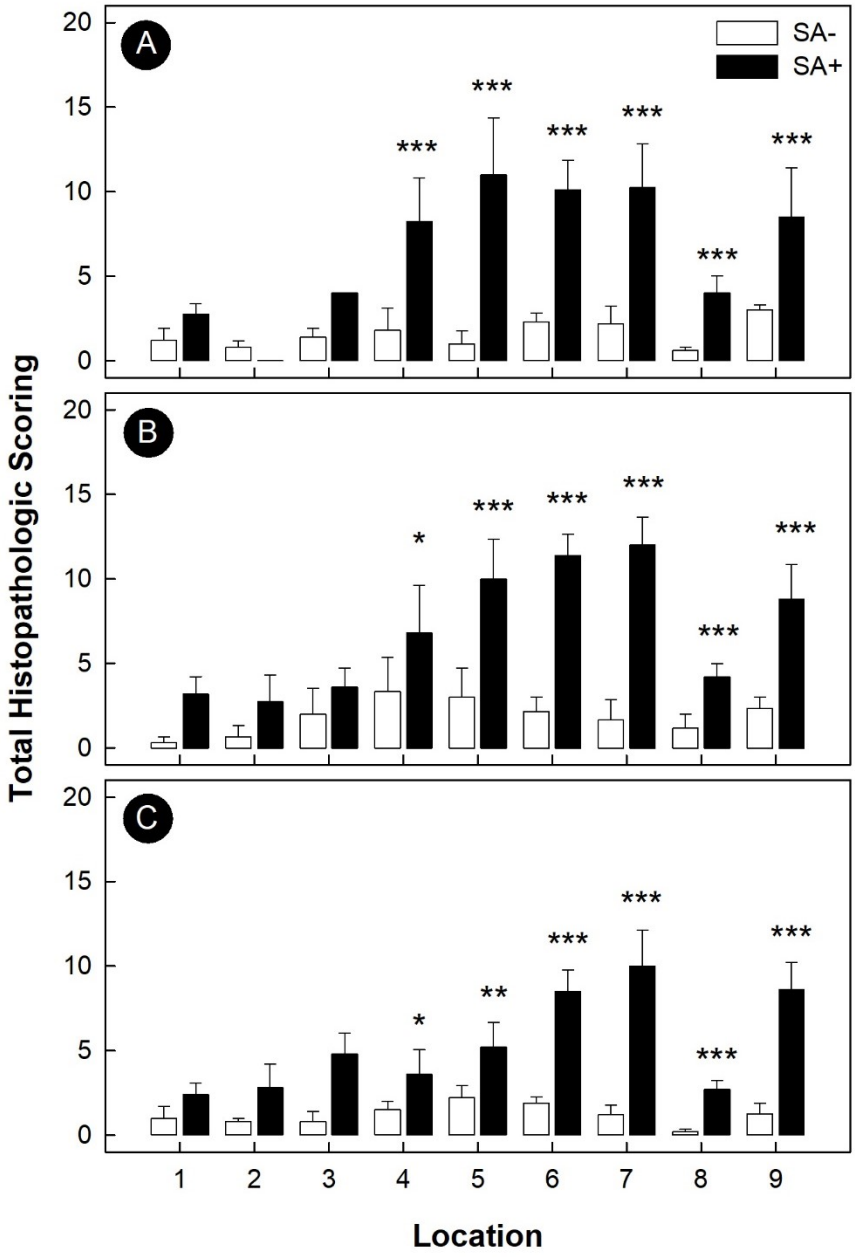


Figure 2.3 Total histopathologic scores in piglets orally inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). Intestinal locations are: (1) duodenum; (2) proximal jejunum; (3) mid- jejunum; (4) distal jejunum; (5) ileum; (6) cecum; (7) ascending colon; (8) spiral colon; and (9) descending colon. (A) Two dpi; (B) 6 dpi; (C) 10 dpi. Vertical lines associated with markers are standard errors of the mean. Histogram bars with asterisks indicate differences (*P<0.050, **P<0.010, ***P<0.001) between treatments at the corresponding intestinal location.

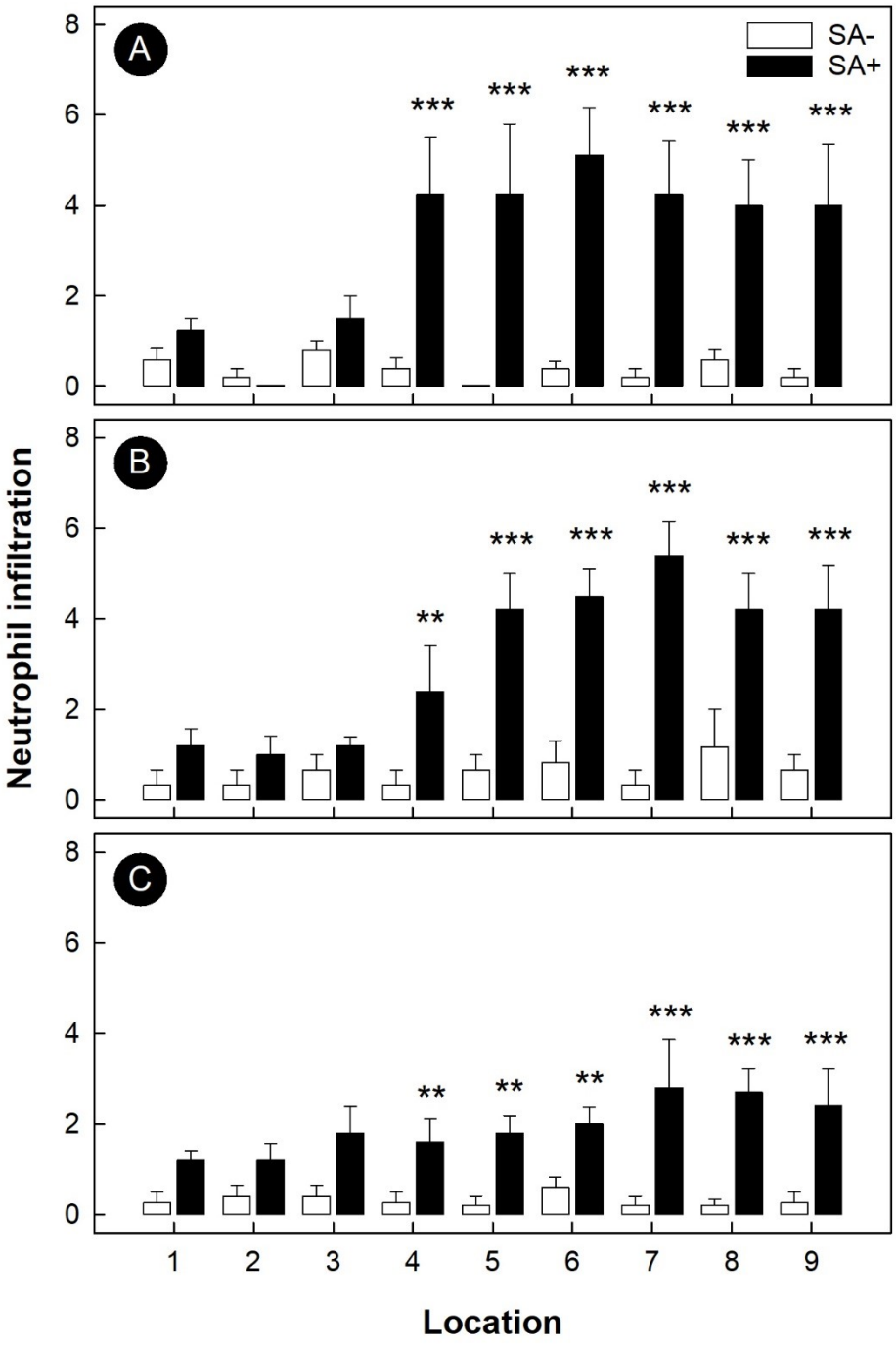


Figure 2.4 Neutrophil infiltration in piglets orally inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). Intestinal locations are: (1) duodenum; (2) proximal jejunum; (3) mid-jejunum; (4) distal jejunum; (5) ileum; (6) cecum; (7) ascending colon; (8) spiral colon; and (9) descending colon. (A) Two dpi; (B) 6 dpi; (C) 10 dpi. Vertical lines associated with markers are standard errors of the mean. Histogram bars with an asterisk indicate a difference (*P<0.050, **P<0.010, ***P<0.001) between the two treatments at the corresponding intestinal location.

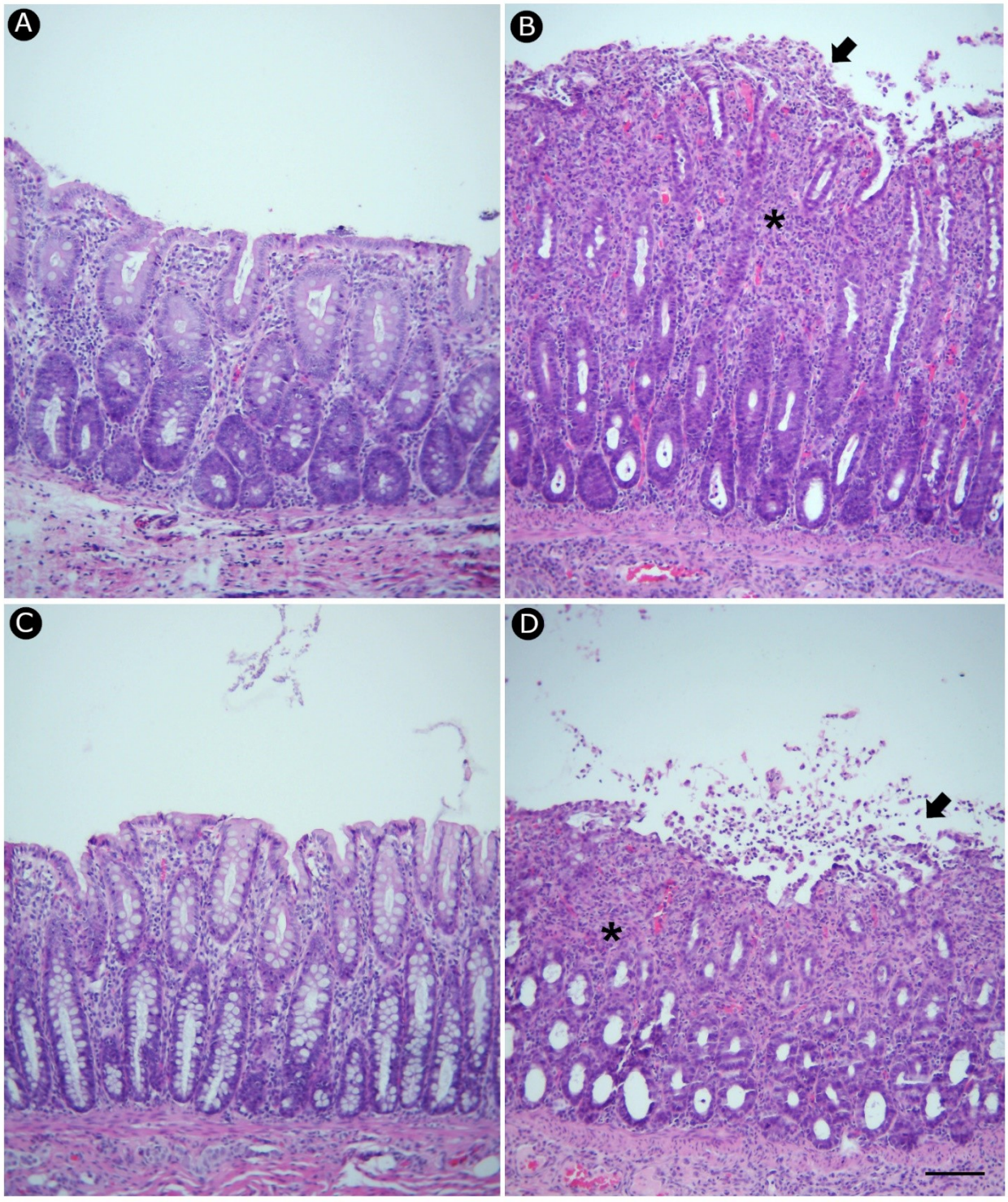


Figure 2.5 Histological representation of intestinal tissue harvested at 2 days post-inoculation from piglets orally inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). Arrows indicate epithelial injury, and asterisks indicate leukocytes infiltrate. (A) Cecum SA-; (B) Cecum SA+; (C) Spiral Colon SA-; (D) Spiral Colon SA+. Bar = 100 µm.

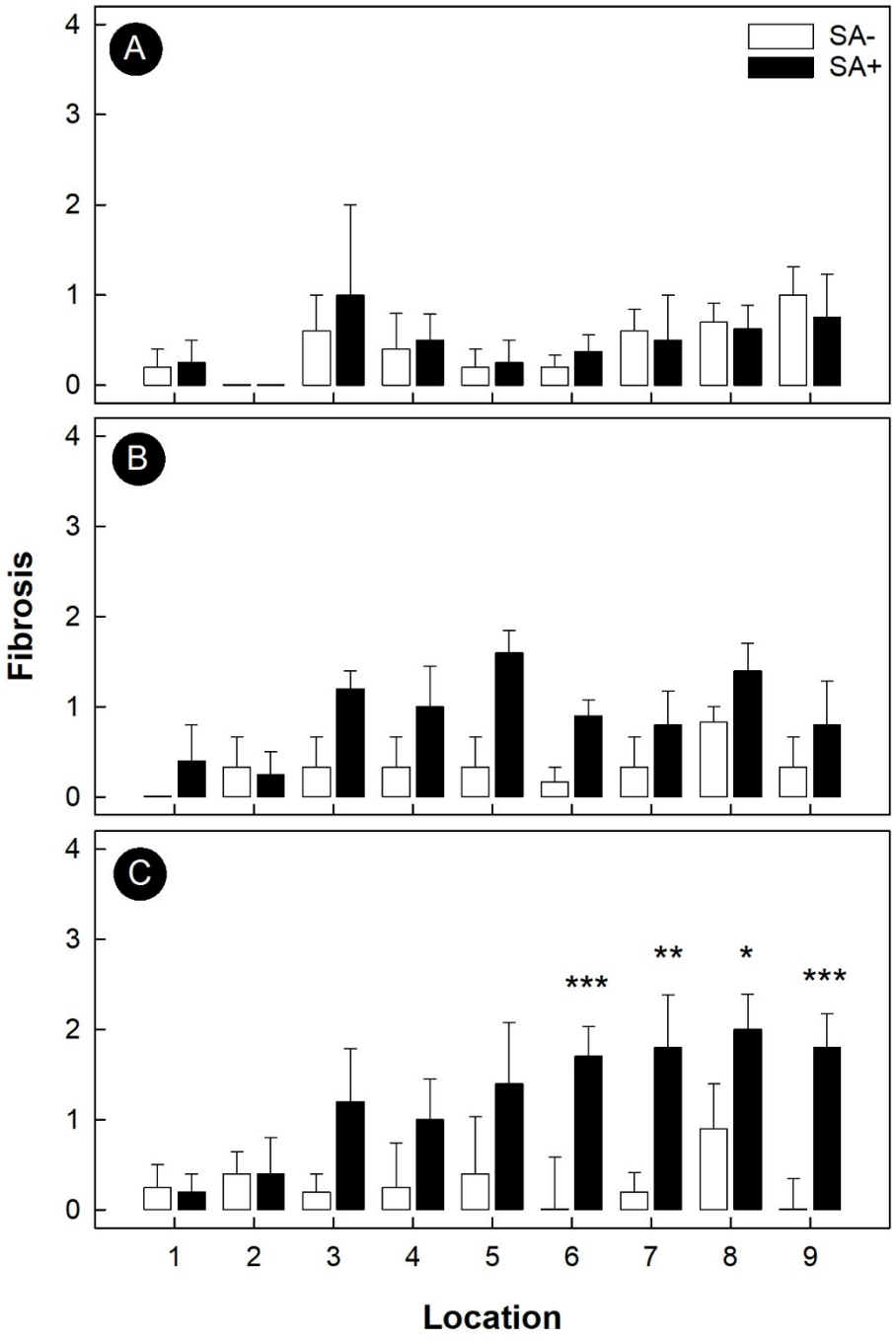


Figure 2.6 Fibrosis in piglets orally inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). Intestinal locations are: (1) duodenum; (2) proximal jejunum; (3) mid-jejunum; (4) distal jejunum; (5) ileum; (6) cecum; (7) ascending colon; (8) spiral colon; and (9) descending colon. (A) Two dpi; (B) 6 dpi; (C) 10 dpi. Vertical lines associated with markers are standard errors of the mean. Histogram bars with an asterisk indicate a difference (*P<0.050, **P<0.010, ***P<0.001) between the two treatments at the corresponding intestinal location.

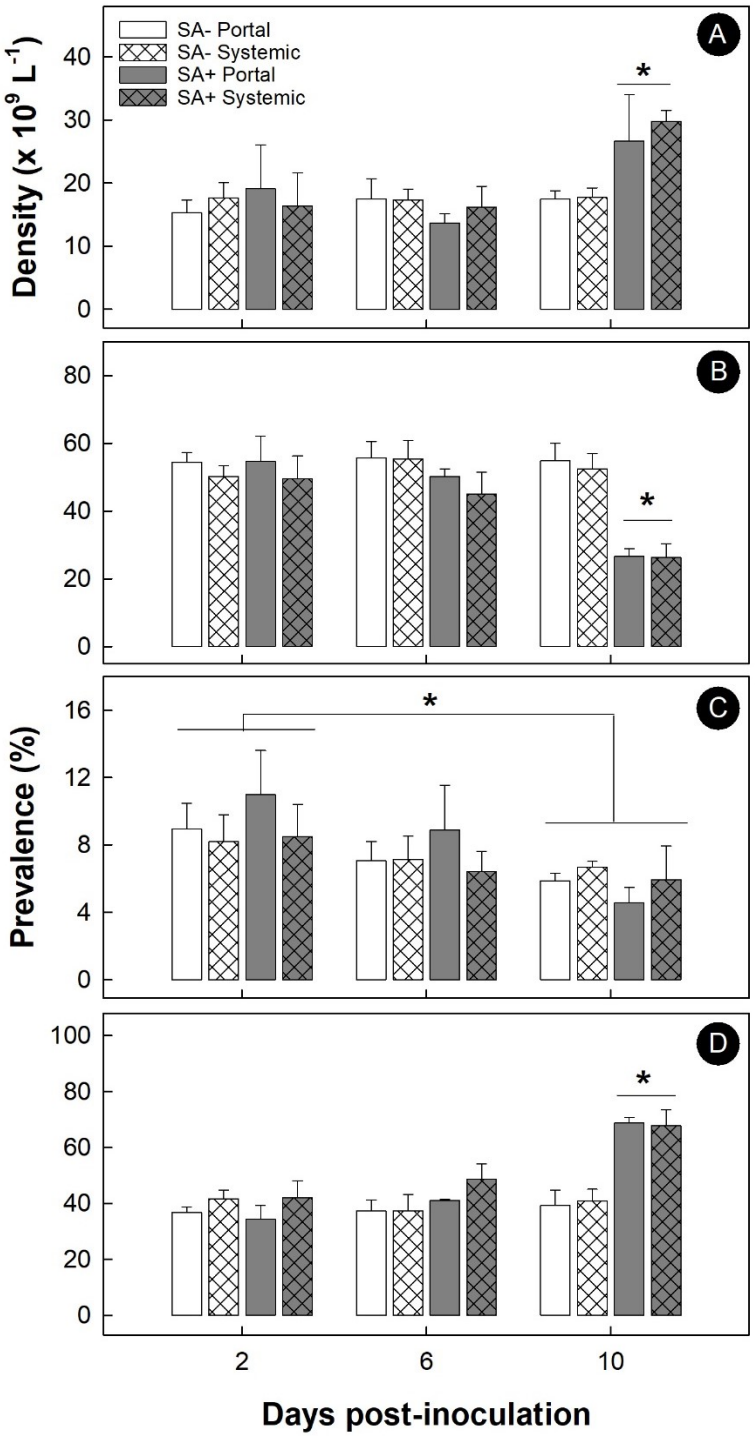


Figure 2.7 Densities of total white blood cells (x $10^9 L^{-1}$), and percentage of lymphocytes, monocytes, and granulocytes in portal vein and cardiac (i.e. systemic) blood of piglets at 2, 6, and 10 dpi with *Salmonella enterica* Typhimurium (SA+) or medium alone (i.e. SA-). (A) White blood cells; (B) lymphocytes; (C) monocytes; (D) granulocytes. Vertical lines associated with histogram bars represent standard error of the means. Histogram bars with an asterisk indicate a difference ($P<0.050$) between treatments.

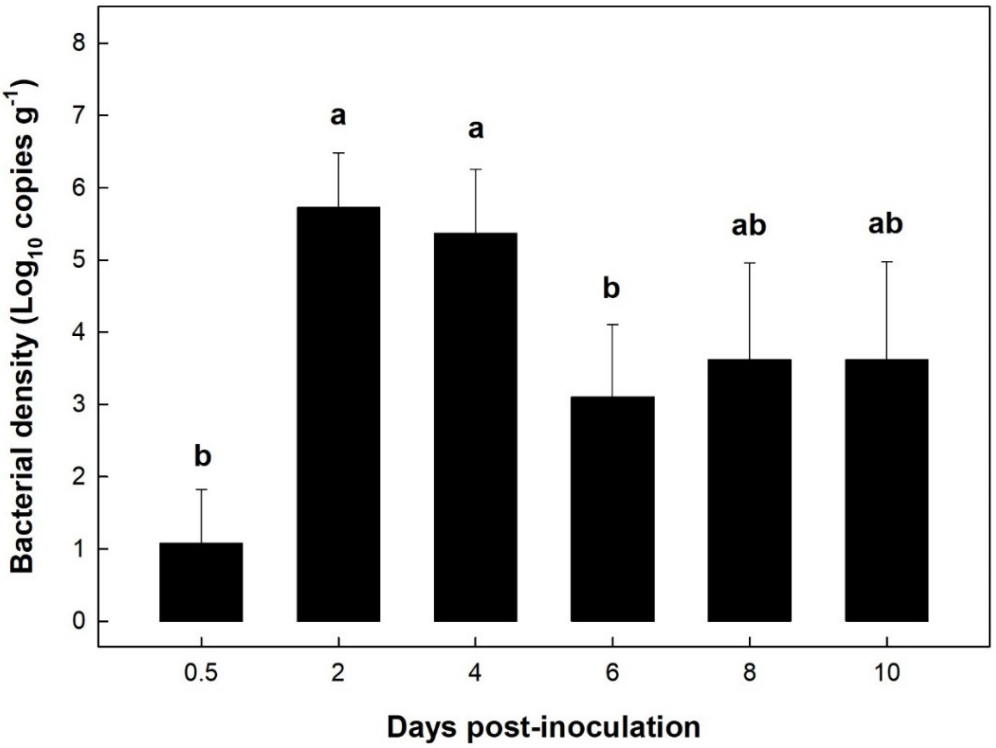


Figure 2.8 Temporal shedding of *Salmonella enterica* Typhimurium in feces from piglets orally inoculated with the pathogen (SA+). Vertical lines associated with histogram bars represent standard errors of the mean. Histogram bars indicated by different letters differ (P<0.050). No *Salmonella* was detected in feces from piglets orally administered medium alone (SA-).

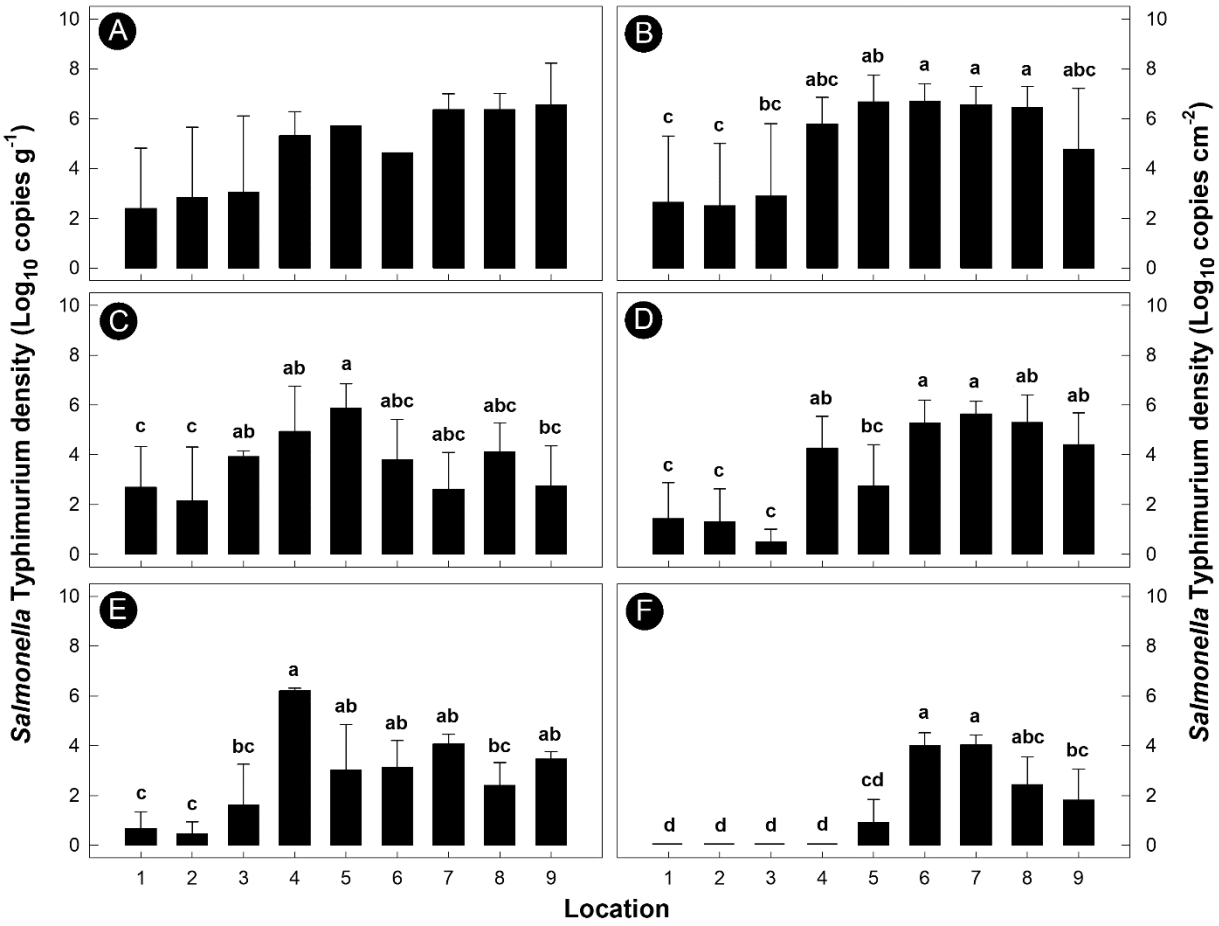


Figure 2.9 *Salmonella* densities from piglets at 2, 6, and 10 dpi with *Salmonella enterica* Typhimurium (SA+). Locations are: (1) duodenum; (2) proximal jejunum; (3) mid-jejunum; (4) distal jejunum; (5) ileum; (6) cecum; (7) ascending colon; (8) spiral colon; and (9) descending colon. Vertical lines associated with histogram bars represent standard error of the means. Histogram bars indicated by different letters at each time post-inoculation differ (P<0.050). (A) Digesta at 2 dpi; (B) mucosa-associated at 2 dpi; (C) digesta at 6 dpi; (D) mucosa-associated at 6 dpi; (E) digesta at 10 dpi; (F) mucosa-associated at 10 dpi. No *Salmonella* was detected in digesta or associated with mucosa from piglets orally administered medium alone (SA-).

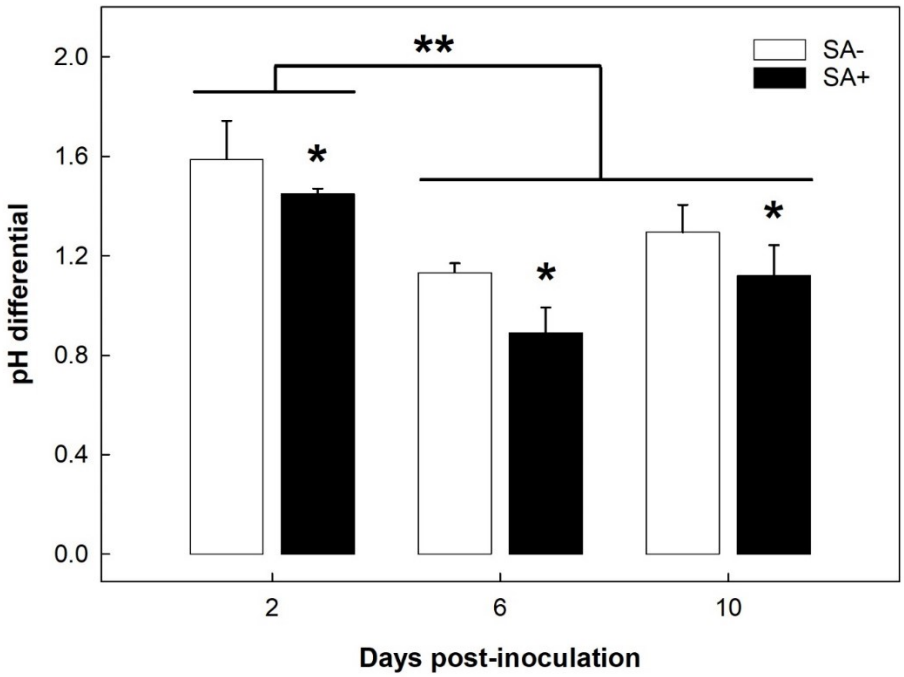


Figure 2.10 pH differential between initial measurement (45 min) and final measurement (24 h post-mortem) in piglets inoculated with *Salmonella* (SA+) or with medium alone (SA-). Vertical lines associated with histogram bars represent standard error of the mean. Histogram bars with an asterisk indicate a difference (*P<0.050, **P<0.010) between treatments.

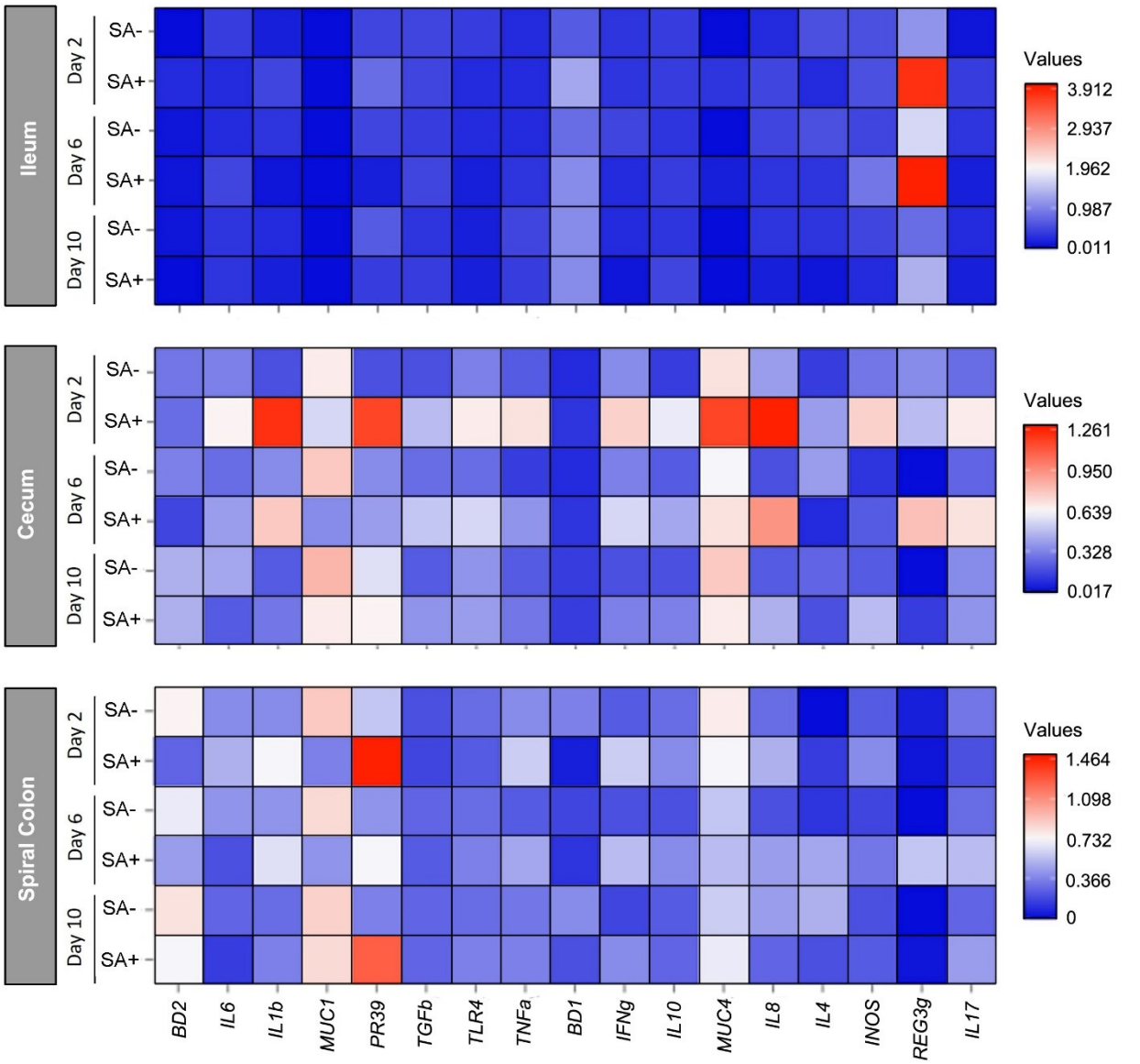


Figure 2.11 Relative gene expression in ileal, cecal, and spiral colonic tissue from piglets inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). Tissues were sampled at 2, 6, and 10 dpi. High to low expression are represented by a change of colours from red to blue, respectively.

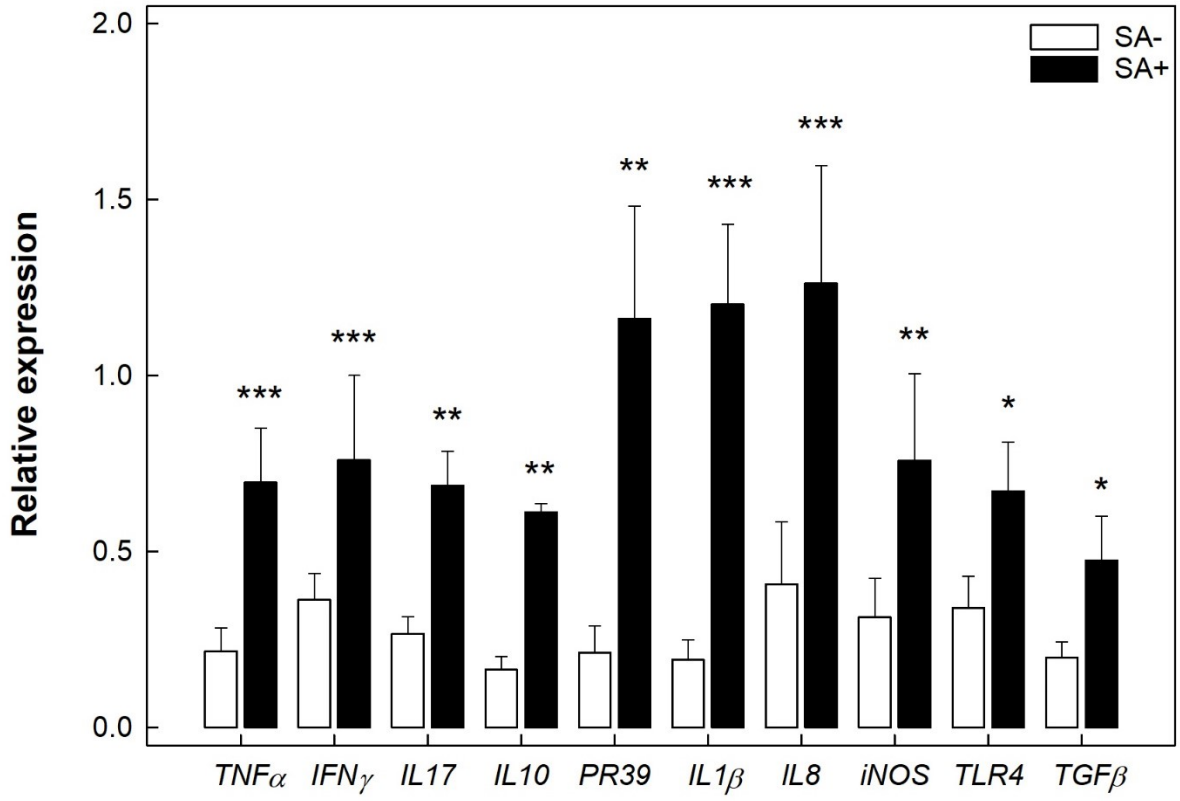


Figure 2.12 Relative gene expression in cecal tissue of piglets orally inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-) at 2 dpi. Vertical lines associated with markers are standard errors of the mean. Histogram bars with asterisks indicate differences (*P<0.050, **P<0.010, ***P<0.001) between the two treatments.

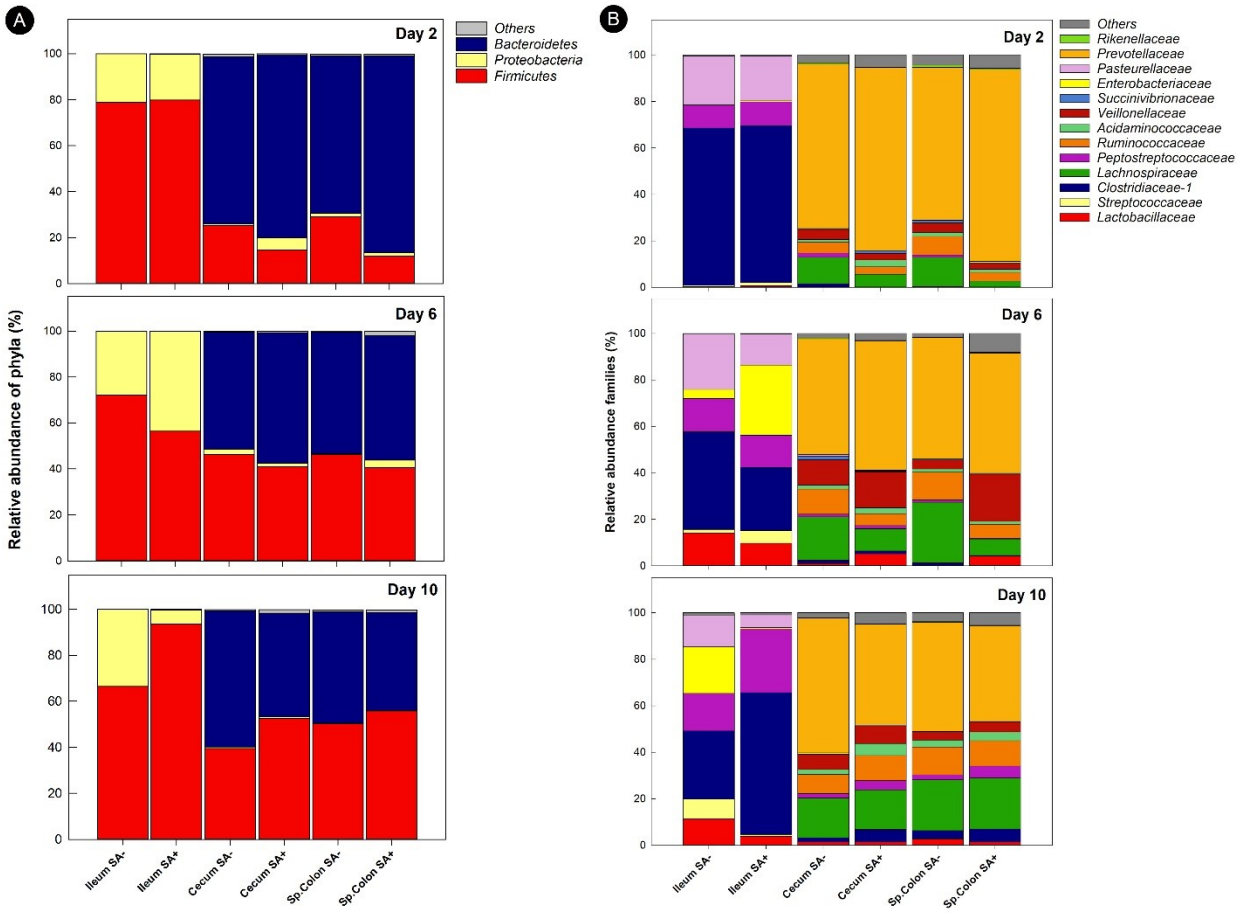


Figure 2.13 Spatial characterization of the main taxa in digesta from the ileum, cecum, and spiral colon of piglets inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). Samples were obtained from piglets 2, 6, and 10 dpi. Relative abundances (%) are represented at different taxonomic levels. (A) Phyla; (B) Families.

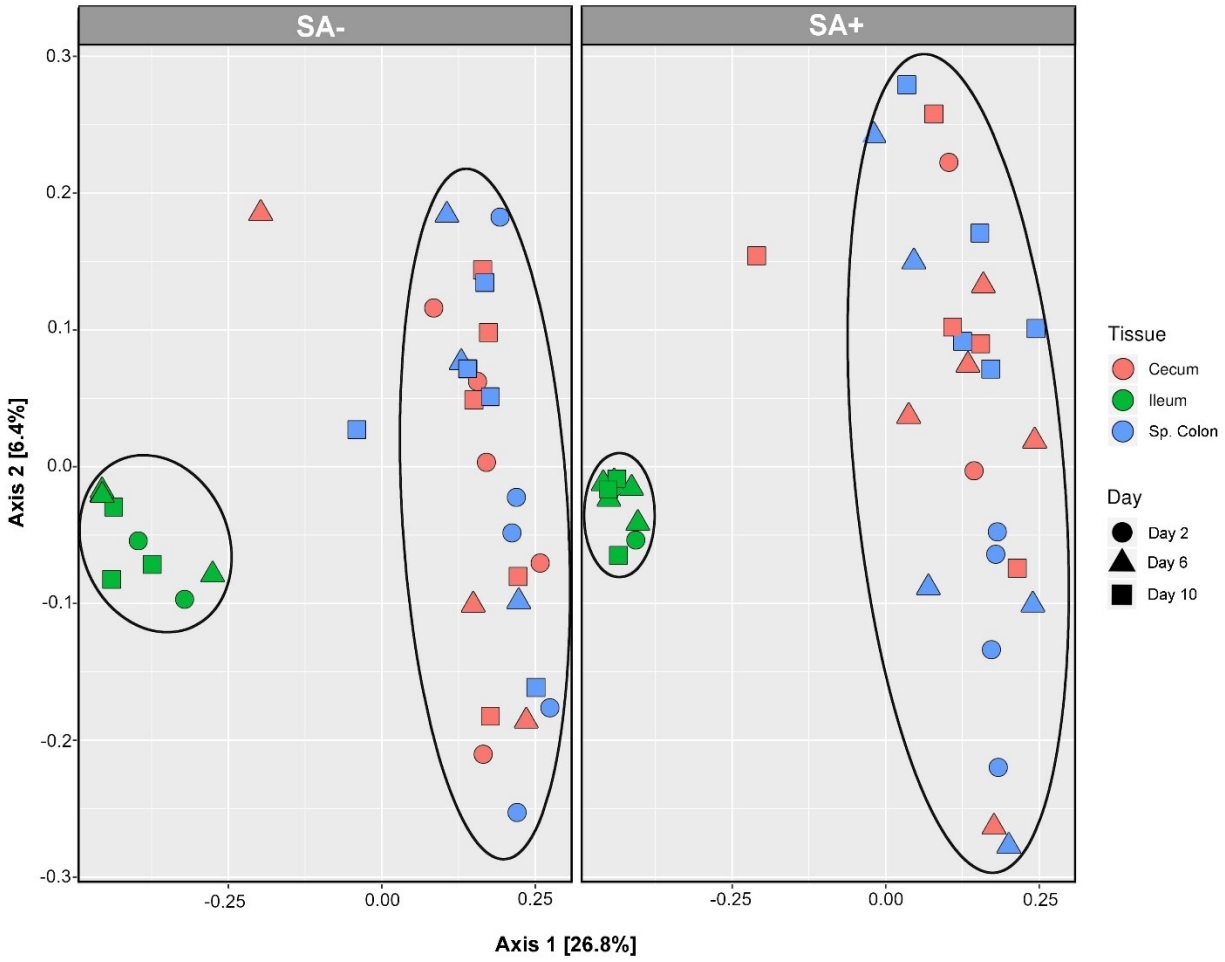


Figure 2.14 PCoA plot based on unweighted UniFrac distances of bacterial communities in digesta from the ileum, cecum and spiral colon of piglets inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). Samples were obtained from piglets at 2, 6, and 10 dpi.

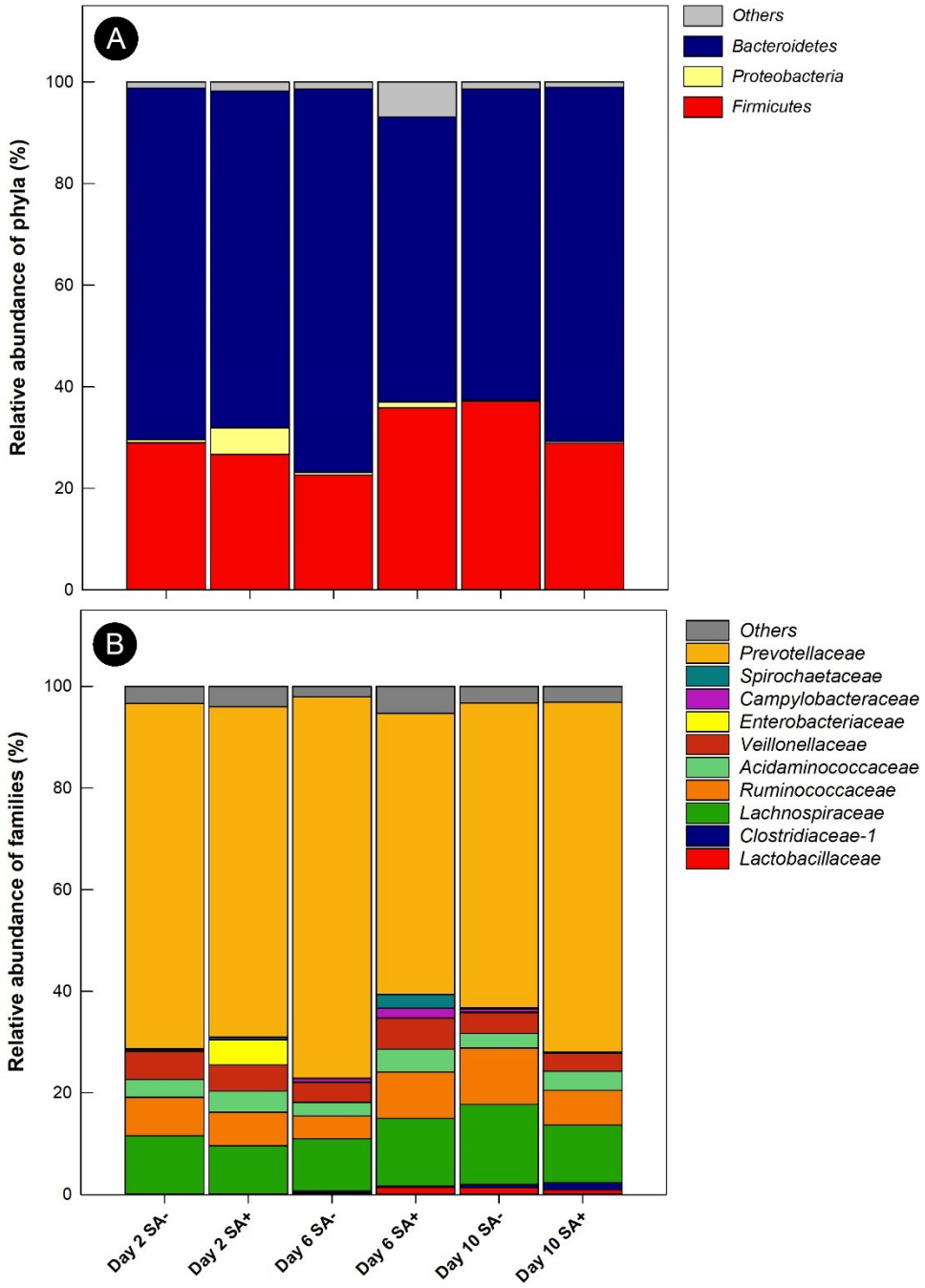


Figure 2.15 Relative abundance (%) of bacterial phyla and families associated with spiral colonic mucosa of piglets inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). Samples were obtained at 2, 6 and 10 dpi. Communities were characterized by Illumina sequencing. (A) Phyla. (B) Families.

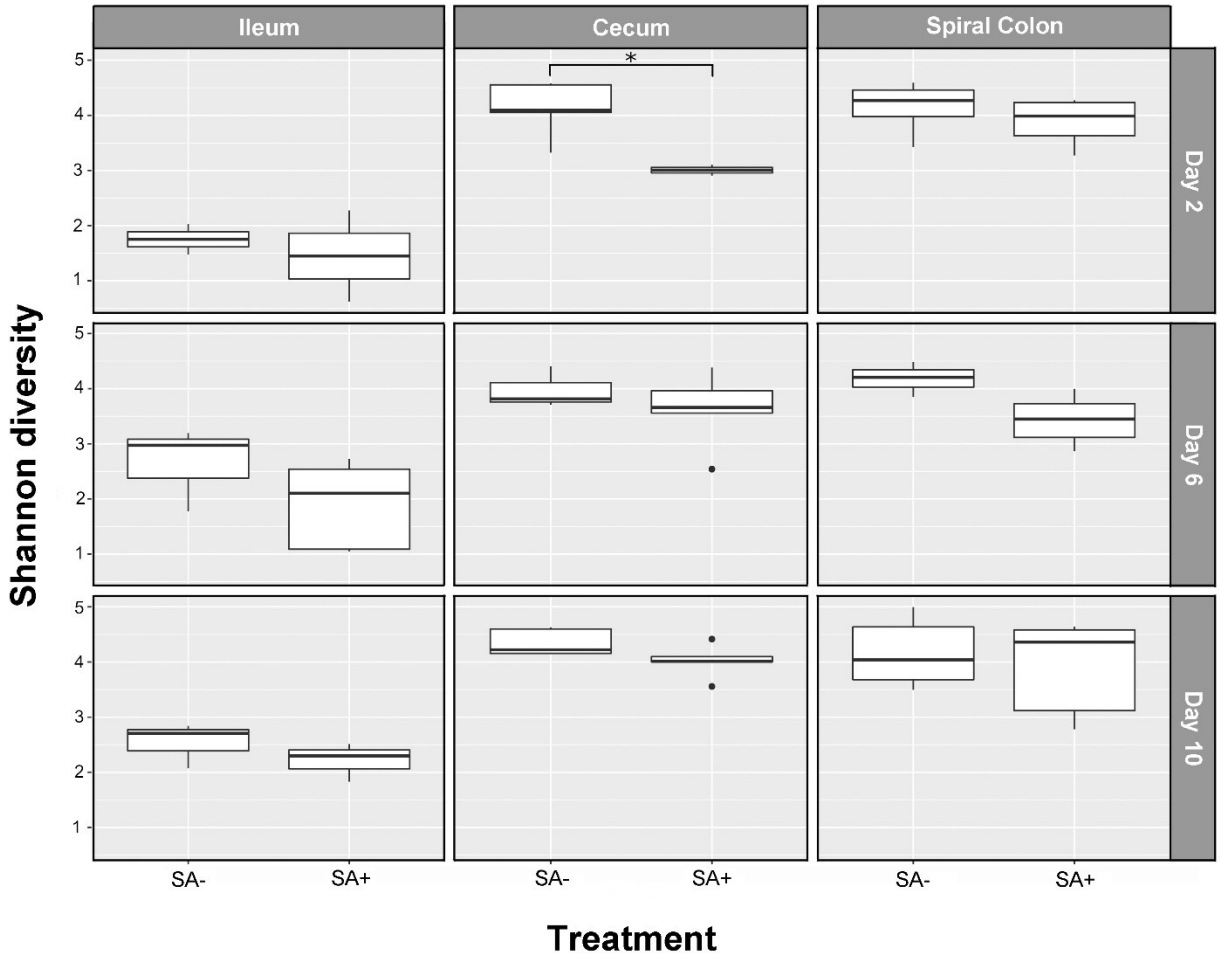


Figure 2.16 Alpha-diversity of bacterial communities in digesta from the ileum, cecum, and spiral colon of piglets inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). Samples were obtained from piglets at 2, 6 and 10 dpi. Values are expressed as means ± standard error. Boxes with an asterisk indicate a difference (*P<0.050) between treatments.

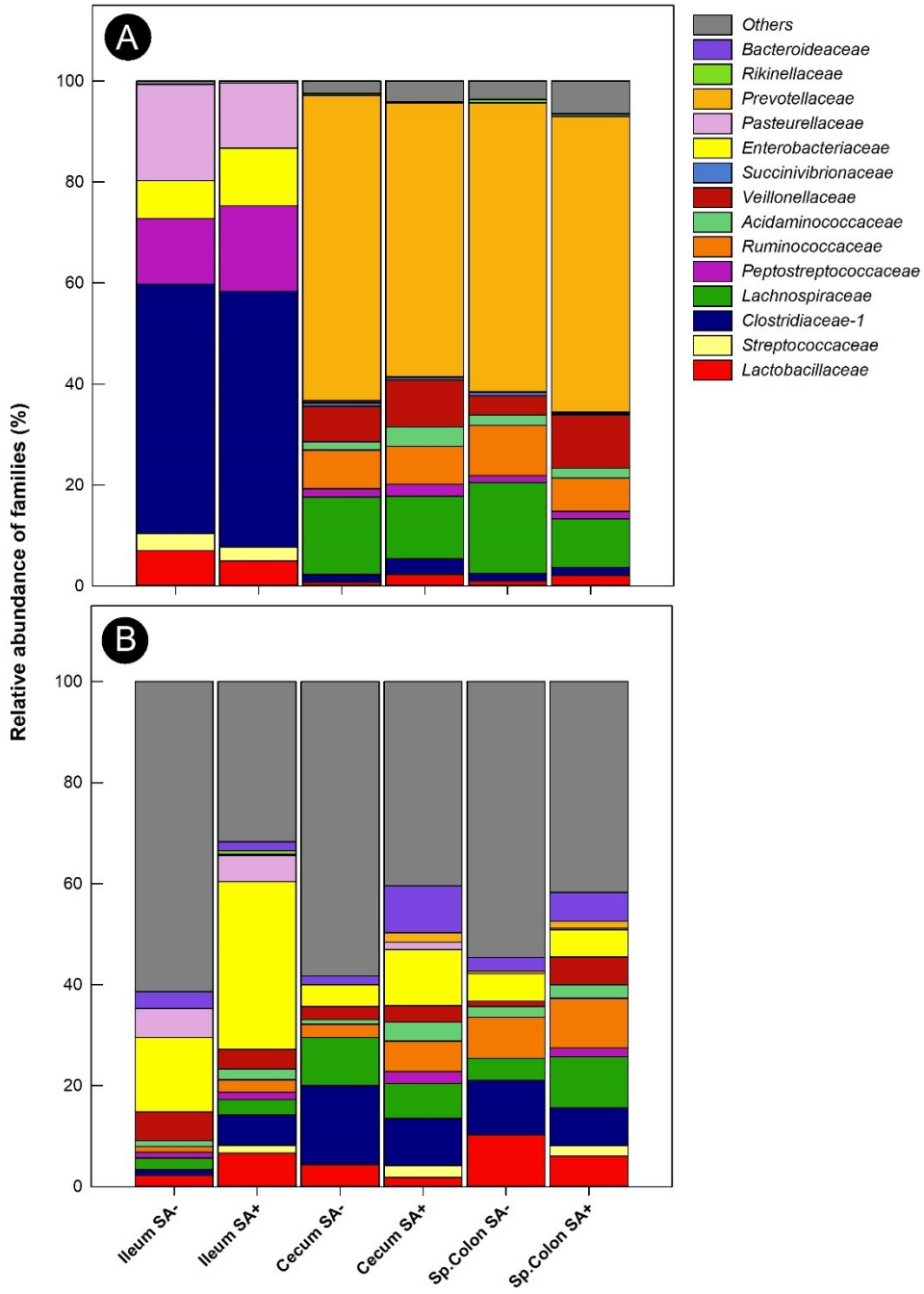


Figure 2.17 Spatial characterization of the main families of bacteria in digesta from the ileum, cecum, and spiral colon of piglets inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). (A) Next-generation sequencing; (B) culturomics. Data were combined across sample times (i.e. 2, 6, and 10 dpi).

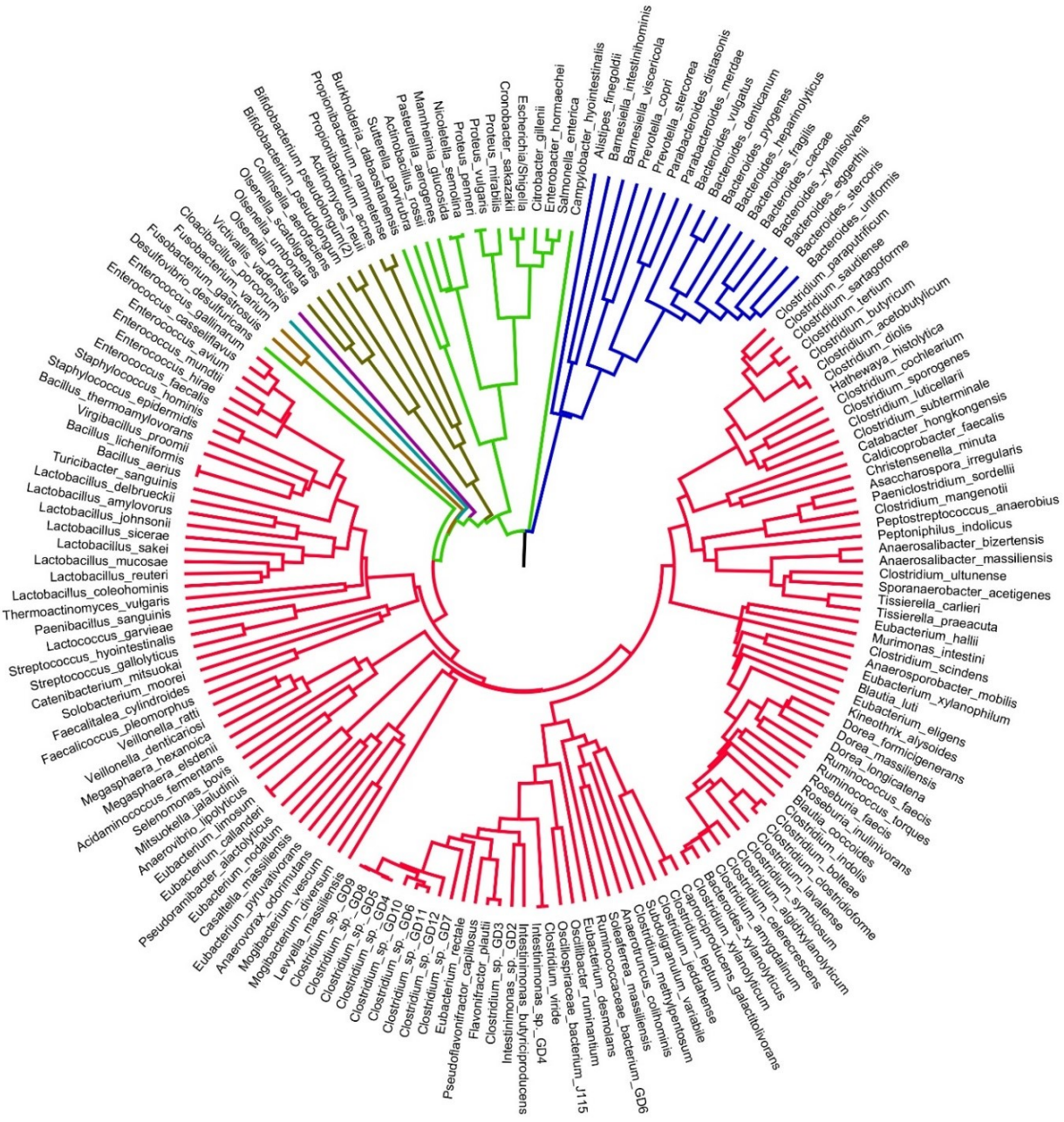


Figure 2.18 Phylogenetic Tree of the 16S rDNA sequence of bacteria isolated from the intestines of piglets. Species identities were determined using the Ribosomal Database Project (RDP). Figure generated by Paul Moote.

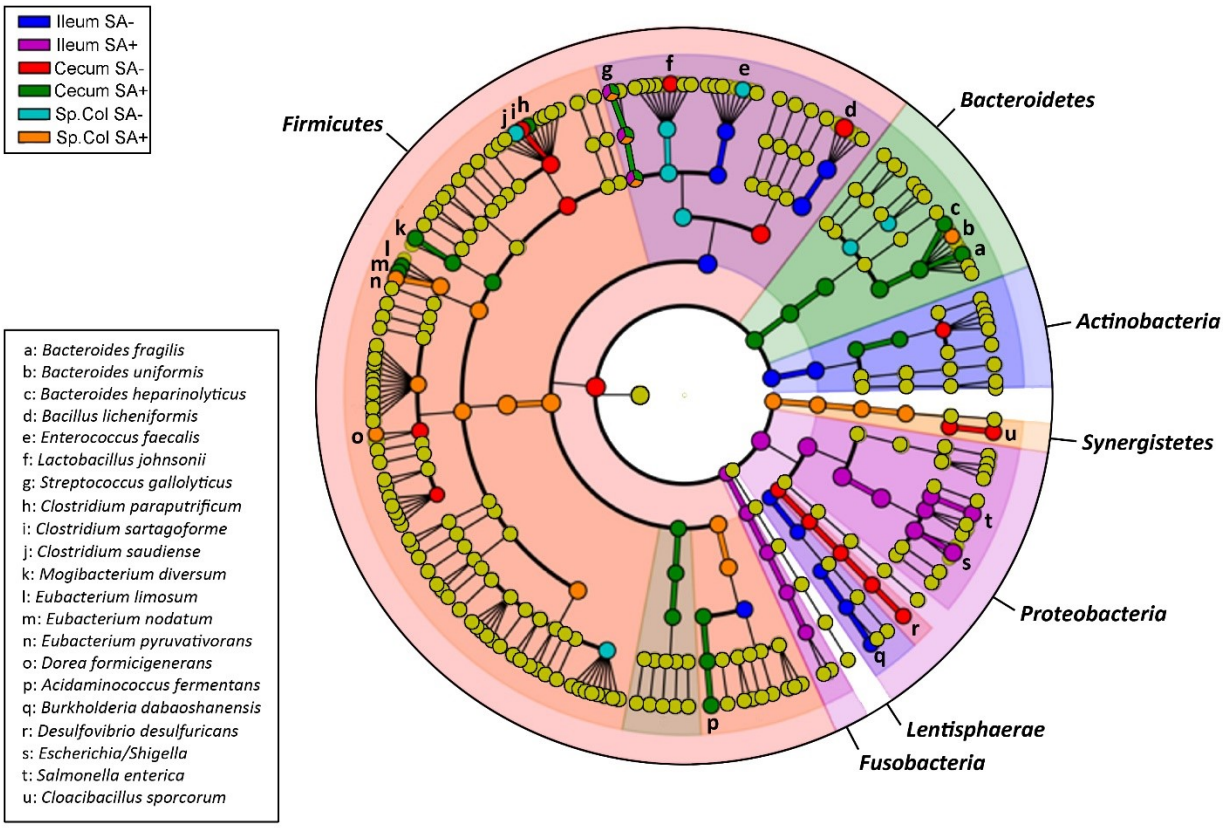


Figure 2.19 Cladogram illustrating abundance of bacterial species isolated from the ileum, cecum, and spiral colon of piglets inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). The cladogram background is color coded to illustrate relative changes in the abundance of isolated bacteria between treatments and intestinal locations. Moreover, 21 bacterial species that were differentially abundant in SA+ and SA- piglets are indicated with colored circles, and are labelled 'a' through 'u'. Gold circles represent bacteria in which no difference in abundance due to infection by *S.* Typhimurium was observed. Figure generated by Paul Moote.

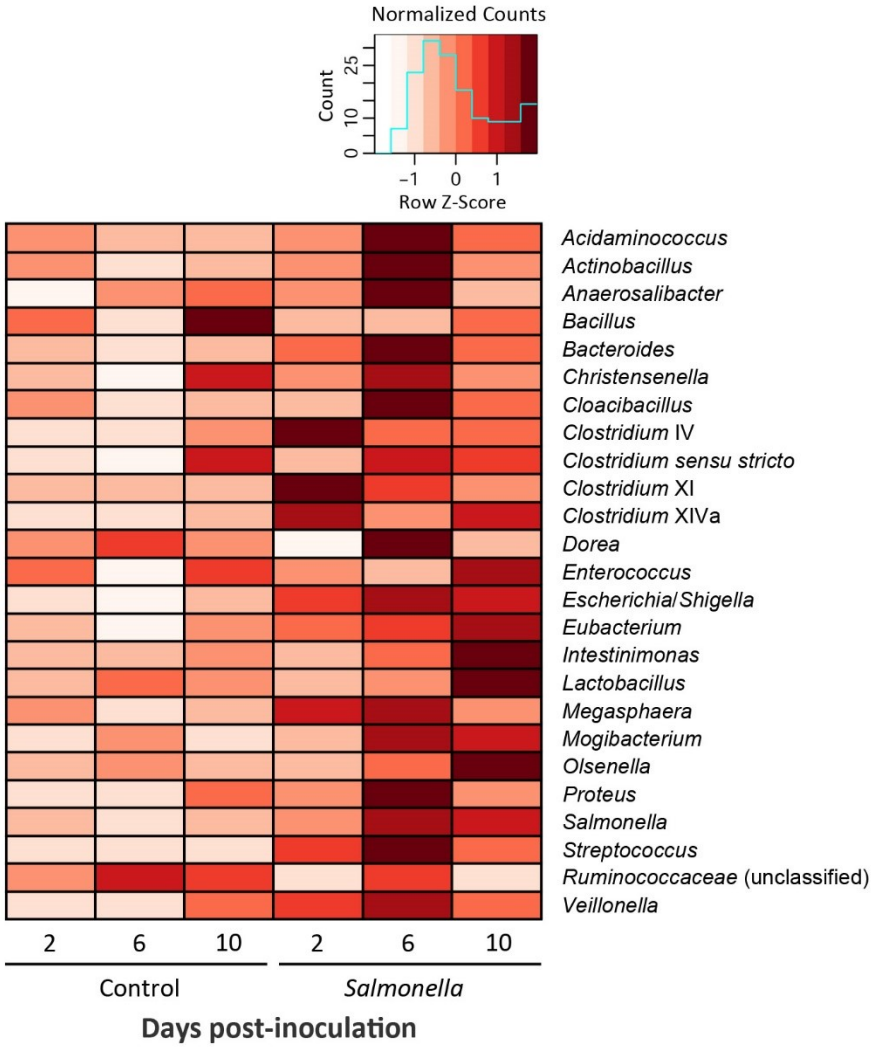


Figure 2.20 Abundance of bacteria isolated from the ileum, cecum, ascending colon, and spiral colon of piglets inoculated with *Salmonella* Typhimurium or buffer alone (control) at 2, 6, and 10 dpi. Cells are colored according to the number of bacteria recovered, and the distribution and color scheme of these counts are indicated in the "normalized counts" plot above the heatmap. The heatmap was generated using the heatmap.2 function contained in the gplots package of R (Warnes *et al.* 2015). Figure generated by Paul Moote.

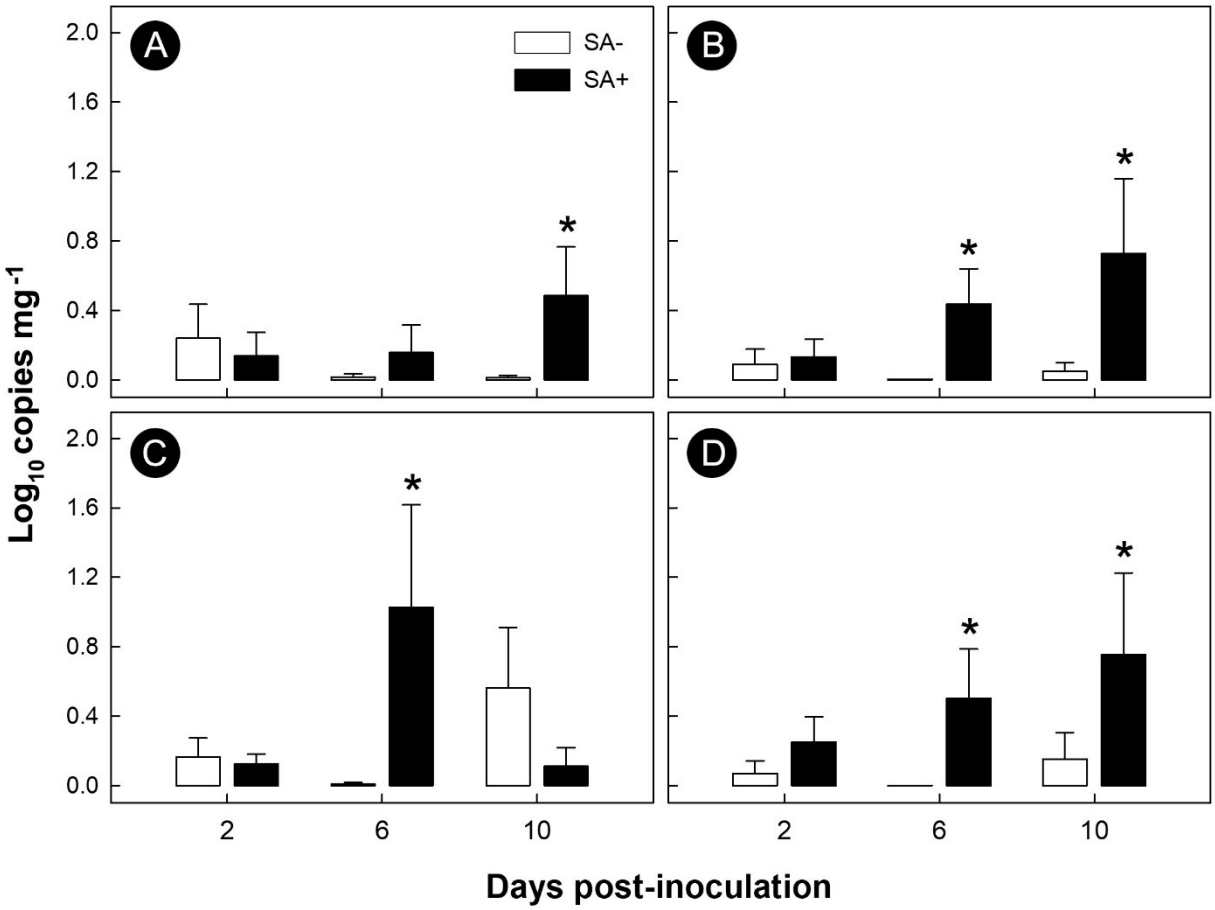


Figure 2.21 Densities of *Bacteroides uniformis* and *Streptococcus gallolyticus* in digesta of piglets inoculated with *Salmonella* Typhimurium (SA+) or medium alone (SA-). (A) *B. uniformis* in the cecum; (B) *S. gallolyticus* in the cecum; (C) *B. uniformis* in the spiral colon; (D) *S. gallolyticus* in the spiral colon. Vertical lines associated with histogram bars represent standard error of the means. Histogram bars with asterisks differ (*P≤0.124) between the SA+ and SA- treatments.

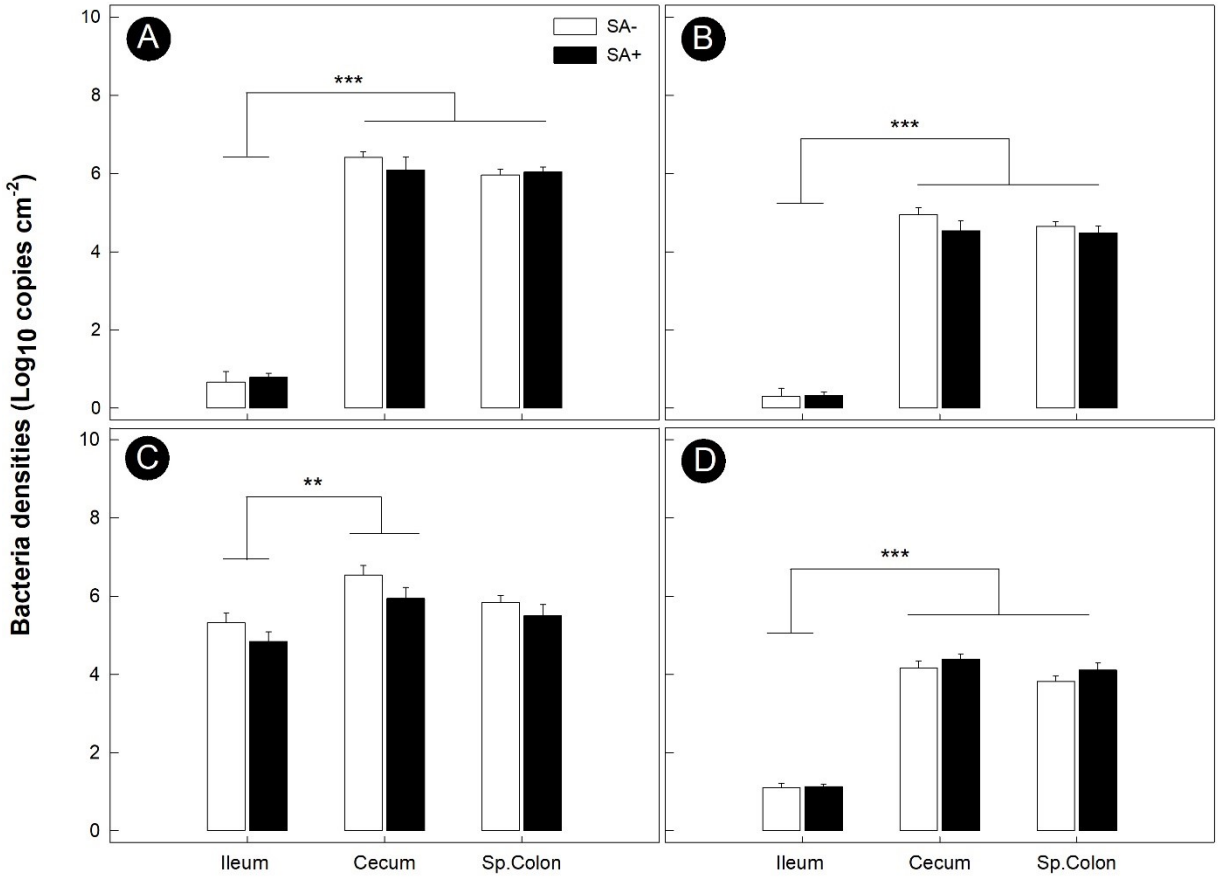


Figure 2.22 Bacteria densities within digesta from the ileum, cecum, and spiral colon of piglets inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-). (A) *Prevotella;* (B) *Ruminoccus;* (C) *Clostridium* cluster I; (D) *Intestinimonas*. Vertical lines associated with histogram bars represent standard error of the means. Histogram bars with an asterisk indicate a difference (*P<0.050, **P<0.010, ***P<0.001) between tissues.

### 2.6 References


Agbor, T. A., and B. A. McCormick. 2011. '*Salmonella* effectors: important players modulating host cell function during infection', *Cell Microbiol*, 13: 1858-69.

Al-Jashamy, K., A. Murad, M. Zeehaida, M. Rohaini, and J. Hasnan. 2010. 'Prevalence of colorectal cancer associated with *Streptococcus bovis* among inflammatory bowel and chronic gastrointestinal tract disease patients', *Asian Pac J Cancer Prev*, 11: 1765-8.

Alain, B. Pajarillo E., J. P. Chae, M. P. Balolong, H. Bum Kim, and D. K. Kang. 2014. 'Assessment of fecal bacterial diversity among healthy piglets during the weaning transition', *J Gen Appl Microbiol*, 60: 140-6.

Allen, H. K., T. Looft, D. O. Bayles, S. Humphrey, U. Y. Levine, D. Alt, and T. B. Stanton. 2011. 'Antibiotics in feed induce prophages in swine fecal microbiomes', *MBio*, 2.

Arguello, H., J. Estelle, S. Zaldivar-Lopez, A. Jimenez-Marin, A. Carvajal, M. A. Lopez-Bascon, F. Crispie, O. O'Sullivan, P. D. Cotter, F. Priego-Capote, L. Morera, and J. J. Garrido. 2018. 'Early *Salmonella* Typhimurium infection in pigs disrupts microbiome composition and functionality principally at the ileum mucosa', *Sci Rep*, 8: 7788.

Balaji, R., K. J. Wright, C. M. Hill, S. S. Dritz, E. L. Knoppel, and J. E. Minton. 2000. 'Acute phase responses of pigs challenged orally with *Salmonella* Typhimurium', *J Anim Sci*, 78: 1885-91.

Bearson, S. M., H. K. Allen, B. L. Bearson, T. Looft, B. W. Brunelle, J. D. Kich, C. K. Tuggle, D. O. Bayles, D. Alt, U. Y. Levine, and T. B. Stanton. 2013. 'Profiling the gastrointestinal microbiota in response to *Salmonella*: low versus high *Salmonella* shedding in the natural porcine host', *Infect Genet Evol*, 16: 330-40.

Berdy, B., A. L. Spoering, L. L. Ling, and S. S. Epstein. 2017. 'In situ cultivation of previously uncultivable microorganisms using the ichip', *Nat Protoc*, 12: 2232-42.

Boleij, A., and H. Tjalsma. 2013. 'The itinerary of *Streptococcus gallolyticus* infection in patients with colonic malignant disease', *Lancet Infect Dis*, 13: 719-24.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodriguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y. X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, 2nd, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vazquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. 'Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2', *Nat Biotechnol*, 37: 852-57.

Boyen, F. Haesebrouck, F. Maes, D. Van Immerseel, F. Ducatelle, and F. R. Pasmans. 2008. 'Non-typhoidal *Salmonella* infections in pigs: a closer look at epidemiology, pathogenesis and control', *Vet Microbiol*, 130: 1-19.

Boyen, F., F. Haesebrouck, A. Vanparys, J. Volf, M. Mahu, F. Van Immerseel, I. Rychlik, J. Dewulf, R. Ducatelle, and F. Pasmans. 2008. 'Coated fatty acids alter virulence properties of *Salmonella* Typhimurium and decrease intestinal colonization of pigs', *Vet Microbiol*, 132: 319-27.

Boyer, P. E., S. D'Costa, L. L. Edwards, M. Milloway, E. Susick, L. B. Borst, S. Thakur, J. M. Campbell, J. D. Crenshaw, J. Polo, and A. J. Moeser. 2015. 'Early-life dietary spray-dried plasma influences immunological and intestinal injury responses to later-life *Salmonella typhimurium* challenge', *Br J Nutr* 113: 783-93.

Brooks SPJ, Green-Johnson J, Inglis GD, Uwiera RRE, Kalmokoff M:. 2011. *Gut microbiology - relatively unexplored domain* (Comprehensive Biotechnology).

Browne, H. P., S. C. Forster, B. O. Anonye, N. Kumar, B. A. Neville, M. D. Stares, D. Goulding, and T. D. Lawley. 2016. 'Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation', *Nature*, 533: 543-6.

Bui, T. P., J. Ritari, S. Boeren, P. de Waard, C. M. Plugge, and W. M. de Vos. 2015. 'Production of butyrate from lysine and the Amadori product fructoselysine by a human gut commensal', *Nat Commun*, 6: 10062.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. Johnson, and S. P. Holmes. 2016. 'DADA2: High-resolution sample inference from Illumina amplicon data', *Nat Methods*, 13: 581-3.

Castillo, M., S. M. Martin-Orue, E. G. Manzanilla, I. Badiola, M. Martin, and J. Gasa. 2006. 'Quantification of total bacteria, enterobacteria and lactobacilli populations in pig digesta by real-time PCR', *Vet Microbiol*, 114: 165-70.

Cherayil, B. J., and D. Antos. 2001. 'Inducible nitric oxide synthase and *Salmonella* infection', *Microbes Infect*, 3: 771-6.

Chirullo, B. , M. Pesciaroli, R. Drumo, J. Ruggeri, E. Razzuoli, C. Pistoia, P. Petrucci, N. Martinelli, L. Cucco, L. Moscati, M. Amadori, C. F. Magistrali, G. L. Alborali, and P. Pasquali. 2015. '*Salmonella* Typhimurium exploits inflammation to its own advantage in piglets', *Front Microbiol*, 6: 985.

Cole, J. R., Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske, and J. M. Tiedje. 2014. 'Ribosomal Database Project: data and tools for high throughput rRNA analysis', *Nucleic Acids Res*, 42: D633-42.

Collado-Romero, M. , C. Arce, M. Ramirez-Boo, A. Carvajal, and J. J. Garrido. 2010. 'Quantitative analysis of the immune response upon *Salmonella* Typhimurium infection along the porcine intestinal gut', *Vet Res*, 41: 23.

Costa, E., N. J. Puhl, L. B. Selinger, and G. D. Inglis. 2009. 'Characterization of mucosa-associated bacterial communities of the mouse intestine by terminal restriction fragment length polymorphism: utility of sampling strategies and methods to reduce single-stranded DNA artifacts', *J Microbiol Methods*, 78: 175-80.

Donaldson, G. P., S. M. Lee, and S. K. Mazmanian. 2016. 'Gut biogeography of the bacterial microbiota', *Nat Rev Microbiol*, 14: 20-32.

Dou, S., P. Gadonna-Widehem, V. Rome, D. Hamoudi, L. Rhazi, L. Lakhal, T. Larcher, N. Bahi-Jaber, A. Pinon-Quintana, A. Guyonvarch, I. L. Huerou-Luron, and L. Abdennebi-Najar. 2017. 'Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea', *PLoS One*, 12: e0169851.

Drumo, R., M. Pesciaroli, J. Ruggeri, M. Tarantino, B. Chirullo, C. Pistoia, P. Petrucci, N. Martinelli, L. Moscati, E. Manuali, S. Pavone, M. Picciolini, S. Ammendola, G. Gabai, A. Battistoni, G. Pezzotti, G. L. Alborali, V. Napolioni, P. Pasquali, and C. F. Magistrali. 2015. '*Salmonella enterica* serovar typhimurium exploits inflammation to modify swine intestinal microbiota', *Front Cell Infect Microbiol*, 5: 106.

Duimstra, J. R., L. L. Myers, J. E. Collins, D. A. Benfield, D. S. Shoop, and W. C. Bradbury. 1991. 'Enterovirulence of enterotoxigenic *Bacteroides fragilis* in gnotobiotic pigs', *Vet Pathol*, 28: 514-8.

Edgar, R. C. 2004. 'MUSCLE: multiple sequence alignment with high accuracy and high throughput', *Nucleic Acids Res*, 32: 1792-7.

Fenske, G. J., S. Ghimire, L. Antony, J. Christopher-Hennings, and J. Scaria. 2020. 'Integration of culture-dependent and independent methods provides a more coherent picture of the pig gut microbiome', *FEMS Microbiol Ecol*, 96.

Fyderek, K., M. Strus, K. Kowalska-Duplaga, T. Gosiewski, A. Wedrychowicz, U. Jedynak-Wasowicz, M. Sladek, S. Pieczarkowski, P. Adamski, P. Kochan, and P. B. Heczko. 2009. 'Mucosal bacterial microflora and mucus layer thickness in adolescents with inflammatory bowel disease', *World J Gastroenterol*, 15: 5287-94.

Gallo, R. L., M. Ono, T. Povsic, C. Page, E. Eriksson, M. Klagsbrun, and M. Bernfield. 1994. 'Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds', *Proc Natl Acad Sci U S A*, 91: 11035-9.

Gantois, I., R. Ducatelle, F. Pasmans, F. Haesebrouck, I. Hautefort, A. Thompson, J. C. Hinton, and F. Van Immerseel. 2006. 'Butyrate specifically down-regulates salmonella pathogenicity island 1 gene expression', *Appl Environ Microbiol*, 72: 946-9.

Garner, C. D., D. A. Antonopoulos, B. Wagner, G. E. Duhamel, I. Keresztes, D. A. Ross, V. B. Young, and C. Altier. 2009. 'Perturbation of the small intestine microbial ecology by streptomycin alters pathology in a *Salmonella enterica* serovar typhimurium murine model of infection', *Infect Immun*, 77: 2691-702.

Godinez, I., T. Haneda, M. Raffatellu, M. D. George, T. A. Paixao, H. G. Rolan, R. L. Santos, S. Dandekar, R. M. Tsolis, and A. J. Baumler. 2008. 'T cells help to amplify inflammatory responses induced by *Salmonella enterica* serotype Typhimurium in the intestinal mucosa', *Infect Immun*, 76: 2008-17.

He, T., Y. H. Zhu, J. Yu, B. Xia, X. Liu, G. Y. Yang, J. H. Su, L. Guo, M. L. Wang, and J. F. Wang. 2019. '*Lactobacillus johnsonii* L531 reduces pathogen load and helps maintain short-chain fatty acid levels in the intestines of pigs challenged with *Salmonella enterica* Infantis', *Vet Microbiol*, 230: 187-94.

Holani, R., C. Shah, Q. Haji, G. D. Inglis, R. R. E. Uwiera, and E. R. Cobo. 2016. 'Proline-arginine rich (PR-39) cathelicidin: structure, expression and functional implication in intestinal health', *Comp Immunol Microbiol Infect Dis*, 49: 95-101.

Hooper, L. V., T. Midtvedt, and J. I. Gordon. 2002. 'How host-microbial interactions shape the nutrient environment of the mammalian intestine', *Annu Rev Nutr*, 22: 283-307.

Inglis, G. D., M. C. Thomas, D. K. Thomas, M L. Kalmokoff, S. P. J. Brooks, and L. B. Selinger. 2012. 'Methods to measure intestinal bacteria: a review', *J AOAC Int*, 95: 5-23.

James Kozich, Patrick Schloss, Niel Baxter, Matt Jenior, Charles Koumpouras, Lucas Bishop. 2013. Access at https://github.com/SchlossLab/MiSeq_WetLab_SOP/blob/master/MiSeq_WetLab_SOP.md. "16S rRNA sequencing with the Illumina MiSeq: library generation, QC, & sequencing." In.

Jiminez, J. A., T. C. Uwiera, D. W. Abbott, R. R. E. Uwiera, and G. D. Inglis. 2017. 'Butyrate supplementation at high concentrations alters enteric bacterial communities and reduces intestinal inflammation in mice infected with *Citrobacter rodentium*', *mSphere*, 2.

Johnson, B. J., Dritz, S. S., Skjolaas-Wilson, K. A., Burkey, T. E., & Minton, J. E. 2005. 'Interactive responses in gut immunity, and systemic and local changes in the insulin-like growth factor system in nursery pigs in response to *Salmonella enterica* serovar Typhimurium.', *J Anim Sci*, 83: E48-E56.

Jung, T. H., J. H. Park, W. M. Jeon, and K. S. Han. 2015. 'Butyrate modulates bacterial adherence on LS174T human colorectal cells by stimulating mucin secretion and MAPK signaling pathway', *Nutr Res Pract*, 9: 343-9.

Kelly, J., K. Daly, A. W. Moran, S. Ryan, D. Bravo, and S. P. Shirazi-Beechey. 2017. 'Composition and diversity of mucosa-associated microbiota along the entire length of the pig gastrointestinal tract; dietary influences', *Environ Microbiol*, 19: 1425-38.

Kumar, R., J. L. Herold, D. Schady, J. Davis, S. Kopetz, M. Martinez-Moczygemba, B. E. Murray, F. Han, Y. Li, E. Callaway, R. S. Chapkin, W. M. Dashwood, R. H. Dashwood, T. Berry, C. Mackenzie, and Y. Xu.

2017. *'Streptococcus gallolyticus subsp. gallolyticus* promotes colorectal tumor development', *PLoS Pathog*, 13: e1006440.

Kumar, R., P. K. Surendran, and N. & Thampuran. 2010. 'Rapid quantification of *Salmonella* in seafood by real-time PCR assay.', *J Microbiol Biotechnol*, N.20: 569-73.

Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. 'MEGA X: molecular evolutionary genetics analysis across computing platforms', *Mol Biol Evol*, 35: 1547-49.

Lagier, J. C., F. Armougom, M. Million, P. Hugon, I. Pagnier, C. Robert, F. Bittar, G. Fournous, G. Gimenez, M. Maraninchi, J. F. Trape, E. V. Koonin, B. La Scola, and D. Raoult. 2012. 'Microbial culturomics: paradigm shift in the human gut microbiome study', *Clin Microbiol Infect*, 18: 1185-93.

Lagier, J. C., S. Khelaifia, M. T. Alou, S. Ndongo, N. Dione, P. Hugon, A. Caputo, F. Cadoret, S. I. Traore, E. H. Seck, G. Dubourg, G. Durand, G. Mourembou, E. Guilhot, A. Togo, S. Bellali, D. Bachar, N. Cassir, F. Bittar, J. Delerce, M. Mailhe, D. Ricaboni, M. Bilen, N. P. Dangui Nieko, N. M. Dia Badiane, C. Valles, D. Mouelhi, K. Diop, M. Million, D. Musso, J. Abrahao, E. I. Azhar, F. Bibi, M. Yasir, A. Diallo, C. Sokhna, F. Djossou, V. Vitton, C. Robert, J. M. Rolain, B. La Scola, P. E. Fournier, A. Levasseur, and D. Raoult. 2016. 'Culture of previously uncultured members of the human gut microbiota by culturomics', *Nat Microbiol*, 1: 16203.

Larsen, N., F. K. Vogensen, F. W. van den Berg, D. S. Nielsen, A. S. Andreasen, B. K. Pedersen, W. A. Al-Soud, S. J. Sorensen, L. H. Hansen, and M. Jakobsen. 2010. 'Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults', *PLoS One*, 5: e9085.

Lee, S. M., G. P. Donaldson, Z. Mikulski, S. Boyajian, K. Ley, and S. K. Mazmanian. 2013. 'Bacterial colonization factors control specificity and stability of the gut microbiota', *Nature*, 501: 426-9.

Li, J., M. Post, R. Volk, Y. Gao, M. Li, C. Metais, K. Sato, J. Tsai, W. Aird, R. D. Rosenberg, T. G. Hampton, F. Sellke, P. Carmeliet, and M. Simons. 2000. 'PR39, a peptide regulator of angiogenesis', *Nat Med*, 6: 49-55.

Litvak, Y., M. X. Byndloss, R. M. Tsolis, and A. J. Baumler. 2017. 'Dysbiotic *Proteobacteria* expansion: a microbial signature of epithelial dysfunction', *Curr Opin Microbiol*, 39: 1-6.

Liu, H., E. Ivarsson, J. Dicksved, T. Lundh, and J. E. Lindberg. 2012. 'Inclusion of chicory (Cichorium intybus L.) in pigs' diets affects the intestinal microenvironment and the gut microbiota', *Appl Environ Microbiol*, 78: 4102-9.

Looft, T., T. A. Johnson, H. K. Allen, D. O. Bayles, D. P. Alt, R. D. Stedtfeld, W. J. Sul, T. M. Stedtfeld, B. Chai, J. R. Cole, S. A. Hashsham, J. M. Tiedje, and T. B. Stanton. 2012. 'In-feed antibiotic effects on the swine intestinal microbiome', *Proc Natl Acad Sci U S A*, 109: 1691-6.

Lupp, C., M. L. Robertson, M. E. Wickham, I. Sekirov, O. L. Champion, E. C. Gaynor, and B. B. Finlay. 2007. 'Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of *Enterobacteriaceae*', *Cell Host Microbe*, 2: 119-29.

Lynch, M. D., and J. D. Neufeld. 2015. 'Ecology and exploration of the rare biosphere', *Nat Rev Microbiol*, 13: 217-29.

Malago, J. J., J. F. Koninkx, P. C. Tooten, E. A. van Liere, and J. E. van Dijk. 2005. 'Anti-inflammatory properties of heat shock protein 70 and butyrate on *Salmonella*-induced interleukin-8 secretion in enterocyte-like Caco-2 cells', *Clin Exp Immunol*, 141: 62-71.

Mandal, S., W. Van Treuren, R. A. White, M. Eggesbo, R. Knight, and S. D. Peddada. 2015. 'Analysis of composition of microbiomes: a novel method for studying microbial composition', *Microb Ecol Health Dis*, 26: 27663.

McCormick, B. A., P. M. Hofman, J. Kim, D. K. Carnes, S. I. Miller, and J. L. Madara. 1995. 'Surface attachment of *Salmonella* Typhimurium to intestinal epithelia imprints the subepithelial matrix with gradients chemotactic for neutrophils', *J Cell Biol*, 131: 1599-608.

Meurens, F. , M. Berri, G. Auray, S. Melo, B. Levast, I. Virlogeux-Payant, C. Chevaleyre, V. Gerdts, and H. Salmon. 2009. 'Early immune response following *Salmonella enterica* subspecies *enterica* serovar Typhimurium infection in porcine jejunal gut loops', *Vet Res*, 40: 5.

Molla, B., A. Sterman, J. Mathews, V. Artuso-Ponte, M. Abley, W. Farmer, P. Rajala-Schultz, W. E. Morrow, and W. A. Gebreyes. 2010. '*Salmonella enterica* in commercial swine feed and subsequent isolation of phenotypically and genotypically related strains from fecal samples', *Appl Environ Microbiol*, 76: 7188-93.

Mon, K. K., P. Saelao, M. M. Halstead, G. Chanthavixay, H. C. Chang, L. Garas, E. A. Maga, and H. Zhou. 2015. '*Salmonella enterica* serovars Enteritidis infection alters the indigenous microbiota diversity in young layer chicks', *Front Vet Sci*, 2: 61.

Mookherjee, N., L. M. Rehaume, and R. E. Hancock. 2007. 'Cathelicidins and functional analogues as antisepsis molecules', *Expert Opin Ther Targets*, 11: 993-1004.

Moote, P. E., S. J. M. Zaytsoff, R. Ortega Polo, D. W. Abbott, R. R. E. Uwiera, and G. D. Inglis. 2020. 'Application of culturomics to characterize diverse anaerobic bacteria from the gastrointestinal tract of broiler chickens in relation to environmental reservoirs', *Can J Microbiol*: 1-15.

Mukherjee, S., and L. V. Hooper. 2015. 'Antimicrobial defense of the intestine', *Immunity*, 42: 28-39.

O'Donnell, H., O. H. Pham, L. X. Li, S. M. Atif, S. J. Lee, M. M. Ravesloot, J. L. Stolfi, S. P. Nuccio, P. Broz, D. M. Monack, A. J. Baumler, and S. J. McSorley. 2014. 'Toll-like receptor and inflammasome signals converge to amplify the innate bactericidal capacity of T helper 1 cells', *Immunity*, 40: 213-24.

Pohlert, T. . 2018. 'PMCMR:Calculate pairwise multiple comparisons of mean rank sums. Available from https://CRAN.R-project.org/package=PMCMR'.

Prouty, A. M., and J. S. Gunn. 2000. '*Salmonella enterica* serovar Typhimurium invasion is repressed in the presence of bile', *Infect Immun*, 68: 6763-9.

Quan, J., G. Cai, J. Ye, M. Yang, R. Ding, X. Wang, E. Zheng, D. Fu, S. Li, S. Zhou, D. Liu, J. Yang, and Z. Wu. 2018. 'A global comparison of the microbiome compositions of three gut locations in commercial pigs with extreme feed conversion ratios', *Sci Rep*, 8: 4536.

Rambaut, A. . 2012. 'FigTree v1. 4'.

Ramirez-Farias, C., K. Slezak, Z. Fuller, A. Duncan, G. Holtrop, and P. Louis. 2009. 'Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*', *Br J Nutr*, 101: 541-50.

Rashidan, M., M. Azimirad, M. Alebouyeh, M. Ghobakhlou, H. Asadzadeh Aghdaei, and M. R. Zali. 2018. 'Detection of B. fragilis group and diversity of bft enterotoxin and antibiotic resistance markers cepA, cfiA and nim among intestinal *Bacteroides fragilis* strains in patients with inflammatory bowel disease', *Anaerobe*, 50: 93-100.

Rastall, R. A. 2004. 'Bacteria in the gut: friends and foes and how to alter the balance', *J Nutr*, 134: 2022S-26S.

Rigottier-Gois, L. 2013. 'Dysbiosis in inflammatory bowel diseases: the oxygen hypothesis', *ISME J*, 7: 1256-61.

Rios-Covian, D., P. Ruas-Madiedo, A. Margolles, M. Gueimonde, C. G. de Los Reyes-Gavilan, and N. Salazar. 2016. 'Intestinal short chain fatty acids and their link with diet and human health', *Front Microbiol*, 7: 185.

Russell, E. G. 1979. 'Types and distribution of anaerobic bacteria in the large intestine of pigs', *Appl Environ Microbiol*, 37: 187-93.

Salzman, N. H., M. A. Underwood, and C. L. Bevins. 2007. 'Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa', *Semin Immunol*, 19: 70-83.

Sansonetti, P. J. 2004. 'War and peace at mucosal surfaces', *Nat Rev Immunol*, 4: 953-64.

Sassone-Corsi, M., and M. Raffatellu. 2015. 'No vacancy: how beneficial microbes cooperate with immunity to provide colonization resistance to pathogens', *J Immunol*, 194: 4081-7.

Scherer, K., I. Szabo, U. Rosler, B. Appel, A. Hensel, and K. Nockler. 2008. 'Time course of infection with *Salmonella* Typhimurium and its influence on fecal shedding, distribution in inner organs, and antibody response in fattening pigs', *J Food Prot*, 71: 699-705.

Sears, C. L., S. Islam, A. Saha, M. Arjumand, N. H. Alam, A. S. Faruque, M. A. Salam, J. Shin, D. Hecht, A. Weintraub, R. B. Sack, and F. Qadri. 2008. 'Association of enterotoxigenic *Bacteroides fragilis* infection with inflammatory diarrhea', *Clin Infect Dis*, 47: 797-803.

Soler, L., I. Miller, K. Nobauer, S. Carpentier, and T. Niewold. 2015. 'Identification of the major regenerative III protein (RegIII) in the porcine intestinal mucosa as RegIIIgamma, not RegIIIalpha', *Vet Immunol Immunopathol*, 167: 51-6.

Song, Y., C. Liu, and S. M. Finegold. 2004. 'Real-time PCR quantitation of clostridia in feces of autistic children', *Appl Environ Microbiol*, 70: 6459-65.

Stecher, B., R. Robbiani, A. W. Walker, A. M. Westendorf, M. Barthel, M. Kremer, S. Chaffron, A. J. Macpherson, J. Buer, J. Parkhill, G. Dougan, C. von Mering, and W. D. Hardt. 2007. '*Salmonella enterica* serovar Typhimurium exploits inflammation to compete with the intestinal microbiota', *PLoS biology*, 5: 2177-89.

Tamura, K., M. Nei, and S. Kumar. 2004. 'Prospects for inferring very large phylogenies by using the neighbor-joining method', *Proc Natl Acad Sci U S A*, 101: 11030-5.

Thiennimitr, Parameth, Sebastian E. Winter, Maria G. Winter, Mariana N. Xavier, Vladimir Tolstikov, Douglas L. Huseby, Torsten Sterzenbach, Renée M. Tsolis, John R. Roth, and and Andreas J. Bäumler. 2011. 'Intestinal inflammation allows *Salmonella* to use ethanolamine to compete with the microbiota.', *Proc Natl Acad Sci U S A*, 108: 17480-85.

Toms, C. , and F. Powrie. 2001. 'Control of intestinal inflammation by regulatory T cells', *Microbes Infect*, 3: 929-35.

Uthe, J. J., A. Royaee, J. K. Lunney, T. J. Stabel, S. H. Zhao, C. K. Tuggle, and S. M. Bearson. 2007. 'Porcine differential gene expression in response to *Salmonella enterica* serovars Choleraesuis and Typhimurium', *Mol Immunol*, 44: 2900-14.

Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman. 2002. 'Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes', *Genome Biol*, 3: RESEARCH0034.

Veldhuizen, E. J., M. Rijnders, E. A. Claassen, A. van Dijk, and H. P. Haagsman. 2008. 'Porcine beta-defensin 2 displays broad antimicrobial activity against pathogenic intestinal bacteria', *Mol Immunol*, 45: 386-94.

Veldhuizen, E. J., V. A. Schneider, H. Agustiandari, A. van Dijk, J. L. Tjeerdsma-van Bokhoven, F. J. Bikker, and H. P. Haagsman. 2014. 'Antimicrobial and immunomodulatory activities of PR-39 derived peptides', *PLoS One*, 9: e95939.

Wang, G. 2014. 'Human antimicrobial peptides and proteins', *Pharmaceuticals (Basel)*, 7: 545-94.

Wang, W., H. Hu, R. T. Zijlstra, J. Zheng, and M. G. Ganzle. 2019. 'Metagenomic reconstructions of gut microbial metabolism in weanling pigs', *Microbiome*, 7: 48.

Wang, Y., L. Qu, J. J. Uthe, S. M. Bearson, D. Kuhar, J. K. Lunney, O. P. Couture, D. Nettleton, J. C. Dekkers, and C. K. Tuggle. 2007. 'Global transcriptional response of porcine mesenteric lymph nodes to *Salmonella enterica* serovar Typhimurium', *Genomics*, 90: 72-84.

Warnes, G. R., B. Bolker, L. Bonebakker, R. Gentleman, W. H. A. Liaw, T. Lumley, M. Maechler, A. Magnusson, S. Moeller, M. Schwartz, and B. Venables. 2015. 'gplots: various R programming tools for plotting data', *https://www.scienceopen.com/document?vid=0e5d8e31-1fe4-492f-a3d8-8cd71b2b8ad9*.

Wexler, H. M. 2007. '*Bacteroides*: the good, the bad, and the nitty-gritty', *Clin Microbiol Rev*, 20: 593-621.

Wilcock, B. P., C. H. Armstrong, and H. J. & Olander. 1976. 'The significance of the serotype in the clinical and pathological features of naturally occurring porcine salmonellosis.', *Can J Comp Med*, 40: 80.

Winter, S. E., P. Thiennimitr, M. G. Winter, B. P. Butler, D. L. Huseby, R. W. Crawford, J. M. Russell, C. L. Bevins, L. G. Adams, R. M. Tsolis, J. R. Roth, and A. J. Baumler. 2010. 'Gut inflammation provides a respiratory electron acceptor for *Salmonella*', *Nature*, 467: 426-9.

Xu, J. Gordon, J. I. 2003. 'Honor thy symbionts', *Proc Natl Acad Sci U S A*, 100: 10452-9.

Yang, H., X. Huang, S. Fang, W. Xin, L. Huang, and C. Chen. 2016. 'Uncovering the composition of microbial community structure and metagenomics among three gut locations in pigs with distinct fatness', *Sci Rep*, 6: 27427.

Yin, F., A. Farzan, Q. C. Wang, H. Yu, Y. Yin, Y. Hou, R. Friendship, and J. Gong. 2014. 'Reduction of *Salmonella enterica* serovar Typhimurium DT104 infection in experimentally challenged weaned pigs fed a lactobacillus-fermented feed', *Foodborne Pathog Dis*, 11: 628-34.

Zeng, M. Y., N. Inohara, and G. Nunez. 2017. 'Mechanisms of inflammation-driven bacterial dysbiosis in the gut', *Mucosal Immunol*, 10: 18-26.

Zhang, L., W. Wu, Y. K. Lee, J. Xie, and H. Zhang. 2018. 'Spatial heterogeneity and co-occurrence of mucosal and luminal microbiome across swine intestinal tract', *Front Microbiol*, 9: 48.

Zhao, W., Y. Wang, S. Liu, J. Huang, Z. Zhai, C. He, J. Ding, J. Wang, H. Wang, W. Fan, J. Zhao, and H. Meng. 2015. 'The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments', *PLoS One*, 10: e0117441

## Chapter 3: The murine cathelicidin-related antimicrobial peptide modulates host responses enhancing *Salmonella enterica* serovar Typhimurium infection

### 3.1 Introduction

Host-defense peptides are an evolutionary conserved component of the innate immune system that play an essential role in protection of the host (Mukherjee *et al.* 2015). Antimicrobial peptides are comprised of defensins, C-type lectins, and cathelicidins (Mukherjee *et al.* 2008). Cathelicidins are peptides characterized by an N-terminal signal peptide, a cathelin-like propeptide, and a variable C-terminal domain, which is cleaved to release the antimicrobial activity (Iimura *et al.* 2005). These antimicrobial peptides have been identified in mammalian species including rats (Travis *et al.* 2000), human beings (Gudmundsson *et al.* 1996), rabbits (Larrick *et al.* 1991), monkeys (Bals *et al.* 2001), pigs (Holani *et al.* 2016), and cows (Zanetti *et al.* 1993), among others. In mice, the only cathelicidin that has been identified is the murine cathelicin-related antimicrobial peptide (mCRAMP) (Gallo *et al.* 1997). mCRAMP was first isolated from the bone marrow and has been the focus of investigation due to the homology in gene sequence, structure, and protein processing that it shares with the human cathelicidin, LL-37/hCAP-18 (Pestonjamasp *et al.* 2001). mCRAMP is mainly expressed in neutrophils; however, its presence has also been observed in testis, lung, urinary tract, and the gastrointestinal tract (Gallo *et al.* 1997). mCRAMP is an amphipathic α-helical structure that binds to negatively charged groups of the outer bacterial membrane, thus altering its structure and permeability with ensuing bactericidal activity (Brogden *et al.* 2005). Several studies using mCRAMP null mice have shown the role that this cathelicidin plays in the protection of skin (Nizet *et al.* 2001), the urinary tract (Chromek *et al.* 2006), and the gastrointestinal tract (GIT) (Iimura *et al.* 2005); however, the studies conducted in the GIT were mainly restricted to the colonic tissue, and targeted colitis incited by *Candida albicans, Escherichia coli* and *Citrobacter rodentium* (Fan *et al.* 2015; Koon *et al.* 2011). Cathelicidins also participate in modulation of immune responses, including chemoattraction of leukocytes by activation of formyl peptide receptors (FPR) (Kurosaka *et al.* 2005), stimulation of degranulation (van Harten *et al.* 2018), enhancement of phagocytosis (Kress *et al.* 2017), and stimulation of humoral adaptive immune responses (Kurosaka *et al.* 2005). mCRAMP has also been shown to stimulate neovascularization of cutaneous wounds (Koczulla *et al.* 2003), and to play a pivotal role in maintaining homeostasis of the colonic microbiota (Yoshimura *et al.* 2018).

It is well known that the commensal microbiota confers protection to the host by competing directly and indirectly with enteric pathogens, referred to as colonization resistance (Lawley *et al.* 2013). The precise mechanisms by which colonization resistance functions are enigmatic at present. However,

direct competition between pathogenic microorganisms and the autochthonous microbiota has been suggested, including competition for nutrients (Momose *et al.* 2008) and niches (Freter *et al.* 1983), delivery of bactericidal effectors by means of the type VI secretion system (Jana *et al.* 2019), and production of anti-microbial compounds, such as bacteriocins (Martinez *et al.* 2013). Indirect mechanisms of colonization resistance include stimulation of short chain fatty acid production (Rios-Covian *et al.* 2016), pro-inflammatory cytokines (Hasegawa *et al.* 2012), and antimicrobial peptides (Fan *et al.* 2015). As a consequence, when the structure of the autochthonous microbiota is affected (i.e. reduction in diversity), the ensuing dysbiosis can result in a loss of homeostasis, including the loss of colonization resistance, with associated negative health implications for the host (Gu *et al.* 2013).

*Salmonella enterica* serovar Typhimurium is a Gram-negative intracellular pathogen that can incite a wide spectrum of clinical manifestations that vary from mild enterocolitis to fulminating septicemia (Coburn *et al.* 2007). In mice, infection by this pathogen produces a typhoid-like fever disease (Gal-Mor *et al.* 2014). Disruption of the commensal microbiota in mice via administration of streptomycin before inoculation with *S.* Typhimurium has been used to mimic and thus study human salmonellosis characterized by typhlitis, colitis, and diarrhea (Kaiser *et al.* 2012; Barthel *et al.* 2003). However, the temporal clearance of the microbiota is an important limitation of this model due to the disruption and suppression of the mechanisms of colonization resistance conferred by the autochthonous microbiota. In this regard, investigations have shown that mice harbouring a disrupted microbiota are highly susceptible to enteric infections by pathogens (Zachar *et al.* 1979; Wadolkowski *et al.* 1988). The importance of the microbiota in modulating host immune responses has been extensively studied. The recognition of commensals by toll-like receptors (TLRs) is essential to maintain intestinal homeostasis (Rakoff-Nahoum *et al.* 2004), and bacteria recognition by TLRs is an important process to induce antimicrobial host defense responses (Salzman *et al.* 2007). mCRAMP released via TLR stimulation has previously been described to impair mouse colitis (Koon *et al.* 2011). However, the role that mCRAMP plays in the initiation and progression of salmonellosis *in vivo*, including colonization resistance against *S.* Typhimurium is unknown.

I hypothesized that the host defense peptide, cathelicidin, aids in protecting the host from salmonellosis by modulating the intestinal microbiota and host immune responses. I comparatively and temporally evaluated the impact of cathelicidin on the enteric microbiota as well as host immune responses, including mucosal responses to *S.* Typhimurium DT104 (SA+) relative to non-infected (SA-) mice. I also ascertained the effects of cathelicidin by measuring responses in cathelicidin deficient ($mCRAMP^{-/-}$) and cathelicidin competent ($mCRAMP^{+/+}$) mice. Moreover, I included a streptomycin sulfate

(ST+) or no streptomycin (ST-) treatment to induce a disruption of the microbiota composition (Stecher *et al.* 2007), and to contrast responses in mice with and without a dysbiosis. My data supported my hypothesis that the absence of mCRAMP predisposed mice to enteric and systemic salmonellosis by triggering an innate immune response and modifying the composition of autochthonous microbiota. In addition, my evidence suggests that the use of mCRAMP knockout mice may be a suitable alternative to the conventional streptomycin dysbiosis model for studying *Salmonella* enterocolitis in mice.

### 3.2 Materials and methods

#### 3.2.1 Ethics statement

The study was carried out in strict accordance with the recommendations established in the Canadian Council on Animal Care Guidelines. The project was reviewed and approved by the Lethbridge Research and Development Centre (LeRDC) Animal Care Committee (Animal Use Protocol Review 1729), and the LeRDC Biosafety and Biosecurity Committee before commencement of the research.

#### 3.2.2 Experimental design

The experiment was designed as a two (± mCRAMP) by two (± *Salmonella*) by two (± streptomycin) by two (sample time) factorial experiment, arranged as a completely randomized design (CRD). Three replicates were conducted on separate occasions to ensure independence. A total of 48 mice were used.

#### 3.2.3 Animal maintenance

Specific-pathogen-free agnotobiotic C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME), and used to establish breeding colonies at LeRDC. The breeding colony of mCRAMP$^{-/-}$ mice was maintained in an isolator (CBClean, Madison, WI), whereas mCRAMP$^{+/+}$ mice were maintained in conventional cages in an adjacent animal room. Individual mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice at 6-weeks-of-age were transferred to individually ventilated cages (IVCs) connected to a HEPA filter unit (Techniplast, Montreal, QC) operated in containment mode. Mice were individually housed, but could see each other through the transparent plastic cages. Mice were acclimatized to the IVCs for 7 days before commencement of the experiment. Mice were maintained on a Prolab RMH 3500, Autoclavable 5P04 diet (LabDiet, St. Louis, MO), and they were permitted to eat and drink *ad libitum*.

#### 3.2.4 Inoculation and streptomycin administration

Mice were intragastrically administered either 100 µl of streptomycin (20 mg µl$^{-1}$) or 100 µl of water alone using a 22G, rigid gavage needle (Western Drug Distribution Centre Ltd, Edmonton, AB). Twenty four h after administration of the antibiotic or water, mice were orally inoculated with a 100 µl of *S. enterica* serovar Typhimurium DT104 (strain SA970934) (Yin *et al.* 2014) or Columbia Broth (CB) alone (VWR, Mississauga, ON) as described above for administration of streptomycin. To produce inoculum,

the bacterium was grown aerobically on MacConkey's agar (MA) (Difco BD, Mississauga, ON) at 37°C for 24 hr. Biomass was removed from the surface of the agar and transferred into CB. Cultures were maintained for 180 to 210 min at 37°C shaking at 150 rpm, until an optical density (600 nm) of 1.2 or greater was obtained. Cultures were centrifuged at 4,000 x g for 15 min, supernatants were removed to a volume of 15 ml, and the optical density was adjusted to a target of $3.0 \times 10^9$ cells $ml^{-1}$. To ascertain *S*. Typhimurium cell densities, the suspension was diluted in a 10-fold dilution series, 100 µl of each dilution was spread in duplicate onto MA, cultures were incubated aerobically at 37°C, and colonies were counted at the dilution yielding 30 to 300 colony forming units after 24 hr.

### 3.2.5 Animal health status and tissue collection

Mice were monitored daily for changes in health status, which included evidence of diarrhea, altered food consumption, and behavioral changes (e.g. restless and depressed). Twenty four and 48 hpi, animals were anaesthetized with isoflurane (Western Drug Distribution Centre Ltd, Edmonton, AB). Under anesthesia, blood was collected intracardially, and animals were humanly euthanized by cervical dislocation. Immediately after death, a laparotomy was completed, and viscera was exposed. The liver, spleen, and intestine were aseptically collected.

### 3.2.6 Histopathology

Tissue samples from the duodenum, jejunum, ileum, cecum, and proximal and distal colon were placed in TrueFlow Macrosette cassettes (Tissue Path; Thermo Fisher Scientific, Edmonton, AB), and submerged in 10% neutral buffered formalin (Surgipath Canada, Inc., Winnipeg, MB). Samples were dehydrated using a tissue processor (Leica TP 1020, Leica Biosystems, Location), and embedded in paraffin (Fisherfinest™ Histoplast PE; Thermo Fisher Scientific) using a Shandon Histocentre 3 (Thermo Fisher Scientific). Using a Shandon Finesse 325 microtome (Thermo Fisher Scientific), 5-µm-thick sections were transferred to positively charged slides (Fisherbrand Superfrost™ Plus Gold; Thermo Fisher Scientific) and allowed to dry prior to being deparaffinized with xylene. Slides were rehydrated in ethanol and stained with H&E using a standard protocol. Histopathologic changes were scored in a blinded fashion as to treatment by board-certified pathologist (V.F.B). The scoring system used was developed from Boyer *et al.* (2015), Garner *et al.* (2009), Koelink *et al.* (2018), and Erben *et al.* (2014) (Table 3.1). Scores were combined across all categories to obtain the total histopathologic score (maximum score of 30).

### 3.2.7 Bacterial genomic DNA extraction

For quantification of *S*. Typhimurium associated with intestinal and liver tissues by quantitative (q)PCR, DNA was extracted from the ileum, cecum, proximal colon, and liver using the Qiagen Blood and

Tissue kit (Qiagen Inc., Toronto, ON) following the gram positive protocol as recommended by the manufacturer. For quantification of *S*. Typhimurium and characterization of bacterial communities within digesta, DNA was extracted from cecal digesta using the Qiagen Fast DNA Stool Mini Kit (Qiagen Inc.) according to the manufacturer's protocol. A bead beating step using 5.0-mm-diam stainless steel beads using a TissueLyser LT (Qiagen Inc.) at 30 Hz was added to ensure efficient release of genomic DNA from both Gram positive and Gram Negative bacteria; the bead beating step was conducted three times (30 sec duration).

### 3.2.8 Quantification of *Salmonella*

Duplicate PCR reactions were prepared. Each reaction contained 10 µl QuantiTect SYBR Green Mastermix (Qiagen Inc.), 0.5 µM of the forward and reverse primers (IDT, San Diego, CA), 2 µg of BSA (Promega, Madison, WI), 2 µl of DNA, and 4 µl of nuclease free water (Qiagen Inc.). The primers used were F-(Sal) and R-(Sal) (Kumar *et al.* 2010). Data was collected using an Mx3005p Realtime PCR instrument (Agilent Technologies Canada Inc. Mississauga, ON). Cycle conditions were 95°C for 15 min, followed by 40 cycles of 94°C for 15 sec, 64°C for 30 sec, and 72°C for 30 sec. A standard curve was prepared with serial dilutions of genomic DNA ($2.6 \times 10^6$ copies $g^{-1}$) extracted from pure cultures of the pathogen. A disassociation curve (55-95°C) was included with each run to verify amplicon specificity. All reactions were run in duplicates, and average Ct values were calculated.

### 3.2.9 Quantification of immune genes

Within 15 min of death, samples from the ileum and cecum (≈0.5 x 0.5 cm) were placed in RNAprotect® (Qiagen Inc.) and stored at -20°C until processed. RNA was extracted from the samples using an RNeasy mini kit (Qiagen Inc.) with a DNase step added to eliminate residual genomic DNA (Qiagen Inc.). RNA quantity and quality was determined using Bioanalyzer 2100 (Agilent Technologies Canada Inc., Mississauga, ON) and 1000 ng of RNA was transcribed to cDNA (Qiagen Inc.). Evaluation of innate immune defenses included expression of host defense peptide (*ßd1*, *RegIIIγ*, *mCramp*), pattern recognition receptor (*Tlr2, Tlr4, Tlr5*), phagocytes immune (*Il18, iNOS, Il1ß*), epithelial barrier (*Kc, Zo1, Occludin, Muc2*), T-helper (*Ifnγ, Il22, Il4*) and T-regulatory (*Il10, Tgfß*) responses. Reactions were run in a 384-well plate containing 5.0 µl QuantiTect SYBR Green Master Mix (Qiagen Inc.), 0.5 µl of each primer (10 µM), 3.0 µl of RNase-free water, and 1.0 µl of cDNA. Quantitative PCR was performed in ABI7900HT thermocycler (Applied Biosystems, Carlsbad, CA). Cycle conditions consisted of 95°C for 15 min, followed by 40 cycles of 95 °C for 15 sec, 58-62°C for 30 sec, and 72°C for 30 sec. A disassociation curve (55-95°C) was included. All reactions were run in triplicate. Average Ct values were used to calculate gene expression normalized to Peptidylprolyl isomerase A (*Ppia*), hypoxanthine-guanine

phosphoribosyltransferase (*Hprt*), and beta-glucuronidase (*Gusβ*) reference genes. These genes were selected using the geNorm algorithm in qbase+ (Biogazelle, Zwijnaarde, Belgium) based on stability among samples.

**3.2.10 Quantification of immune peptides and proteins**

Blood collected by intracardiac puncture was directly transferred to a BD Microtainer® SST tubes (BD, Franklin Lake, NJ) and processed according to the manufacturer's directions. Serum was aliquoted into two 2 ml cryovials and stored at -80°C until processed. The left lateral lobe of the liver and a section of ileum were snap frozen in liquid nitrogen, and stored at -80°C until processing. To homogenize tissues, samples from each mouse were suspended in immunoprecipitation buffer at a ratio of 1:5 (w/v). The buffer was adapted from Burgos-Ramos *et al.* (2011), and consisted of 50 mM of $NaH_2PO_4$, 100 mM $Na_2PO_4$, 0.1% sodium dodecyl sulfate, 0.5% NaCl, 1% Triton X-100, and 5 mg $mL^{-1}$ sodium deoxycholate, with the addition of a 1% Protease Inhibitor Cocktail (Sigma Aldrich, St. Louis, MO). Frozen samples were immediately homogenized using a Tissue-Tearor® model 398 homogenizer (Biospec Products, Bartlesville, OK), and centrifuged at 14,000 × g for 30 min at 4°C. Supernatants were collected and stored at -80°C until analysis. Cytokine concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using freshly thawed serum and tissue homogenates. Regulatory cytokines included in the analysis were active and total transforming growth factor beta-1 (TGFβ1) and interleukin-10 (IL10). Pro-inflammatory cytokines and chemokines included neutrophil chemoattractant CXCL1/KC, macrophage inflammatory protein 2 (CXCL2/MIP2), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFNγ), tumour necrosis factor alpha (TNFα), IL22, and IL2. In addition, neutrophil enzyme myeloperoxidase (MPO) and murine-cathelicidin related antimicrobial peptide (mCRAMP) were measured. Cytokines, chemokines and enzyme were quantified using mouse DuoSet ELISA Development kits according to the manufacturer's protocols (R&D Systems, Minneapolis, MN). ELISAs were performed using 96-well high-binding half area microplates (Greiner Bio-One, Frickenhausen, Germany). Quantification of mCRAMP was carried out using a mouse Camp ELISA Kit following the manufacturer's instructions (Aviva Systems Biology, San Diego, CA). Optical density of the reactions were determined at a wavelength of 450 nm on a Synergy HT multi-detection microplate reader (BioTek Instruments Inc, Winooski, VT) with Gen5 analysis software (BioTek Instruments Inc., Winooski, VT).

**3.2.11 Flow cytometric analysis of splenic immune cell populations**

Excised spleens were placed on a sterile 70 µm cell strainer and crushed using a 3 ml syringe plunger with RMPI-1640 cell medium containing 10% fetal bovine serum (FBS). Splenic cells were pelleted by

centrifugation and red blood cells were lysed using one times RBC Lysis Buffer (Invitrogen, Carlsbad CA). Cells were subjected to viability staining using Ghost Dye™ Red 780 (Tonbo Biosciences, San Diego, CA) followed by an Fc blocking step with 10% heat-inactivated FBS. Samples were divided into three separate panels with $1 \times 10^6$ cells each in an effort to maximize the amount of cell populations analyzed. The first panel included anti-CD3-PerCP and anti-IL23R-PE (R&D Systems) with anti-CD4 PE-Cy7, anti-CD8-FITC and anti-TCR-β-APC (Tonbo Biosciences). The second panel utilized anti-CD3-PerCP, anti-CD161-PE-Cy7 (Invitrogen), anti-CD11b-FITC and anti-CD11c-PE (Tonbo Biosciences). The third panel included anti-CD45-PE-Cy7, anti-CD11b-FITC and anti-Ly-6G-APC (Tonbo Biosciences) with anti-CD18-PE and anti-Ly-6C-PerCP (BioLegend, San Diego, CA). Cells were subsequently fixed with Intracellular Fixation and Permeabilization Buffer (Invitrogen) according to the manufacturer's instructions, and the second panel subset was further stained intracellularly with anti-CD68-APC or its corresponding IgG2aκ isotype control (BioLegend). Samples were acquired on a BD FACSCanto II (BD Biosciences, San Jose, CA) flow cytometer equipped with blue 488 nm and red 633 nm lasers, and cell populations were analyzed using FlowJo v10 (BD Biosciences, Ashland, OR).

### 3.2.12 Analysis of bacterial communities

DNA extracted for community analysis was quantified with a Qubit (Thermo Fisher Scientific). Library preparation, next generation sequencing and quality control was performed by McGill University and Genome Quebec Innovation Centre (Montreal, QC). Bacterial 16S rRNA libraries were amplified with the Illumina index adaptor primers 341F (5'-CCTACGGGNGGCWGCAG-3'), 805R (5'-GACTACHVGGGTATCTAATCC-3') and run on an Illumina MiSeq platform. QIIME2 (Bolyen *et al.* 2019) was used to classify bacterial reads from digesta communities. Raw reads were denoised with DADA 2 (Callahan *et al.* 2016), and representative sequences and ASVs were generated. A phylogenetic tree of ASVs sequences was generated, and the taxonomy of each ASV was identified by using a machine learning classifier pre-trained with the reference SILVA 132 database (silva-132-99-341-806-nb-classifier.qza). Alpha diversity metrics including number of taxa observed, Pielou's evenness, Shannon's index of diversity, and the Faith's index were calculated. The phyloseq package (version 1.28.0) of R version 3.6.1 was used to evaluate beta-diversity with a principal coordinate analysis (PCoA) of the calculated unweighted and weighted UniFrac distances, generating ordination plots. Detection of differential abundance between tissues was done with Gneiss in QIIME2 (Morton *et al.* 2017).

### 3.2.13 Metabolomics

Samples from the right medial lobe of liver were collected, fast frozen in liquid nitrogen and stored at -80°C. In order to extract water-soluble metabolites, samples were thawed on ice, 100 mg of tissue

was mixed with 4 ml $g^{-1}$ of methanol and 1.6 ml $g^{-1}$ of deionized $H_2O$, and vortexed (high setting) until thoroughly mixed. Samples were then homogenized with a TissueLyser LT (Qiagen Inc.) and 5-mm-diam stainless steel beads for 5 min at 50 Hz, vortexed for 1 min, and process repeated two times. Chloroform (4 ml $g^{-1}$) and deionized $H_2O$ (4 ml $g^{-1}$) were added to the homogenate and mixed thoroughly by vortexing. Samples were kept at 4°C for 15 min, centrifuged at 4°C for 15 min at 1,000 x g, the supernatant (600 µl) transferred to a new tube, and the tube with the lid removed was maintained in an operating fume hood for 4 days to allow the solvents to evaporate. The remaining pellet was resuspended in 480 µl of metabolomics buffer (0.125 M $KH_2PO_4$, 0.5 M $K_2HPO_4$, 0.00375 M $NaN_3$, and 0.375 M KF; pH 7.4). A 120 µl aliquot of deuterium oxide containing 0.05% v/v trimethylsilylpropanoic acid (TMSP) was added to each sample (final total volume of 600 µl); TMPS was used as a chemical shift reference for 1H-NMR spectroscopy. A 550 µl aliquot was then loaded into a 5 mm NMR tube and run on a 700 MHz Bruker Avance III HD spectrometer (Bruker, ON, Canada) for spectral collection. Data acquisition and processing were followed as previously described (Paxman *et al.* 2018).

**3.2.14 Statistical analysis**

Statistical analyses for gene and protein expression, histopathologic measurements, *S.* Typhimurium quantification, cell populations and immune protein quantification were performed using Statistical Analysis Software (SAS Institute Inc. Cary, NC). With the exception of histopathologic data, normality was confirmed, and data was analyzed using the MIXED procedure of SAS. In the event of a main effect ($P \leq 0.050$), the least squares means test was used to compare treatments within factors. Histopathologic measurement data was analyzed using the pairwise Fisher's exact test in SAS. Data is represented by mean ± standard error of the mean (SEM).

Metabolomics NMR spectra were exported to MATLAB (Math Works, MA, USA) where they underwent spectral peak alignment and binning using Recursive Segment Wise Peak Alignment (Veselkov *et al.* 2009) and Dynamic Adaptive Binning (Anderson *et al.* 2010), respectively. After these analyses the dataset was then normalized to the total metabolome, excluding the region containing the water peak, and pareto scaled. The MetaboanalystR package was used to perform univariate and multivariate statistics including calculation of fold changes of specific metabolites, principal component analysis, heat map creation, and hierarchical clustering analysis (Chong *et al.* 2019). These tests were carried out using the bins identified as significant by univariate tests in order to observe group separation. Univariate measures included the t-test and the Mann-Whitney U test. Both tests determine if there is a significant difference between the means of the two groups; however, the t-test and the Mann-Whitney U test are applied in the case where the data is normally distributed (parametric) or not,

respectively. The test for data normality was carried out using a decision tree algorithm as described by Goodpaster *et al.* (2010). All p-values obtained from analysis were Bonferroni-Holm corrected for multiple comparisons. Metabolites were then identified using Chenomx 8.2 NMR Suite (Chenomx Inc., AB, Canada).

### 3.3 Results

#### 3.3.1 mCRAMP modulated histopathologic damage caused by *Salmonella* Typhimurium

Histopathologic injury was observed in the intestines of SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, and SA+/ST+/mCRAMP$^{+/+}$). In the ileum, cecum, and colon, histopathological changes were higher (P≤0.004) at 48 hours post-inoculation (hpi) than at 24 hpi. Although intestinal damage extended distally from ileum to the colon, the highest level of intestinal damage was observed within the cecum (Figure 3.1-3.2). In the cecum, equivalent damage (P=0.680) was observed in SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{+/+}$ mice. Although less intestinal damage was observed in SA+/ST-/mCRAMP$^{-/-}$ than in SA+/ST+/mCRAMP$^{-/-}$ mice (P=0.050), these animals showed conspicuously higher (P<0.001) histopathological scores than in SA+/ST-/mCRAMP$^{+/+}$ mice. When mice were not pretreated with streptomycin, substantive leukocyte infiltration, epithelial hyperplasia, goblet cell loss, cryptitis, irregular crypts, crypt loss, and epithelial injury were observed only in SA+/ST-/mCRAMP$^{-/-}$ mice (Figure 3.2C-D).

#### 3.3.2 mCRAMP influenced the immune response triggered by *Salmonella* Typhimurium

Differential mRNA expression was evaluated in ileal and cecal samples of mice. In the ileum, *Kc* (P<0.001), *Inos* (P<0.001), *Il10* (P=0.004), *RegIIIγ* (P<0.001), and *Il18* (P=0.002) were upregulated in all SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{+/+}$) at 48 hpi (*data not shown*). In the cecum at 48 hpi, higher (P=0.051) levels of gene expression were observed in both SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{+/+}$ mice relative to SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST-/mCRAMP$^{+/+}$ mice (Figure 3.3A-B). Upregulation of *Ifnγ* (P=0.044), *Kc* (P=0.041), *Inos* (P=0.049), *RegIIIγ* (P=0.024), *Il22* (P=0.015), and *Il1β* (P=0.045) was observed in the ceca of SA+/ST-/mCRAMP$^{-/-}$ relative to SA+/ST-/mCRAMP$^{+/+}$ mice at 48 hpi (Figure 3.3A). *Il10* (P=0.051) and *Il1β* (P=0.049) were upregulated in SA+/ST+/mCRAMP$^{-/-}$ relative to SA+/ST+/mCRAMP$^{+/+}$ mice (Figure 3.3B). A higher expression of *Ifnγ* (P≤0.014), *Kc* (P≤0.007), *Inos* (P≤0.010), *RegIIIγ* (P≤0.006), *Il22* (P≤0.038), *Il10* (P≤0.001), *Tlr4* (P≤0.002), and *Il1β* (P≤0.018) was observed in SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST-/mCRAMP$^{-/-}$ mice relative to SA-/ST-/mCRAMP$^{-/-}$ (Figure 3.4A). Only SA+/ST+/mCRAMP$^{+/+}$ mice showed a higher expression of *Ifnγ* (P=0.048), *Kc* (P<0.001), *Inos* (P<0.001), and *Il1β* (P=0.002) relative to SA-/ST-/mCRAMP$^{+/+}$ animals (Figure 3.4B). Moreover, no difference (P≥0.530) was observed in expression of

immune genes among SA+/ST-/mCRAMP$^{+/+}$, SA-/ST+/mCRAMP$^{+/+}$, SA-/ST-/mCRAMP$^{+/+}$, SA-/ST+/mCRAMP$^{-/-}$, and SA-/ST-/mCRAMP$^{-/-}$ mice. Relative expression of *mCramp* was evaluated in the ileum and cecum at 24 and 48 hpi, and no expression of the gene was observed in mCRAMP$^{-/-}$ mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA+/ST-/ mCRAMP$^{-/-}$, and SA+/ST+/ mCRAMP$^{-/-}$)(Figure 3.5). At 24 hpi, no differences (P≤0.907) in levels of expression of *mCramp* were observed in ileum or cecum of SA-/ST-/mCRAMP$^{+/+}$, SA-/ST+/mCRAMP$^{+/+}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$ mice (*data not shown*). However, higher expression (P=0.001) of *mCramp* was observed in the cecum of SA+/ST-/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{+/+}$ mice as compared to SA-/ST-/mCRAMP$^{+/+}$ and SA-/ST+/mCRAMP$^{+/+}$ mice at 48 hpi (Figure 3.5). No significant differences (P≥0.266) were observed among treatments in the expression of *Il4, Tgfβ, Tlr2, Tlr5, Zo1*, *Occludin* and *Muc2*.

### 3.3.3 Streptomycin and mCRAMP modified *Salmonella* Typhimurium densities within digesta and associated with mucosa in the cecum, but not in the ileum, proximal colon, or liver

No *S.* Typhimurium was isolated from digesta, mucosa, or liver samples of SA- mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, and SA-/ST+/mCRAMP$^{+/+}$). In contrast, the pathogen was isolated from all collected tissues of SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$), with the highest densities (P=0.057) observed in digesta and associated with mucosa of the cecum at 24 hpi. Densities of *S.* Typhimurium within digesta and associated with the mucosa in the ileum, cecum, and proximal colon tended to decrease between the 24 and 48 hpi end points (*data not shown*). In contrast, densities of the bacterium in the liver increased (P=0.011) between the 24 and 48 hpi end points (*data not shown*). Higher densities of *S.* Typhimurium (P≤0.001) were observed in SA+/ST+/mCRAMP$^{-/-}$and SA+/ST+/mCRAMP$^{+/+}$ mice as compared to SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST-/mCRAMP$^{+/+}$ mice (Figure 3.6). No differences (P≥0.418) were observed in *S.* Typhimurium densities between SA+/ST+/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{-/-}$ mice at either 24 or 48 hpi. In contrast, higher (P≤0.017) densities of *S.* Typhimurium were observed in SA+/ST-/mCRAMP$^{-/-}$ as compared to SA+/ST-/mCRAMP$^{+/+}$ mice at 24 hpi (Figure 3.6A-B). There was no effect (P≥0.909) of mCRAMP or antibiotic administration on densities of *S*. Typhimurium within the ileum (*data not shown*). In contrast, higher densities (P<0.001) of *S.* Typhimurium were observed in proximal colon of SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{+/+}$ mice (*data not shown*). No differences (P≥0.381) were observed in densities of *S*. Typhimurium in the liver among SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) (*data not shown*).

### 3.3.4 *Salmonella* Typhimurium infection modified immune proteins in the small intestine, and serum

Cytokines and chemokine concentrations were measured in serum as well as in the liver and ileal tissues. In the ileum, higher concentrations (P≤0.004) of myeloperoxidase (MPO) and keratinocyte-derived chemokine (KC) were observed in SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) (Figure 3.7), and concentrations increased (P≤0.025) at 48 hpi. No differences (P≥0.729) in the concentrations of MPO or KC were observed between SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST-/mCRAMP$^{-/-}$ mice. In contrast, a higher (P=0.020) concentration of KC was observed in the ileum of SA+/ST-/mCRAMP$^{-/-}$ mice as compared to SA+/ST-/mCRAMP$^{+/+}$ mice at 48 hpi. In the serum, higher (P≤0.001) concentrations of MPO were observed in SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$), and concentrations were higher (P≤0.007) at 48 hpi (Figure 3.8). mCRAMP was not detected in the serum or ileum of mCRAMP$^{-/-}$ mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{-/-}$, and SA+/ST+/mCRAMP$^{-/-}$). No differences (P≥0.991) were observed in mCRAMP concentration in serum or ileum among mCRAMP$^{+/+}$ mice (i.e. SA-/ST-/mCRAMP$^{+/+}$, SA-/ST+/mCRAMP$^{+/+}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) (*data not shown*). In the liver, no differences (P≥0.113) in the concentrations of IFNγ, MIP2, TNFα, IL10, IL2, GM-CSF, and Active-TGFβ were observed among any of the treatments (*data not shown*).

### 3.3.5 Splenic immune cell populations varied over time

Immune cell populations within the spleen were comparatively analyzed for proportions of T cell subsets, NK cells, and differentiated monocytes and neutrophils. The proportion of splenic CD45$^{+}$ leukocytes expressing CD18 in mCRAMP$^{-/-}$ mice at 24 hpi showed no difference (P≥0.482) among treatments (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{-/-}$, and SA+/ST+/mCRAMP$^{-/-}$) (Figure 3.9C). In contrast, at 24 hpi, the population of CD45$^{+}$ leukocytes expressing CD18 was reduced (P≤0.035) in SA+/ST+/mCRAMP$^{+/+}$ and SA-/ST+/mCRAMP$^{+/+}$ mice (Figure 3.9C). At 48 hpi, this same population was conspicuously reduced (P≤0.014) in SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) as compared to SA- mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, and SA-/ST+/mCRAMP$^{+/+}$) (Figure 3.9D). The percentage of CD18$^{+}$CD11b$^{+}$ leukocytes increased (P≤0.001) in SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) relative to SA- mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, and SA-/ST+/mCRAMP$^{+/+}$) at 24 and 48 hpi (Figure 3.9A-B). The proportion of these leukocytes was reduced (P≤0.023) at 48 hpi relative to 24 hpi

independent of treatment (Figure 3.9A-B). The percentage of $CD18^{+}CD11b^{+}$ $Ly\text{-}6C^{+}Ly\text{-}6G^{+}$ neutrophils increased ($P \leq 0.012$) in SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) relative to SA- mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, and SA-/ST+/mCRAMP$^{+/+}$) (Figure 3.9E-F). No differences ($P \geq 0.164$) were observed in other immune cell populations (Table 3.2).

### 3.3.6 The liver metabolite profile was modified by *Salmonella* Typhimurium infection

Water-soluble metabolites were extracted from the right medial lobe of the liver and analyzed by H-Nuclear Magnetic Resonance (NMR) spectroscopy to evaluate changes in the metabolome associated with streptomycin administration, *Salmonella* infection, and absence of mCRAMP. A comparison between 24 hpi and 48 hpi showed no differences among SA-/ST-/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, SA-/ST+/mCRAMP$^{-/-}$, and SA-/ST+/mCRAMP$^{+/+}$ mice (*data not shown*). There was no separation in metabolite profiles between SA-/ST-/mCRAMP$^{-/-}$ and SA-/ST-/mCRAMP$^{+/+}$ mice (Figure 3.10A). There was also no separation in profiles between SA-/ST+/mCRAMP$^{-/-}$ and SA-/ST+/mCRAMP$^{+/+}$ mice (Figure 3.10B). In contrast, SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST-/mCRAMP$^{+/+}$ mice showed separation in metabolite profiles at 24 hpi (Figure 3.10C), but not at 48 hpi. In this regard, increases of cadaverine ($P=0.022$), taurine ($P=0.047$), valine ($P=0.037$), and leucine ($P=0.038$) were observed in SA+/ST-/mCRAMP$^{-/-}$ at 24 hpi (Figure 3.11A). A temporal comparison of metabolite profiles in SA+/ST-/mCRAMP$^{+/+}$ mice showed increases of phenylalanine ($P=0.028$), taurine ($P=0.003$), cadaverine ($P=0.014$), and carnitine ($P=0.030$) at 48 hpi relative to 24 hpi (Figure 3.11B). Metabolic profiles of SA+/ST+/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{-/-}$ mice showed separation at 24 hpi (Figure 3.10D). Evaluation of specific metabolites showed increases of taurine ($P \leq 0.033$) and carnitine ($P \leq 0.010$) in SA+/ST+/mCRAMP$^{-/-}$ mice at 24 and 48 hpi as compared to SA+/ST+/mCRAMP$^{+/+}$ (*data not shown*). Evaluation of metabolite profiles over time in SA+/ST+/mCRAMP$^{-/-}$ mice showed more alterations at 48 hpi, including increases in phenylalanine ($P=0.026$), taurine ($P=0.013$), cadaverine ($P=0.028$), and carnitine ($P=0.004$) (Figure 3.11C).

### 3.3.7 The composition of the bacterial community, but not diversity in cecal digesta, was subtly different in mCRAMP-knockout mice not inoculated with *Salmonella* Typhimurium or administered streptomycin sulfate

Characterization of bacterial communities in the cecum digesta was carried out by next generation sequencing (NGS) using an Illumina MiSeq platform. The composition of the microbiota differed subtly between SA-/ST-/mCRAMP$^{+/+}$ and SA-/ST-/mCRAMP$^{-/-}$ mice. The microbiota was dominated by *Firmicutes*, representing 51.3% and 69.2% of the community for SA-/ST-/mCRAMP$^{+/+}$ and SA-/ST-/mCRAMP$^{-/-}$ mice, respectively (Figure 3.12A). A higher relative abundance of *Bacteroidetes* was

observed in SA-/ST-/mCRAMP$^{+/+}$ mice (34.3%) as compared to SA-/ST-/mCRAMP$^{-/-}$ mice (7.1%) (Figure 3.12A). At the family level of resolution, these differences were reflected by a higher relative abundance of *Bacteroideaceae* in SA-/ST-/mCRAMP$^{+/+}$ mice and a higher abundance of *Muribaculaceae* in SA-/ST-/mCRAMP$^{-/-}$ mice (Figure 3.12B).

There were no differences in Shannon's index of alpha diversity, Pielou's evenness, number of amplicon sequence variants (ASVs) or Faith phylogenetic diversity between SA-/ST-/mCRAMP$^{-/-}$ and SA-/ST-/mCRAMP$^{+/+}$ mice at either 24 hpi (P≥0.248) or 48 hpi (P≥0.126) (Figure 3.13). Additionally, there were no differences in beta diversity as evaluated by unweighted and weighted principal coordinate analysis between SA-/ST-/mCRAMP$^{-/-}$ and SA-/ST-/mCRAMP$^{+/+}$ mice at 24 hpi (P≥0.201) or 48 hpi (P≥0.175) (Figure 3.14).

### 3.3.8 Administration of streptomycin sulfate modified the composition and diversity of the cecal digesta microbiota in mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice

The administration of streptomycin sulfate conspicuously affected both the diversity and composition of the enteric microbiota. ASV counts (P≤0.006), Shannon's index (P<0.003) (Figure 3.13), Pielou's evenness (P<0.003), and Faith phylogenetic diversity (P≤0.010) were reduced in ST+ mice (i.e. SA-/ST+/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) as compared to ST- mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, and SA+/ST-/mCRAMP$^{+/+}$). Additionally, weighted (P≤0.009) and unweighted (P≤0.008) (Figure 3.14) principal coordinate analysis showed differences between ST+ mice (i.e. SA-/ST+/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) with ST- mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, and SA+/ST-/mCRAMP$^{+/+}$). At a phyla level of resolution, SA-/ST+/mCRAMP$^{+/+}$ mice showed a decrease in the relative abundance of *Bacteroidetes* and *Firmicutes* with an increase in the relative abundance of *Proteobacteria* and *Verrucomicrobia* (Figure 3.12A). In contrast, SA-/ST+/mCRAMP$^{-/-}$ mice exhibited a higher abundance of *Firmicutes* and *Verrucomicrobia* phyla (Figure 3.12A). SA+/ST+/mCRAMP$^{-/-}$ mice showed a conspicuous shift in the composition of the microbiota, with a higher abundance of *Proteobacteria* (97.5%) as compared to SA+/ST+/mCRAMP$^{+/+}$ mice (65%) (Figure 3.12A). All of the bacterial ASV's identified as *Proteobacteria* in infected mice were members of the *Enterobacteriaceae* (Figure 3.12B). No differences (P≥0.295) were observed in alpha or beta diversity between SA+/ST+/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{-/-}$ mice.

### 3.3.9 Bacterial communities differed between mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice infected with *Salmonella* Typhimurium and not administered streptomycin

Gneiss analysis revealed that bacterial communities in the ceca of SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST-/mCRAMP$^{+/+}$ mice differed. In this regard, SA+/ST-/mCRAMP$^{-/-}$ mice had 32 taxa that differed in cecal abundance in comparison to SA+/ST-/mCRAMP$^{+/+}$ mice. Of these taxa, *Akkermansia* and *Anaeroplasma* were conspicuously more abundant in SA+/ST-/mCRAMP$^{-/-}$ mice (Figure 3.12B). Differences in alpha diversity corresponded to bacterial composition; SA+/ST-/mCRAMP$^{+/+}$ mice exhibited a higher Shannon diversity index (P=0.017) (Figure 3.13) and Faith's phylogeny diversity (P=0.017) than SA+/ST-/mCRAMP$^{-/-}$ mice. In addition, a difference in beta diversity was observed between SA+/ST-/mCRAMP$^{+/+}$ and SA+/ST-/mCRAMP$^{-/-}$ mice for both unweighted UniFrac principal coordinate analysis (P=0.003) (Figure 3.14), as well as weighted UniFrac principal coordinate analysis (P=0.003).

## 3.4 Discussion

The protective role of host-defense peptides has been widely studied (Chromek *et al.* 2006; Fan *et al.* 2015; Gallo *et al.* 2012). Murine cathelicidin-related antimicrobial peptide (mCRAMP) has been shown to impair infection by invasive pathogens within the skin (Nizet *et al.* 2001), urinary tract (Chromek *et al.* 2006), and gastrointestinal tract (Iimura *et al.* 2005). *In vitro* studies have shown an antimicrobial effect of mCRAMP directed to *S.* Typhimurium (Gallo *et al.* 1997), and additionally, the intracellular expression of this cathelicidin in macrophages has been shown to impair *S.* Typhimurium replication (Rosenberger CM *et al.* 2004). However, no studies to my knowledge have been conducted *in vivo* to evaluate the interplay among cathelicidin, the host, the microbiota, and *S.* Typhimurium. Since secretion of mCRAMP has been reported to modulate the composition of the enteric microbiota (Yoshimura *et al.* 2018), understanding the role that cathelicidin has on the host-pathogen-microbiota interaction is required to evaluate its role in colonization resistance. In the current study, I inoculated mice with a highly virulent *S.* Typhimurium DT104 strain (Yin *et al.* 2014) or buffer alone (i.e. uninfected control treatment). To ascertain the role that mCRAMP has on salmonellosis, I temporally evaluated immune responses, the structure of the enteric microbiota, and metabolome of the liver in mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice ± oral administration of streptomycin sulfate.

Treatment of mice with streptomycin sulfate to create a dysbiosis is required to generate salmonellosis that is characteristically similar to enteric inflammation observed in human beings (Uzzau *et al.* 2000). Importantly, I observed that SA+/ST+ mice (i.e. SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{+/+}$) developed enteric salmonellosis. Intestinal damage was more prominent in the large intestine, especially in the cecum, which is consistent with previous studies (Stecher *et al.* 2005).

At 48 h post-inoculation, the architecture of the cecal mucosa was conspicuously disrupted in SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{+/+}$ mice, which included epithelial hyperplasia, reduced numbers of goblet cells, epithelial injury, and marked polymorphonuclear infiltration of the submucosa, lamina propria and epithelial layer. These histopathologic changes to cecal tissues have previously been described in dysbiotic mice infected with the bacterium (Barthel *et al.* 2003). Significantly, I observed the same degree of intestinal damage in SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST+/ mCRAMP$^{-/-}$ mice relative to SA+/ST+/mCRAMP$^{+/+}$ mice, which suggested that mCRAMP-deficient mice were more susceptible to the bacterium.

Cathelicidins have been shown to participate in host immune responses by inducing chemoattraction of granulocytes or by enhancing the adaptive immune system (Kurosaka *et al.* 2005). Therefore, evaluation of immune marker modulation is essential to understand *S.* Typhimurium infection and the role of cathelicidin in this enteric disease. Although the immune responses triggered after infection by *S.* Typhimurium in mice have been previously described (Broz *et al.* 2012; Stecher *et al.* 2007), to the best of my knowledge no studies have yet been conducted to describe the role of mCRAMP on immune responses developed locally (intestine) and systemically (spleen and liver) due to infection by *S*. Typhimurium. Moreover, I characterized immune responses at gene, protein, and cellular levels. Expression of RNA transcripts were evaluated in T cells including Th1 (*Ifnγ*), Th2 (*Il4*), Th17 (*Il22*) and T-regulatory (*Il10, Tgfβ*) cells. In addition, I assessed the expression of cytokines mediated by phagocytes (*Il1ß, Inos, Il1β*), secretion of host defense peptides (*RegIIIγ*, *mCramp*), regulation of pattern recognition receptors (*Tlr2, Tlr4, Tlr5*), and functionality of the epithelial barrier (*Kc, Zo1, Occludin, Muc2*). The greatest histopathologic changes were observed in the cecum, and thus I evaluated mRNA expression in the cecum as well as in the ileum. Upregulation of immune markers was observed in the cecum of SA+/ST+/mCRAMP$^{+/+}$, SA+/ST-/mCRAMP$^{-/-}$, and SA+/ST+/mCRAMP$^{-/-}$ mice at 48 hpi. More specifically, I observed upregulation of the pattern recognition receptor *Tlr4*, which plays an essential role in recognition of *Salmonella* lipopolysaccharide and the consequent elimination of the pathogen (Arpaia *et al.* 2011). At 48 hpi, pro-inflammatory cytokines were highly upregulated in the cecal mucosa of SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$). Higher expression of *Il1ß, Ifnγ, Kc,* and *Il22* were identified in both SA+/ST+/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{-/-}$ mice. Additionally, SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{-/-}$ mice showed higher expression of these cytokines relative to SA+/ST-/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{+/+}$ mice. Higher expression of *Il1ß, Ifnγ, Kc,* and *Il22* was also observed in SA+/ST-/mCRAMP$^{-/-}$ as compared to SA+/ST-/mCRAMP$^{+/+}$ mice . The higher expression of these cytokines in

SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{-/-}$ correlates with the severe injury observed in the cecum of these mice, further supporting an increased susceptibility of mCRAMP-deficient mice to salmonellosis. Mice deficient in IL-1β succumb more easily to *Salmonella* infection, highlighting the importance of this cytokine in the mucosal immune response against this pathogen (Raupach *et al.* 2006). Upregulation of *Ifnγ* and *Il22* have been previously reported in *S.* Typhimurium infected mice that were pretreated with streptomycin, illustrating the pivotal role they play in the amplification of the intestinal immune response (Godinez *et al.* 2008). This amplification is mainly associated with neutrophil chemoattraction to the site of infection (Raffatellu *et al.* 2008) following the release of keratinocyte-derived chemokine (*Kc*) (Dubin *et al.* 2007), and secretion of nitric oxide synthase (*Inos*) (Godinez *et al.* 2008). In the current study, I observed upregulation of both *Kc* and *Inos* in all SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$). However, higher levels of both cytokines were observed in SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{-/-}$ mice as compared to SA+/ST-/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{+/+}$ mice. Elevated presence of neutrophils in the submucosa, lamina propria, and epithelial layer were observed in SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, and SA+/ST+/mCRAMP$^{+/+}$), which corresponds with the higher expression of the chemoattractant *Kc* observed. No differences between SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) and SA- mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, and SA-/ST+/mCRAMP$^{+/+}$) were observed in *Il18* expression in the current study. Although this cytokine can have an essential role in the host resistance to the serotype Typhimurium, this function is mainly associated to the systemic phase of the disease, particularly in splenic and liver tissues (Raupach *et al.* 2006).

Higher expression of *mCramp* was observed in the cecum of SA+/ST-/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{+/+}$ mice at 48 hpi. Local expression of *mCramp* has been described to be limited to the colonic epithelium (Gallo *et al.* 1997). However, I was able to demonstrate the presence of this antimicrobial peptide in the cecal and ileal mucosa, with the former being the location where salmonellosis was primarily manifested. The secretion of mCRAMP by neutrophils has been previously reported (Gallo *et al.* 1997). Therefore, the higher expression that I observed at 48 hpi as compared to 24 hpi is likely associated with the elevated cecal infiltration of neutrophils observed in SA+/ST+/mCRAMP$^{+/+}$ mice. Additionally, I observed upregulation of *RegIIIγ* in the cecum of all SA+ mice with higher expression in SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{-/-}$ mice as compared to SA+/ST-/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{+/+}$ mice. Higher expression of this host defense peptide in response to *Salmonella* infection has been previously described (Godinez *et al.* 2009). Interestingly, murine RegIII

lectin family has been described to enhance *Salmonella* infection by altering the mouse microbiota and modifying the gut luminal metabolite profile (Miki *et al.* 2017). In addition to proinflammatory responses, I also conducted evaluation of anti-inflammatory cytokines. I showed that SA+ mice (SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) had a higher expression of *Il10* in the cecal mucosa at 48 hpi. The upregulation of this cytokine could be likely directed to control the exacerbated inflammatory response triggered by the pathogen (Artis *et al.* 2008).

Proteins involved in immune function were examined in the ileum and in serum, which showed that keratinocyte-derived chemokine (KC) and myeloperoxidase (MPO) were highly concentrated in ileum of SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) at 24 hpi, and increased over time. Similar results were observed for MPO in the serum of SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$). The higher concentration of MPO observed in SA+ mice can be directly associated with *S.* Typhimurium infection, since this enzyme produces hypochlorous acid (HOCl), a key cytotoxic antimicrobial product released by neutrophils against the pathogen (Trivedi *et al.* 2015). Additionally, the higher concentration of both proteins observed in the intestine at 48 hpi likely correlates to the higher infiltration of neutrophils observed at this time point. Elevated serum levels of MPO could also be associated with the higher load of *Salmonella* travelling through the blood at this time, which is consistent with previous studies (Barthel *et al.* 2003).

In ST- mice, *Salmonella* Typhimurium colonizes Peyer's patches and mesenteric lymph nodes, and then quickly spreads to the spleen and liver at which point the infection is considered systemic (Carter *et al.* 1974; Hohmann *et al.* 1978). A previous study showed that mice administered streptomycin prior to *S.* Typhimurium inoculation present extensive infiltration of CD18$^{+}$ cells into the lamina propria, and this flux of cells exhibiting a phagocytic/antigen-presenting cell phenotype has been suggested as a mechanism by which the pathogen breaches the intestinal barrier to incite a systemic response (Barthel *et al.* 2003). In the current study, the overall splenic population of CD18$^{+}$ leukocytes decreased in SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{+/+}$ mice at 48 hpi, suggesting this population migrated from the spleen in both genotypes. The CD18$^{+}$CD11b$^{+}$ phenotype represents inflammatory cells capable of phagocytosis and antigen presentation, and this combination of cell surface receptors is required for the process of extravasation (Nunes *et al.* 2010; Spees *et al.* 2014). The CD18$^{+}$CD11b$^{+}$ cell phenotype was higher in SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$), and splenic populations decreased at 48 hpi, further suggesting migration of CD18$^{+}$CD11b$^{+}$ cells from the spleen. Overall, SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$,

SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) maintained higher proportions of neutrophils than SA- mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, and SA-/ST+/mCRAMP$^{+/+}$), further demonstrating the impact of the infection on immune cell populations in the spleen. After 48 hpi, minimal differences were observed between the splenic immune cell populations of SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$ mice, suggesting the pretreatment with streptomycin and infection by *S*. Typhimurium had greater influences on splenic cell population structure than the presence of functional cathelicidin.

*Salmonella* Typhimurium densities directly correspond to histopathological changes and to the modification of the immune responses that were observed in the current study. Higher densities of the pathogen were observed at 24 hpi in the digesta and cecum mucosa of SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$), decreasing over time. Disruption of the mucosal layer is associated with higher internalization of the bacterium into the blood stream (Barthel *et al.* 2003; Que *et al.* 1985). Therefore, the lower densities that I observed in the digesta and mucosa at 48 hpi could be directly associated with systemic dissemination (Barthel *et al.* 2003). This is also in line with the increment of pathogen densities that I observed in the liver of SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$) at 48 hpi. Additionally, the lower bacterial densities observed at 48 hpi in the mucosa of SA+ mice could also be the consequence of the immune response already established against the pathogen (Broz *et al.* 2012).

I used metabolomics to characterize the effects of *S.* Typhimurium infection and streptomycin administration on the liver due to systemic infection, and importantly, to determine the role that mCRAMP plays in these changes. Results showed that the liver metabolome of SA- mice (i.e. SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, and SA-/ST+/mCRAMP$^{+/+}$) did not differ. In contrast, the liver metabolome differed significantly in SA+ mice (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$). When the microbiota is not altered by a broad spectrum antibiotic, naïve mice inoculated with *S.* Typhimurium develop a typhoid-like disease (Santos RL *et al.* 2001), characterized by parenchymal necrosis of the liver and spleen (Nakoneczna (1980)). To address if SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST-/mCRAMP$^{+/+}$ mice differed in the development of typhoid-like fever, I compared their liver metabolic profiles. I observed that the liver metabolome of SA+/ST-/mCRAMP$^{-/-}$ mice was conspicuously more affected than SA+/ST-/mCRAMP$^{+/+}$ mice at 24 hpi, but the difference between the treatments abated by 48 hpi. SA+/ST+/mCRAMP$^{-/-}$ mice also showed a higher degree of change in the liver metabolome relative to SA+/ST+/mCRAMP$^{+/+}$ at 24 and 48 hpi. Moreover, an increase in valine, leucine, taurine, and cadaverine was observed in the livers

of SA+/ST-/mCRAMP$^{-/-}$ relative to SA+/ST-/mCRAMP$^{+/+}$ mice at 24 hpi. While lower plasma levels of branched-chain amino-acids (e.g. valine, leucine) have previously been reported in mice with septicemia, such as typhoid fever (Vente *et al.* 1989; Wannemacher *et al.* 1977), the same amino acids tend to increase in liver under infection (Wannemacher *et al.* 1971). An elevated concentration of taurine in the intestine is associated with higher levels of oxidants, and this may aid in preventing tissue injury under conditions of inflammation (Mochizuki *et al.* 2004; Schuller-Levis *et al.* 2004; Sukhotnik *et al.* 2016). A study conducted in intestinal epithelial cells showed a correlation between the production of the polyamine cadaverine and inhibition of polymorphonuclear transmigration induced by *Shigella* infection via avoidance of the immune response reaction triggered by epithelial cells (Fernandez *et al.* 2001). Additionally, the production of piperidine, a metabolite produced from cadaverine, has been described to inhibit intestinal disease incited by *S.* Typhimurium (Kohler *et al.* 2002). *Salmonella* dissemination and liver damage triggered by the bacterium progress over time (Que *et al.* 1985), and the significant time effect that I observed in the liver metabolome is consistent with this finding. More specifically, I observed that SA+/ST-/mCRAMP$^{+/+}$ and SA+/ST+/mCRAMP$^{-/-}$ mice exhibited higher levels of phenylalanine, taurine, cadaverine, and carnitine at 48 hpi relative to the same treatments at 24 hpi. The concentration of phenylalanine in livers has previously been described to increase during periods of bacterial infection (Wannemacher *et al.* 1977; Wannemacher *et al.* 1976). This could be explained by an increase in the uptake of phenylalanine from the liver to produce acute phase proteins (Wannemacher 1977; Barnes *et al.* 2002). Carnitine is an essential component of cellular metabolism in charge of transporting activated long-chain fatty acids across the mitochondrial membrane for β-oxidation (Reuter *et al.* 2012). However, this metabolite has also been observed to modulate polymorphonuclear activities and the production of radical oxygen species (Fattorossi *et al.* 1993). The alterations to the liver metabolome that I observed in the current study are consistent with my hypothesis that the absence of mCRAMP increases susceptibility to *Salmonella* infection.

The primary mouse model used to study *S.* Typhimurium enterocolitis in human beings is based on the disruption of the microbiota by streptomycin sulfate (Barthel *et al.* 2003). As such, a principal goal of my study was to comparatively characterize the composition of the microbiota of mice receiving different treatments (i.e. SA+/ST-/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA+/ST-/mCRAMP$^{+/+}$, SA+/ST+/mCRAMP$^{+/+}$, SA-/ST-/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{-/-}$, SA-/ST-/mCRAMP$^{+/+}$, and SA-/ST+/mCRAMP$^{+/+}$). To achieve this goal, NGS analysis of the microbiota in the digesta of the cecum, the intestinal region with the highest levels of inflammation was conducted. A comparison of SA-/ST-/mCRAMP$^{+/+}$ and SA-/ST-/mCRAMP$^{-/-}$ mice revealed no difference in diversity, but a subtle difference in

the composition of the microbiota between genotypes, which was attributed to the requisite segregated housing of the two genotypes. In contrast, substantive alterations in the composition and diversity of the microbiota were observed in ST+ mice (i.e. SA-/ST+/mCRAMP$^{-/-}$, SA+/ST+/mCRAMP$^{-/-}$, SA-/ST+/mCRAMP$^{+/+}$, and SA+/ST+/mCRAMP$^{+/+}$), as has been reported previously (Stecher *et al.* 2007). In SA+/ST+/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{+/+}$ mice, the bacterial community was dominated by the phylum, *Proteobacteria*, and family, *Enterobacteriaceae*, as expected. This was attributable to the dysbiosis generated by streptomycin and the ensuing loss of colonization resistance, which primarily benefits members of the *Proteobacteria* including *Salmonella* (Stecher *et al.* 2007). The disproportionate increase of *Proteobacteria* is a hallmark of dysbiosis and epithelial injury (Litvak *et al.* 2017); higher levels of oxygen in the intestinal lumen are normally found in inflamed tissues (Rigottier-Gois *et al.* 2013) attributable to reactive oxygen species and oxidative burst established by neutrophils under infection (Winterbourn *et al.* 2016). The diversity of bacteria observed in the cecal digesta of SA+/ST-/mCRAMP$^{-/-}$ mice was reduced relative to SA+/ST-/mCRAMP$^{+/+}$, which I attributed to the higher levels of mucosal inflammation and an ensuing increase in oxygen that benefited *Proteobacteria* over obligate anaerobes (Winter *et al.* 2010). It has previously been proposed that *Salmonella* induces inflammation to reduce colonization resistance, thereby allowing it to colonize the intestine and infect the host (Lupp *et al.* 2007). The higher abundance of *Enterobacteriaceae*, the increased degree of inflammation, and the severity of mucosal damage observed in the cecum of SA+/ST-/mCRAMP$^{-/-}$ mice could be the result of direct or indirect changes, or both, associated with the mCRAMP deficiency.

The mechanisms of colonization resistance delivered by the enteric microbiota are essential to the well being of the host. When studying an intestinal pathology, the presence of a balanced microbiota is crucial to elucidate the complex interactions among the host, the autochthonous microbiota, and the pathogen (Lawley *et al.* 2013). Although the mechanisms of colonization resistance are enigmatic at present, the putative mechanisms have been divided into direct and indirect competition (Sassone-Corsi *et al.* 2015). Indirect mechanisms of colonization resistance include production of short-chain fatty acids (den Besten *et al.* 2013), modulation of cytokine release (Hasegawa *et al.* 2012), and stimulation of cathelicidin secretion (Fan *et al.* 2015; Iimura *et al.* 2005) to avoid colonization. Additionally, the endogenous production of mCRAMP has also been observed to play an essential role in maintaining a balanced microbiota (i.e. a homeostasis) in the colon (Yoshimura *et al.* 2018). In the current study, I observed that alpha and beta diversity of the enteric microbiota did not differ between SA-/ST-/mCRAMP$^{-/-}$ and SA-/ST-/mCRAMP$^{+/+}$ mice. This indicates that the complex microbiota necessary to induce colonization resistance against *S.* Typhimurium (Brugiroux *et al.* 2016) was conserved in mCRAMP

deficient mice. However, in SA+/ST-/mCRAMP$^{-/-}$ mice a significantly lower Shannon diversity was detected in relation to SA+/ST-/mCRAMP$^{+/+}$ mice indicating that a deficiency of mCRAMP may increase susceptibility to *Salmonella*, at least in part, due to the altered structure of the microbiota. Mice with a defined microbiota that were inoculated with *Akkermansia muciniphila* and *S.* Typhimurium showed a higher degree of inflammation (Ganesh *et al.* 2013). This was attributed to the degradation of the mucus layer by the commensal *A. muciniphila*, which facilitated *Salmonella* attachment to the epithelium. It is noteworthy that in the current study, I observed that cecal digesta of SA+/ST-/mCRAMP$^{-/-}$ mice possessed a higher abundance of the family *Akkermansiaceae* compared to SA+/ST-/mCRAMP$^{+/+}$ mice. Since these bacteria were also present in SA+/ST-/mCRAMP$^{+/+}$ mice however, suggests that they are not entirely responsible for the predisposition to infection that I observed in mCRAMP deficient mice. The administration of mCRAMP to the distal colon of mice has been previously demonstrated to attenuate dextran sulfate sodium-induced colitis by enhancing the mucus layer (Wu *et al.* 2010). Additionally, expression of LL-37, the human homologue of mCRAMP has been observed to enhance mucus production in airways (Zhang *et al.* 2014). Therefore, the inability of mice to produce cathelicidin may have enhanced salmonellosis by directly or indirectly disrupting the mucus layer. Additionally, this disruption of the mucus layer could have benefited the observed proliferation of *Akkermansiaceae* in mCRAMP deficient mice aggravating the infection.

In conclusion, I evaluated the progression of salmonellosis in mCRAMP$^{+/+}$ and mCRAMP$^{-/-}$ mice by characterizing histopathologic changes, host immune responses, and alterations to the liver metabolome and enteric microbiota. The higher upregulation of pro-inflammatory genes (*Ifnγ, Kc, Inos, Il1β*) and the higher concentration of immune proteins (MPO, KC) that I observed in SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{-/-}$ mice are clear indications of a higher susceptibility to salmonellosis when cathelicidin is absent. Moreover, the metabolic profiles of the livers of mice infected with *S.* Typhimurium differed between the mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ genotypes. This evidence demonstrated that mCRAMP deficient mice were predisposed to *Salmonella* infection both locally and systemically. Additionally, I demonstrated that mCRAMP plays a role in host response to infection by *S.* Typhimurium by altering the microbiota. In this regard, the higher abundance of *A. muciniphila* that I observed in SA+/ST-/mCRAMP$^{-/-}$ and SA+/ST+/mCRAMP$^{-/-}$ mice could be an indicator of a disruption to the mucus layer resulting from the absence of mCRAMP. Future studies should further elucidate mechanisms, and could involve studies in which mCRAMP is delivered to sites of intestinal inflammation incited by *S.* Typhimurium in mCRAMP$^{-/-}$ mice.

### 3.5 Tables and figures

Table 3.1 Histopathologic scoring system

| Score | Inflammatory cell infiltrate | | Epithelial changes | | | Mucosal architecture | |
|---|---|---|---|---|---|---|---|
| | **Severity** | **Extent** | **Hyperplasia** | **Goblet cell loss** | **Epithelial injury** | **Irregular crypt*** | **Crypt loss** |
| 0 | normal | normal | normal | normal | none | _ | normal |
| 1 | Minimal: <10% | Mucosal | Minimal: <25% | Minimal <20% | rare (i.e. < 10 surface epithelial cells shedding) | _ | decrease of <10% |
| 2 | Mild: 10-25%; scattered neutrophils | Mucosal and submucosal | Mild: 25-35% | Mild 21-35% | mild (i.e. focal epithelial erosions; 11-50 surface epithelial cells shedding) | _ | decrease of ≥10% |
| 3 | Moderate: 26-50% | Mucosal, submucosal and transmural | Moderate: 36-50%; mitoses in middle/upper third of crypt epithelium, distant from crypt base | Moderate 36-50% | moderate (i.e. multi focal surface epithelium erosion; < 50 of surface epithelial cells shedding) | _ | |
| 4 | Marked: >51%; dense infiltrate | _ | Marked: >51%; mitoses in upper third of crypt epithelium, distant from crypt base | Marked >50% | severe (i.e. multi focal to coalescing surface epithelium erosions; >50 of surface epithelial cells shedding). | Yes | |
| 5 | _ | _ | | | | Yes | |

*****Non-parallel crypts, variable crypt diameters, bifurcation and branched crypts

Table 3.2 Splenic immune cell populations at 48 hpi.

| Cell phenotype | mCRAMP$^{+/+}$ | | | | | | | | mCRAMP$^{-/-}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SA- ST- | | SA- ST+ | | SA+ ST- | | SA+ ST+ | | SA- ST- | | SA- ST+ | | SA+ ST- | | SA+ ST+ | |
| | M | SEM | M | SEM | M | SEM | M | SEM | M | SEM | M | SEM | M | SEM | M | SEM |
| CD3$^+$ T cells | 1.21 | 0.2 | 1.45 | 0.39 | 1.59 | 0.28 | 1.55 | 0.36 | 0.94 | 0.3 | 0.76 | 0.06 | 1.16 | 0.14 | 0.97 | 0.09 |
| CD4$^+$ Th cells | 38.0 | 6.15 | 44.3 | 1.05 | 43.7 | 5.08 | 52.0 | 8.47 | 42.36 | 5.24 | 49.33 | 9.70 | 45.96 | 7.87 | 53.13 | 5.34 |
| CD8$^+$ Tc cells | 3.4 | 1.20 | 2.42 | 0.55 | 4.10 | 1.44 | 4.20 | 0.92 | 4.88 | 2.57 | 4.04 | 1.60 | 5.06 | 1.91 | 5.43 | 1.76 |
| CD3$^+$CD4$^+$TCR$^+$ γδ T cells | 90.9 | 0.68 | 87.9 | 1.96 | 94.0 | 2.70 | 94.6 | 0.77 | 89.26 | 2.23 | 89.9 | 1.70 | 91.56 | 3.73 | 93.93 | 0.56 |
| IL-23R$^+$ γδ T cells | 43.5 | 12.7 | 32.0 | 15.1 | 34.3 | 16.7 | 24.2 | 10.1 | 34.6 | 13.1 | 34.9 | 7.3 | 29.73 | 10.8 | 17.3 | 8.27 |
| CD3$^-$CD161$^+$ NK cells | 4.00 | 0.30 | 3.06 | 0.18 | 3.16 | 0.34 | 2.46 | 0.14 | 3.72 | 0.18 | 3.56 | 0.11 | 3.48 | 0.49 | 3.03 | 0.34 |
| CD3$^-$ CD11b$^+$CD11c$^-$ mon | 0.10 | 0.07 | 0.86 | 0.64 | 0.21 | 0.11 | 0.35 | 0.30 | 0.11 | 0.08 | 0.07 | 0.05 | 0.94 | 0.89 | 0.66 | 0.56 |
| CD3$^-$ CD11b$^+$CD11c$^+$ mDC | 0.17 | 0.12 | 0.20 | 0.09 | 0.13 | 0.08 | 0.10 | 0.07 | 0.14 | 0.11 | 0.16 | 0.13 | 0.10 | 0.05 | 0.10 | 0.06 |
| CD3$^-$CD11b$^-$ CD11c$^+$ pDC | 0.20 | 0.03 | 0.19 | 0.01 | 0.26 | 0.04 | 0.29 | 0.11 | 6.10 | 5.95 | 0.15 | 0.04 | 0.27 | 0.13 | 0.22 | 0.03 |
| CD68$^+$ macrophages | 0.16 | 0.12 | 0.10 | 0.07 | 0.13 | 0.08 | 0.20 | 0.09 | 0.14 | 0.10 | 0.16 | 0.13 | 0.09 | 0.05 | 0.10 | 0.06 |

M, Mean; SEM, standard error of the mean
pDC, plasmacytoid dendritic cells; mDC, myeloid dendritic cells; mon, monocytes

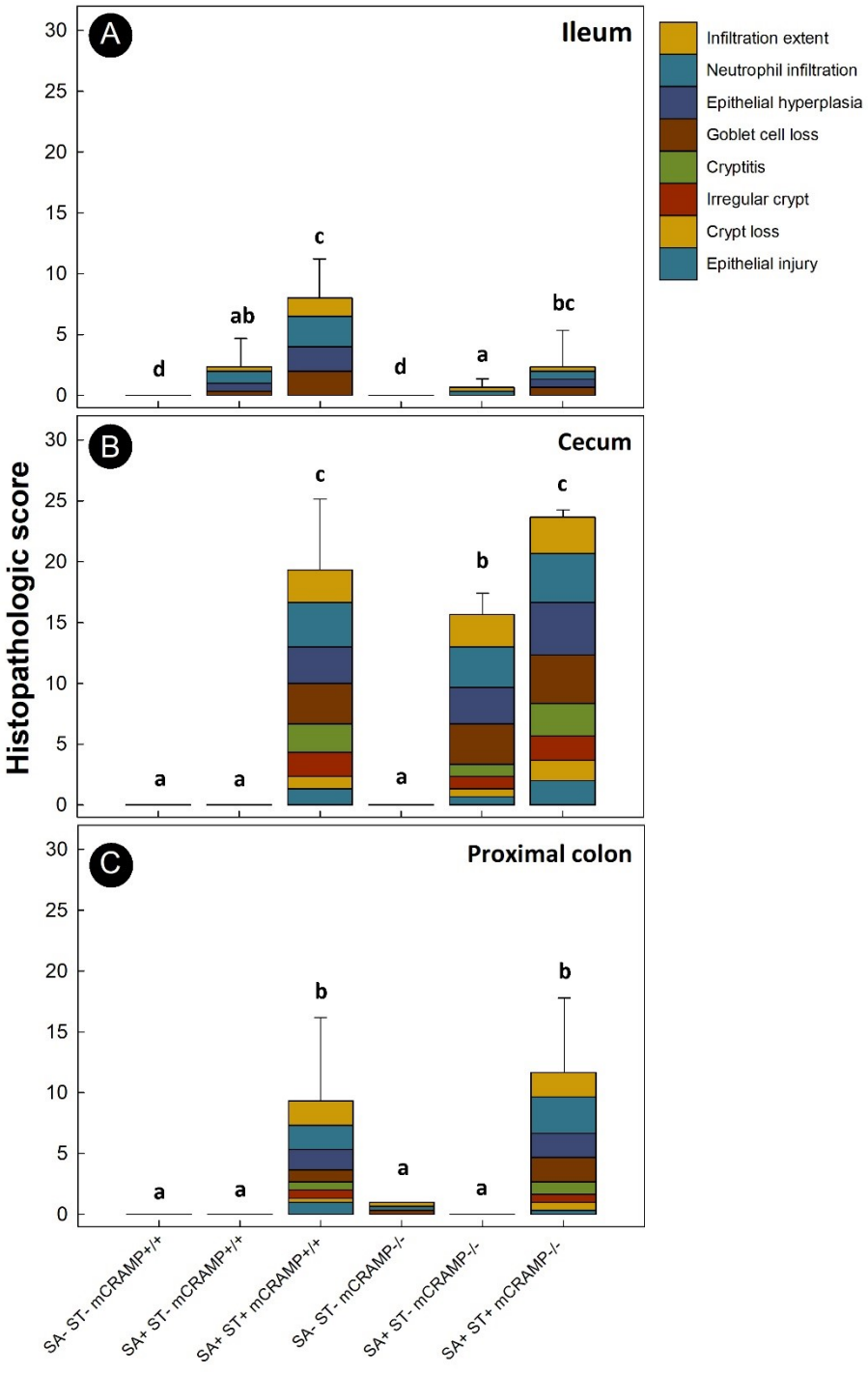


Figure 3.1 Total histopathologic scores in mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice that were inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-), and pretreated with streptomycin (ST+) or water alone (ST-) at 48 hpi. (A) Ileum; (B) Cecum; (C) Proximal Colon. Vertical lines associated with markers are standard errors of the mean. Histograms not indicated with same letter differ (P≤0.050).

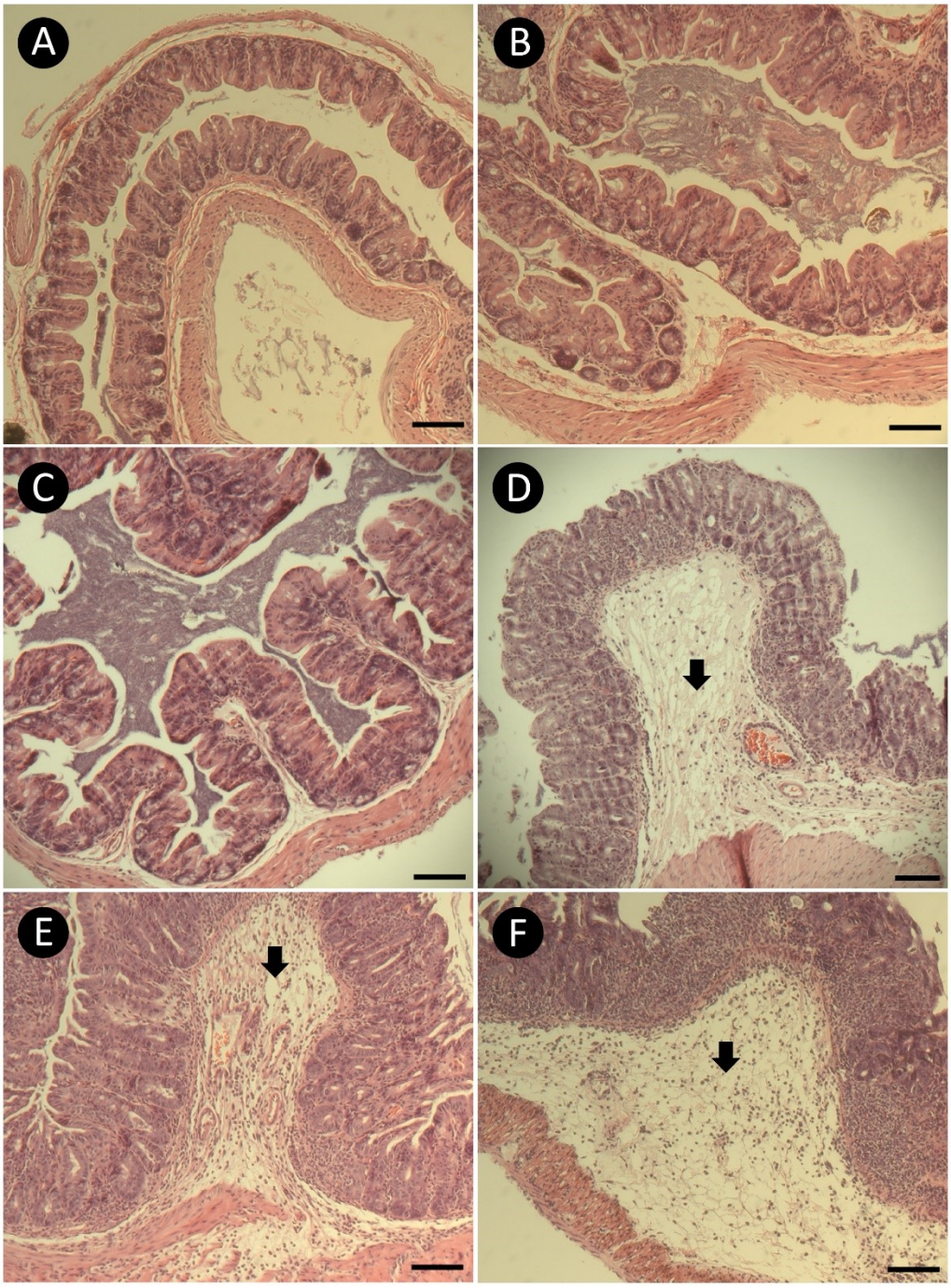


Figure 3.2 Histological representation of cecum tissue from mCRAMP$^{+/+}$ or mCRAMP$^{-/-}$ mice that were inoculated *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-), and pretreated with streptomycin (ST+) or water alone (ST-) at 48 hpi. Arrows indicate leukocyte infiltration. (A) SA-/ST-/mCRAMP$^{+/+}$ mice; (B) SA-/ST-/mCRAMP$^{-/-}$ mice; (C) SA+/ST-/mCRAMP$^{+/+}$ mice; (D) SA+/ST-/mCRAMP$^{-/-}$ mice; (E) SA+/ST+/mCRAMP$^{+/+}$ mice; (F) SA+/ST+/mCRAMP$^{-/-}$ mice. Bar = 1.0 mm.

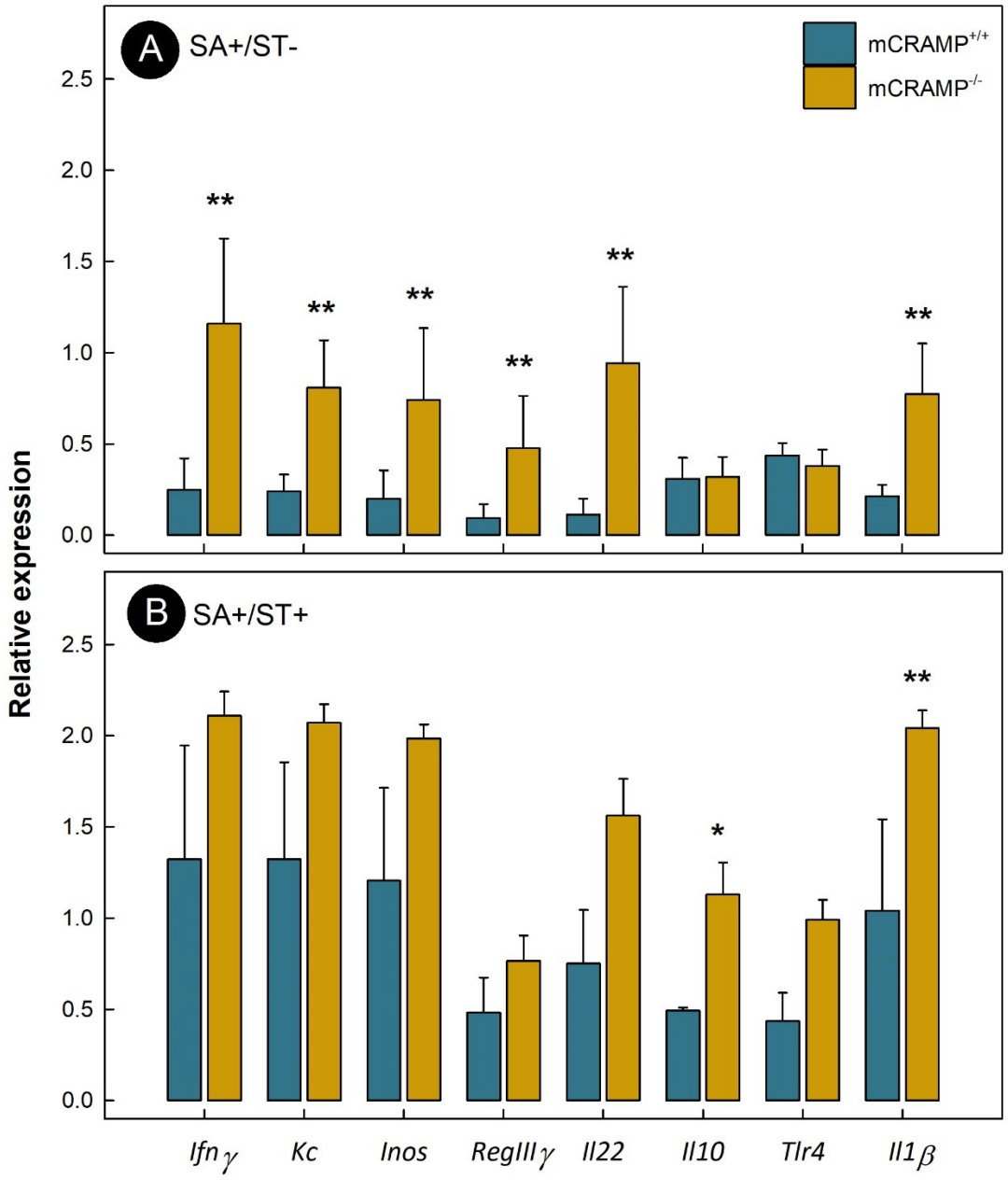


Figure 3.3 Relative gene expression in cecum of mice mCRAMP$^{+/+}$ and mCRAMP$^{-/-}$ mice that were inoculated with *Salmonella enterica* Typhimurium (SA+) at 48 hpi. (A) Mice not administered streptomycin (ST-); (B) mice pretreated with streptomycin (ST+). Vertical lines associated with markers are standard errors of the mean. Histogram bars with asterisks indicate that treatments differ (*P<0.050, **P<0.010).

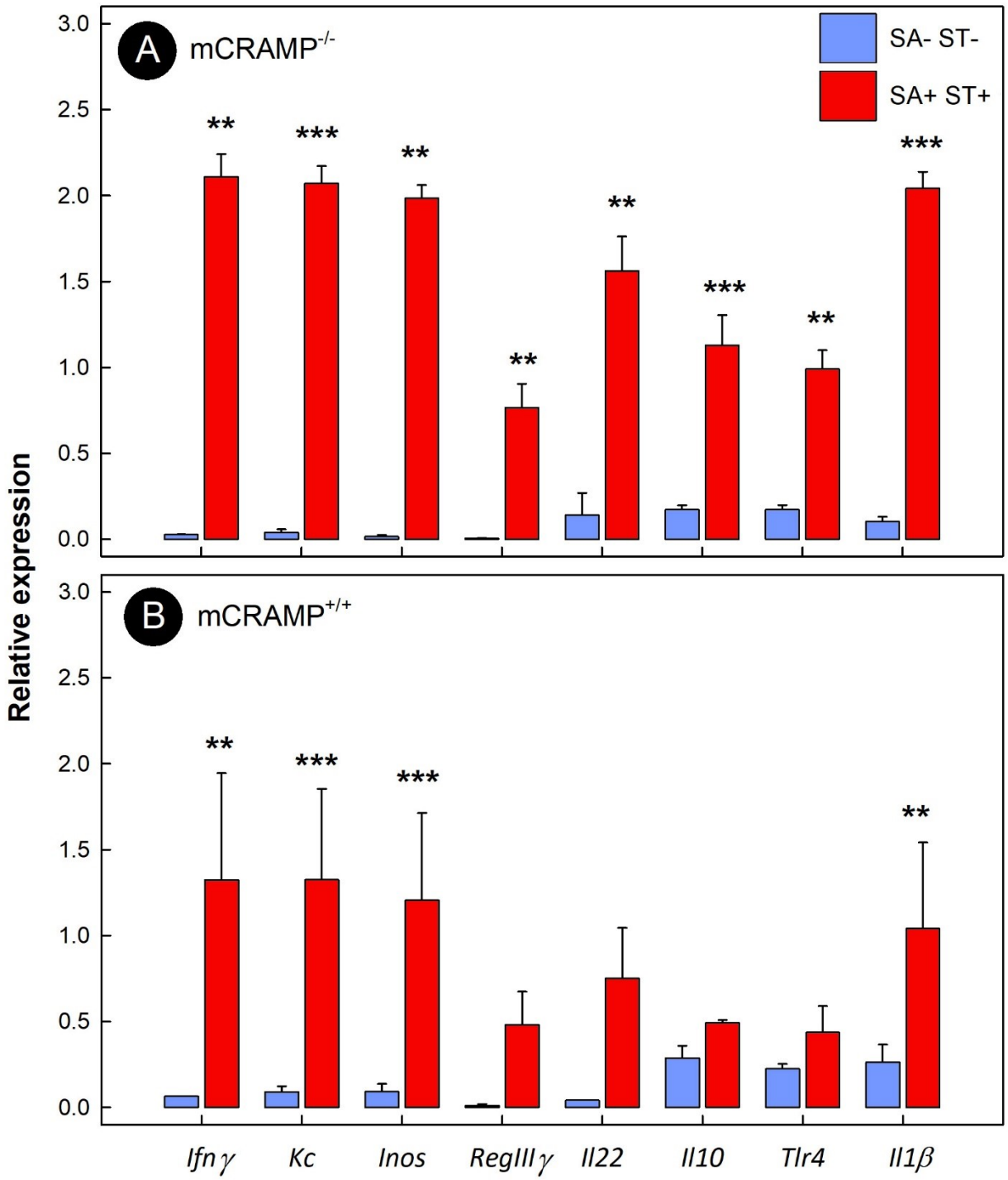


Figure 3.4 Relative gene expression in cecum of mice mCRAMP$^{+/+}$ and mCRAMP$^{-/-}$ mice that were inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-) and pretreated with streptomycin (ST+) or water alone (ST-) at 48 hours post-inoculation. (A) mCRAMP$^{-/-}$ (B) mCRAMP$^{+/+}$. Vertical lines associated with markers are standard errors of the mean. Histogram bars with asterisks indicate that treatments differ (*P<0.050, **P<0.010).

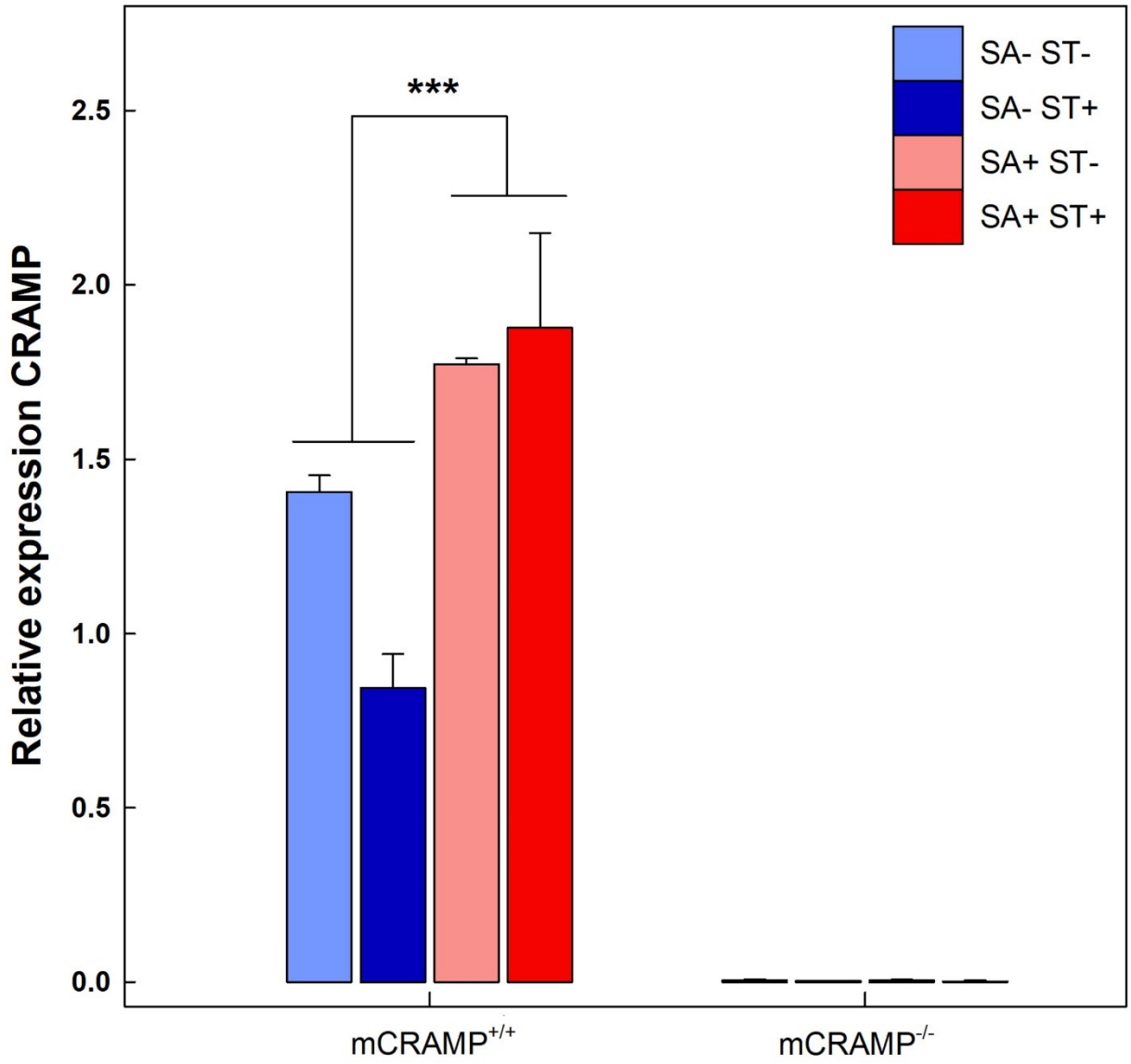


Figure 3.5 Relative gene expression of CRAMP in the cecum of mCRAMP$^{+/+}$ and mCRAMP$^{-/-}$ mice that were inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-), and pretreated with streptomycin (ST+) or water alone (ST-) at 48 hpi. Vertical lines associated with markers are standard errors of the mean. Histogram bars with asterisks indicate that treatments differ (***P<0.001).

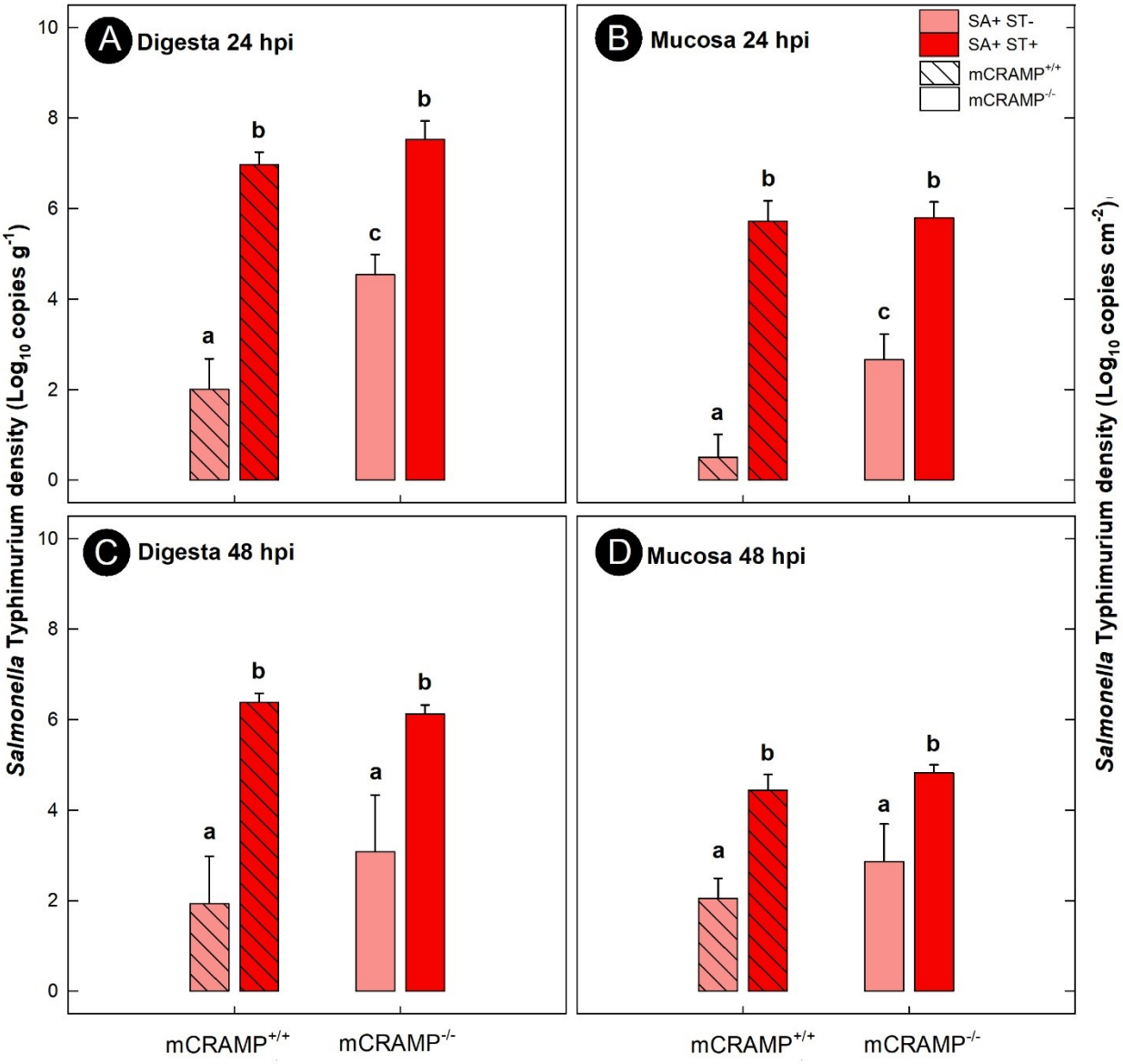


Figure 3.6 *Salmonella* densities from cecal digesta and associated with mucosa of mice 24 and 48 hpi with *Salmonella enterica* Typhimurium (SA+), and pretreated with streptomycin (ST+) or water alone (ST-). (A) Digesta at 24 hpi; (B) mucosa-associated at 24 hpi; (C) digesta at 48 hpi; (D) mucosa-associated at 48 hpi. No *Salmonella* was detected in cecal digesta or associated with mucosa of SA- mice. Vertical lines associated with histogram bars represent standard error of the means. Histogram bars not indicated with the same letter differ ($P<0.050$).

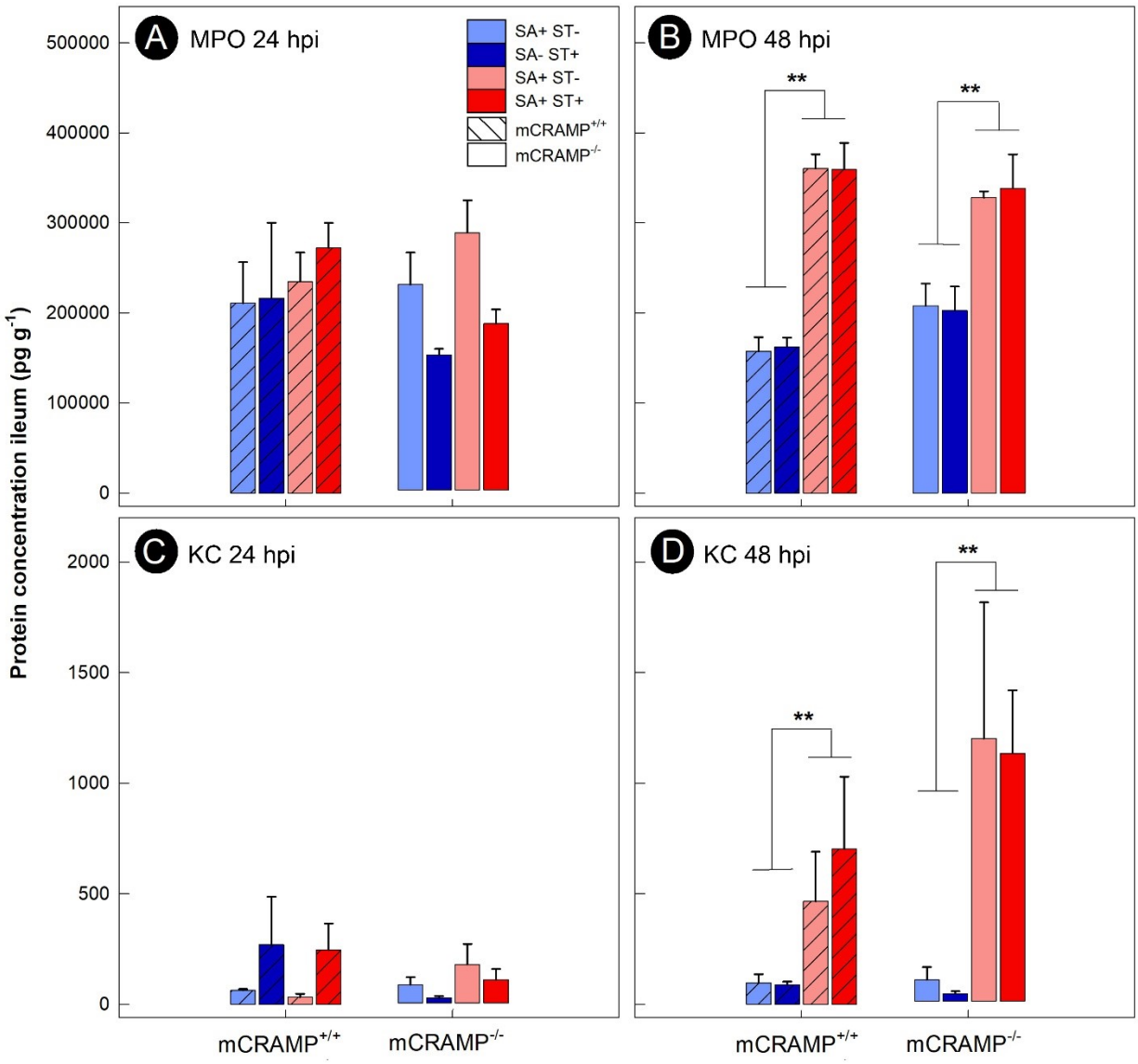


Figure 3.7 Protein concentrations of MPO and KC in the ileum of mCRAMP$^{+/+}$ and mCRAMP$^{-/-}$ mice that were orally inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-), and pretreated with streptomycin (ST+) or water alone (ST-). (A) MPO at 24 hpi; (B) MPO at 48 hpi; (C) KC at 24 hpi; (D) KC at 48 hpi. Vertical lines associated with markers are standard errors of the mean. Histogram bars with asterisks indicate that treatments differ (*P<0.050, **P<0.010, ***P<0.001).

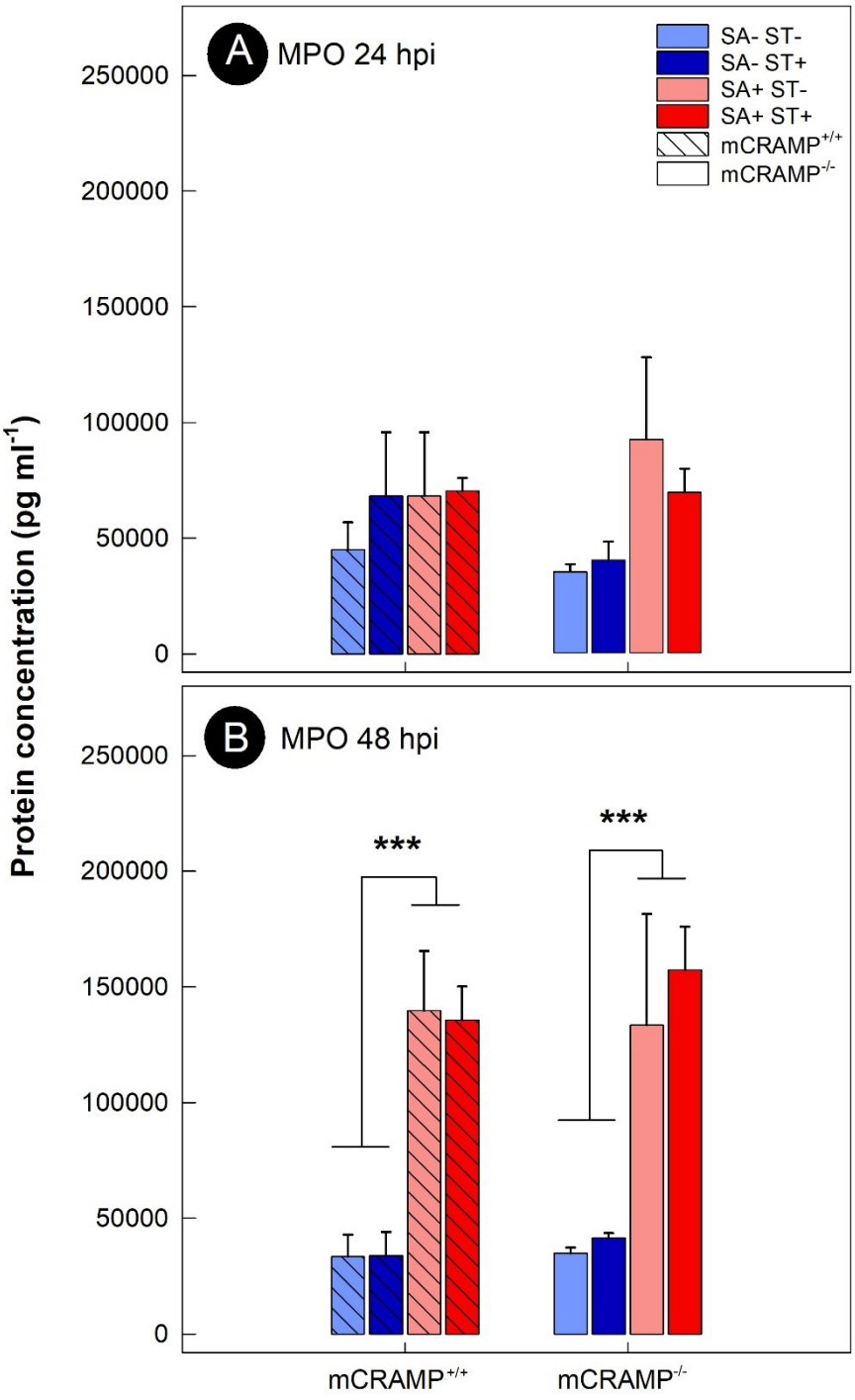


Figure 3.8 Protein concentrations of MPO in the serum of mCRAMP$^{+/+}$ and mCRAMP$^{-/-}$ mice that were orally inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-), and pretreated with streptomycin (ST+) or water alone (ST-). (A) MPO at 24 hours post-inoculation (hpi); (B) MPO at 48 hpi. Vertical lines associated with markers are standard errors of the mean. Histogram bars with asterisks indicate that treatments differ (*P<0.050, **P<0.010, ***P<0.001).

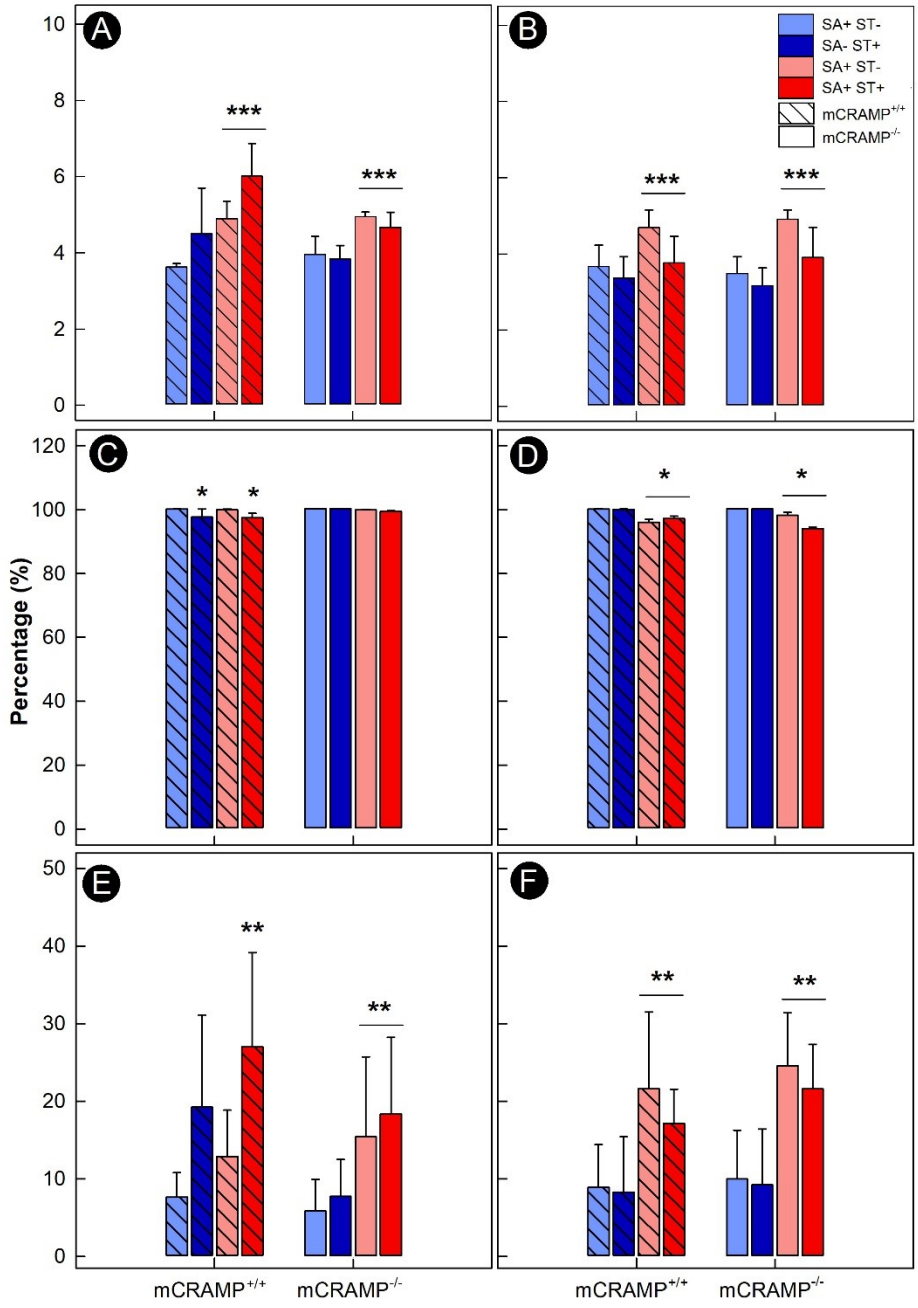


Figure 3.9 Percentage of splenic immune cell populations in mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice, that were orally inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-), and pretreated with streptomycin (ST+) or water alone (ST-). (A) CD45$^{+}$CD18$^{+}$CD11b$^{+}$ leukocytes at 24 hpi; (B) CD45$^{+}$CD18$^{+}$CD11b$^{+}$ leukocytes at 48 hpi; (C) CD45$^{+}$CD18$^{+}$ leukocytes at 24 hpi; (D) CD45$^{+}$CD18$^{+}$ leukocytes at 48 hpi; (E) CD18$^{+}$CD11b$^{+}$ Ly-6C$^{+}$Ly-6G$^{+}$ neutrophils at 24 hpi; (F) CD18$^{+}$CD11b$^{+}$ Ly-6C$^{+}$Ly-6G$^{+}$ neutrophils at 48 hpi. Vertical lines associated with markers are standard errors of the mean. Histogram bars with asterisks indicate that treatments differ (*P<0.050, **P<0.010, ***P<0.001).

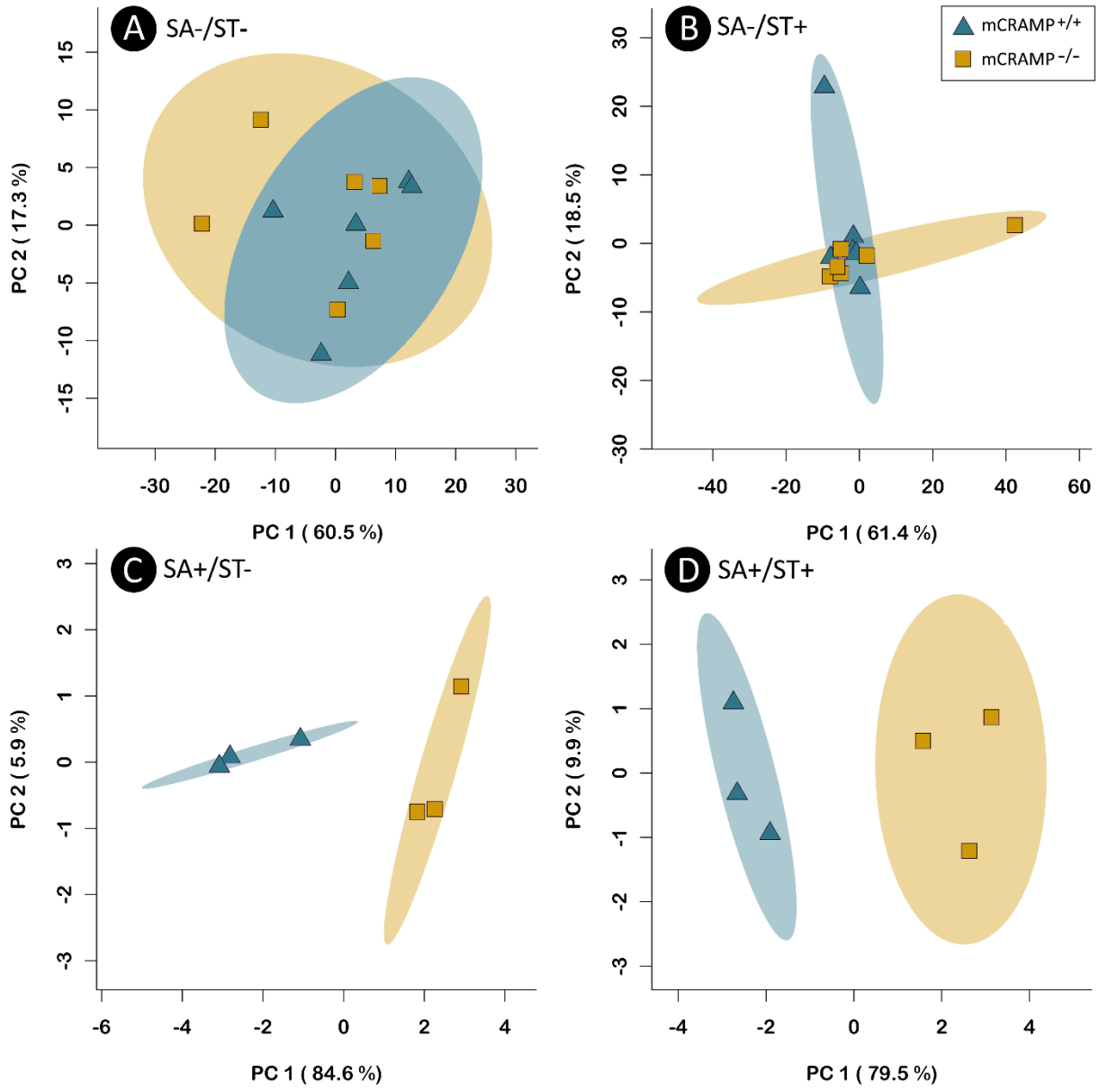


Figure 3.10 Metabolite profiles of mice livers. Principal component analysis plots showing separation between mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice, inoculated with *Salmonella* Typhimurium (SA+) or medium alone (SA-), and pretreated with streptomycin (ST+) or water (ST-). (A) SA-/ST-/mCRAMP$^{-/-}$ vs SA-/ST-/mCRAMP$^{+/+}$ mice at 24 and 48 hpi; (B) SA-/ST+/mCRAMP$^{-/-}$ vs SA-/ST+/mCRAMP$^{+/+}$ mice at 24 hpi and 48 hpi; (C) SA+/ST-/mCRAMP$^{-/-}$ vs SA+/ST-/mCRAMP$^{+/+}$ mice at 24 hpi; (D) SA+/ST+/mCRAMP$^{-/-}$ vs SA+/ST+/mCRAMP$^{+/+}$ mice at 24 hpi. Figure generated by Kate Brown.

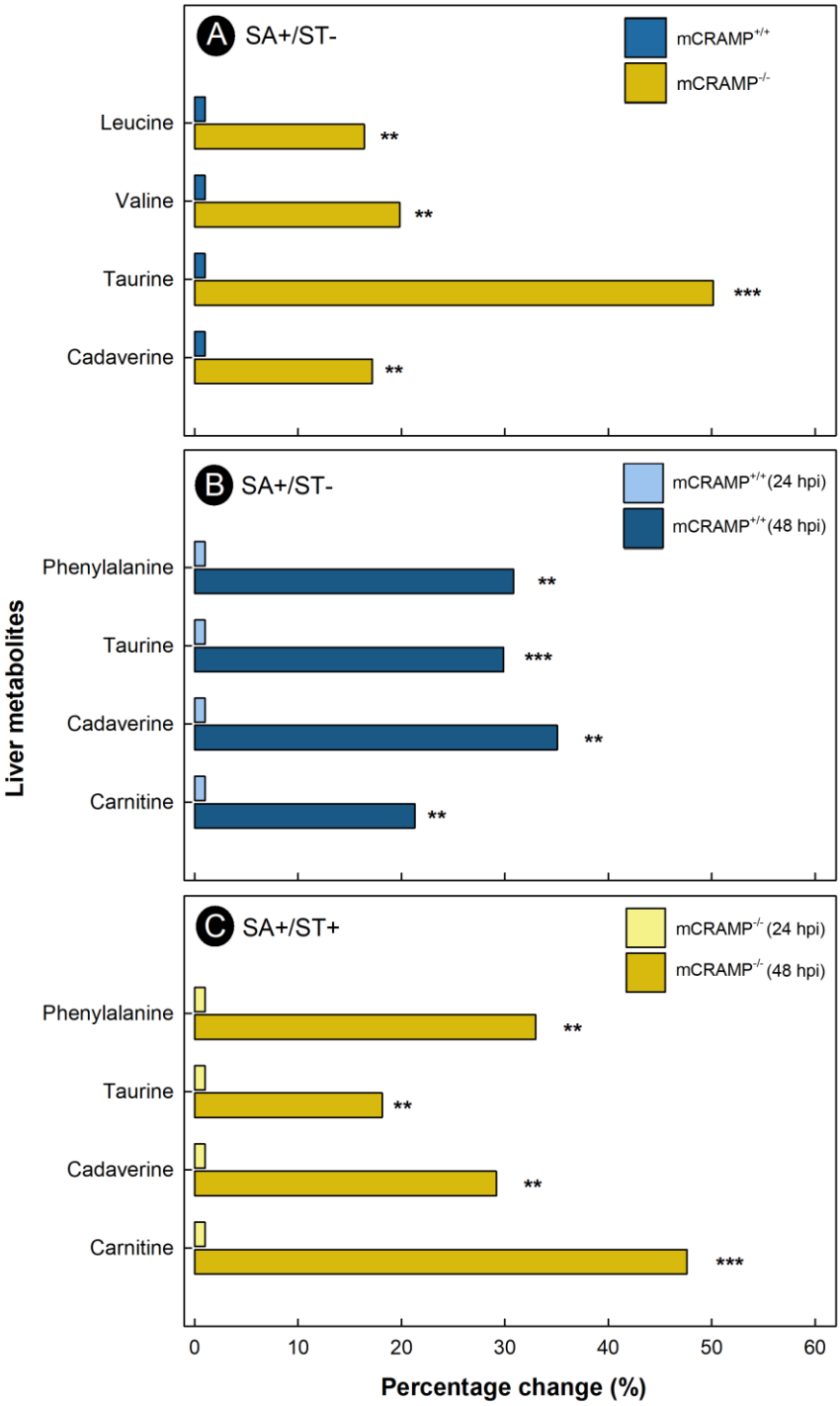


Figure 3.11 Percentage change of discriminated liver metabolites in mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice that were inoculated with *Salmonella* Typhimurium (SA+), and pretreated with streptomycin (ST+) or water alone (ST-). (A) SA+/ST-/mCRAMP$^{-/-}$ vs SA+/ST-/mCRAMP$^{+/+}$ mice at 24 hpi. (B) SA+/ST-/mCRAMP$^{+/+}$ mice at 24 and 48 hpi. (C) SA+/ST+/mCRAMP$^{-/-}$ mice at 24 and 48 hpi. Histogram bars with asterisks indicate that treatments differ (*P<0.050, **P<0.010).

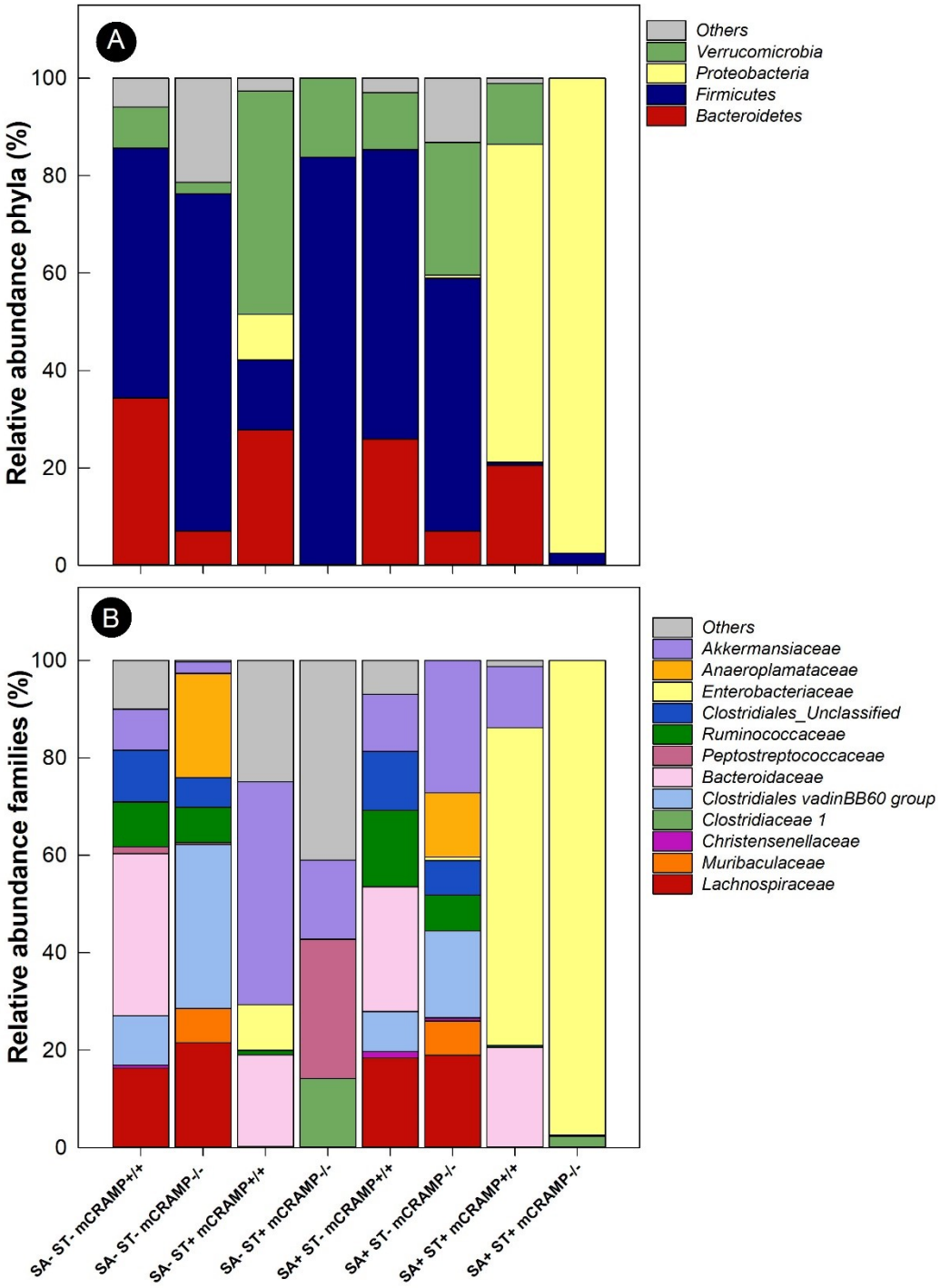


Figure 3.12 Relative abundance (%) of bacterial phyla and families in cecum digesta of mCRAMP$^{+/+}$ and mCRAMP$^{-/-}$ mice that were orally inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-), and pretreated with streptomycin (ST+) or water alone (ST-) at 48 hpi. (A) Phyla; (B) Families.

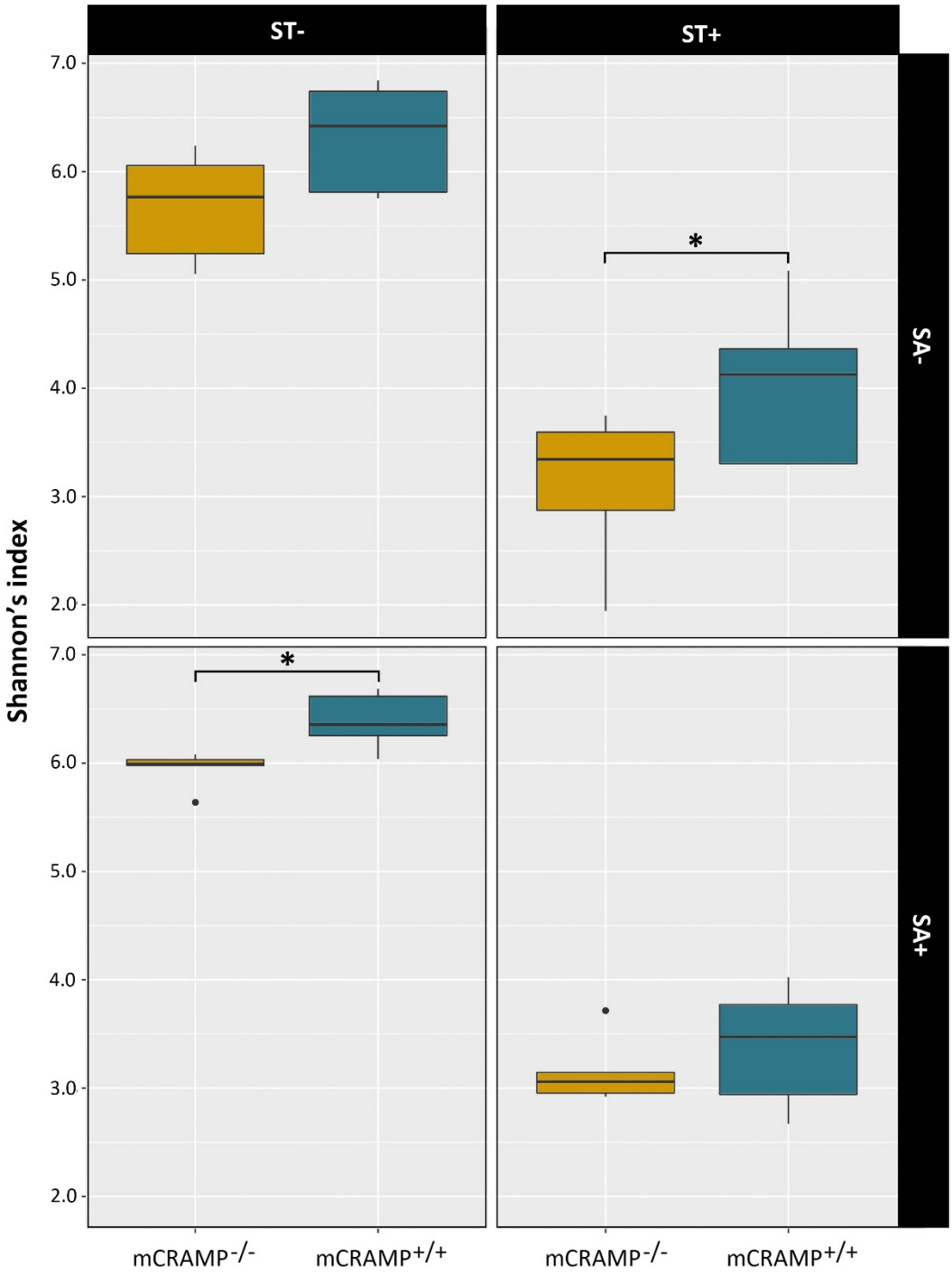


Figure 3.13 Alpha-diversity of bacterial communities in digesta from cecum of mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice that were inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-), and pretreated with streptomycin (ST+) or water alone (ST-). Samples were obtained from mice at 24 and 48 hpi. Values are expressed as means ± standard error. Boxes with an asterisk indicate that treatments differ (*P<0.050).

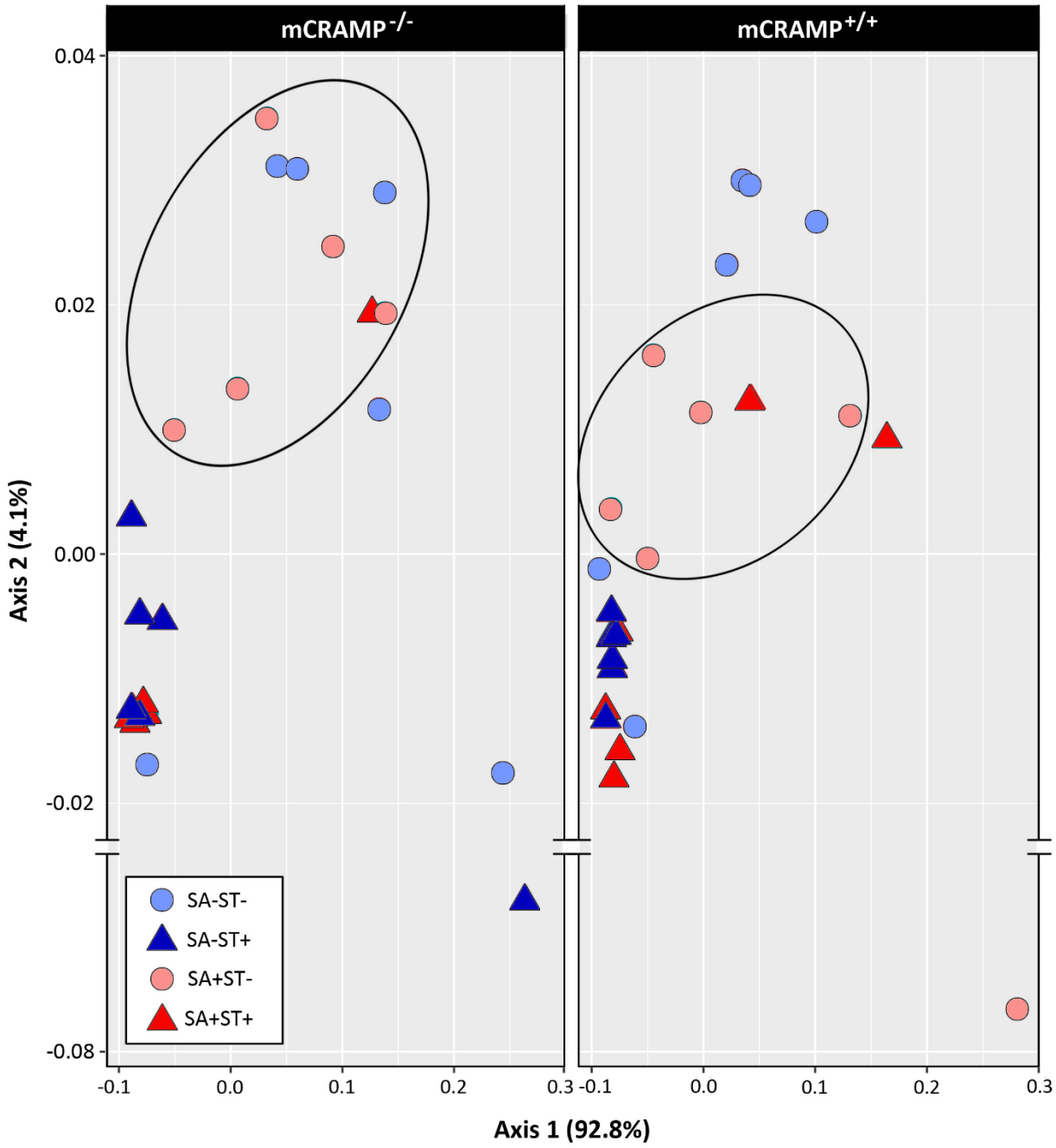


Figure 3.14 Principal coordinate analysis showing unweighted UniFrac distances of bacterial communities in cecal digesta of mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice that were inoculated with *Salmonella enterica* Typhimurium (SA+) or medium alone (SA-), and pretreated with streptomycin (ST+) or water alone (ST-). Samples were obtained from mice at 24 and 48 hpi. Values are expressed as means ± standard error.

### 3.6 References


Anderson, Paul E., Deirdre A. Mahle, Travis E. Doom, Nicholas V. Reo, Nicholas J. DelRaso, and Michael L. Raymer. 2010. 'Dynamic adaptive binning: an improved quantification technique for NMR spectroscopic data', *Metabolomics*, 7: 179-90.

Arpaia, N., J. Godec, L. Lau, K. E. Sivick, L. M. McLaughlin, M. B. Jones, T. Dracheva, S. N. Peterson, D. M. Monack, and G. M. Barton. 2011. 'TLR signaling is required for *Salmonella* Typhimurium virulence', *Cell*, 144: 675-88.

Artis, D. 2008. 'Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut', *Nat Rev Immunol*, 8: 411-20.

Bals, R., C. Lang, D. J. Weiner, C. Vogelmeier, U. Welsch, and J. M. Wilson. 2001. 'Rhesus monkey (Macaca mulatta) mucosal antimicrobial peptides are close homologues of human molecules', *Clin Diagn Lab Immunol*, 8: 370-5.

Barnes, D. M., Z. Song, K. C. Klasing, and W. Bottje. 2002. 'Protein metabolism during an acute phase response in chickens', *Amino Acids*, 22: 15-26.

Barthel, M. , S. Hapfelmeier, L. Quintanilla-Martinez, M. Kremer, M. Rohde, M. Hogardt, K. Pfeffer, H. Russmann, and W. D. Hardt. 2003. 'Pretreatment of Mice with Streptomycin Provides a *Salmonella enterica* Serovar Typhimurium Colitis Model That Allows Analysis of Both Pathogen and Host', *Infect Immun*, 71: 2839-58.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodriguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y. X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, 2nd, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vazquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. 'Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2', *Nat Biotechnol*, 37: 852-57.

Boyer, P. E., S. D'Costa, L. L. Edwards, M. Milloway, E. Susick, L. B. Borst, S. Thakur, J. M. Campbell, J. D. Crenshaw, J. Polo, and A. J. Moeser. 2015. 'Early-life dietary spray-dried plasma influences immunological and intestinal injury responses to later-life *Salmonella* typhimurium challenge', *Br J Nutr*, 113: 783-93.

Brogden, K. A. 2005. 'Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?', *Nat Rev Microbiol*, 3: 238-50.

Broz, P., M. B. Ohlson, and D. M. Monack. 2012. 'Innate immune response to *Salmonella* Typhimurium, a model enteric pathogen', *Gut Microbes*, 3: 62-70.

Brugiroux, S., M. Beutler, C. Pfann, D. Garzetti, H. J. Ruscheweyh, D. Ring, M. Diehl, S. Herp, Y. Lotscher, S. Hussain, B. Bunk, R. Pukall, D. H. Huson, P. C. Munch, A. C. McHardy, K. D. McCoy, A. J. Macpherson, A. Loy, T. Clavel, D. Berry, and B. Stecher. 2016. 'Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium', *Nat Microbiol*, 2: 16215.

Burgos-Ramos, E., J. A. Chowen, E. Arilla-Ferreiro, S. Canelles, J. Argente, and V. Barrios. 2011. 'Chronic central leptin infusion modifies the response to acute central insulin injection by reducing the interaction of the insulin receptor with IRS2 and increasing its association with SOCS3', *J Neurochem*, 117: 175-85.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. Johnson, and S. P. Holmes. 2016. 'DADA2: High-resolution sample inference from Illumina amplicon data', *Nat Methods*, 13: 581-3.

Carter, P. B., and F. M. Collins. 1974. 'The route of enteric infection in normal mice', *J Exp Med*, 139: 1189-203.

Chong, J., M. Yamamoto, and J. Xia. 2019. 'MetaboAnalystR 2.0: From Raw Spectra to Biological Insights', *Metabolites*, 9.

Chromek, M., Z. Slamova, P. Bergman, L. Kovacs, L. Podracka, I. Ehren, T. Hokfelt, G. H. Gudmundsson, R. L. Gallo, B. Agerberth, and A. Brauner. 2006. 'The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection', *Nat Med*, 12: 636-41.

Coburn, B. , G. A. Grassl, and B. B. Finlay. 2007. '*Salmonella*, the host and disease: a brief review', *Immunol Cell Biol*, 85: 112-8.

den Besten, G., K. van Eunen, A. K. Groen, K. Venema, D. J. Reijngoud, and B. M. Bakker. 2013. 'The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism', *J Lipid Res*, 54: 2325-40.

Dubin, P. J., and J. K. Kolls. 2007. 'IL-23 mediates inflammatory responses to mucoid *Pseudomonas aeruginosa* lung infection in mice', *Am J Physiol Lung Cell Mol Physiol*, 292: L519-28.

Erben, Ulrike, Christoph Loddenkemper, Katja Doerfel, Simone Spieckermann, Dirk Haller, Markus M. Heimesaat, Martin Zeitz, Britta Siegmund, and and Anja A. Kühl. 2014. 'A guide to histomorphological evaluation of intestinal inflammation in mouse models.', *Int J Clin Exp Pathol*, 7: 4557.

Fan, D., L. A. Coughlin, M. M. Neubauer, J. Kim, M. S. Kim, X. Zhan, T. R. Simms-Waldrip, Y. Xie, L. V. Hooper, and A. Y. Koh. 2015. 'Activation of HIF-1alpha and LL-37 by commensal bacteria inhibits *Candida albicans* colonization', *Nat Med*, 21: 808-14.

Fattorossi, A., R. Biselli, A. Casciaro, S. Tzantzoglou, and C. de Simone. 1993. 'Regulation of normal human polyrnorphonuclear leucocytes by carnitine', *Mediators Inflamm*, 2: S37-41.

Fernandez, I. M., M. Silva, R. Schuch, W. A. Walker, A. M. Siber, A. T. Maurelli, and B. A. McCormick. 2001. 'Cadaverine prevents the escape of *Shigella flexneri* from the phagolysosome: a connection between bacterial dissemination and neutrophil transepithelial signaling', *J Infect Dis*, 184: 743-53.

Freter, R., H. Brickner, M. Botney, D. Cleven, and A. Aranki. 1983. 'Mechanisms that control bacterial populations in continuous-flow culture models of mouse large intestinal flora', *Infect Immun*, 39: 676-85.

Gal-Mor, O., E. C. Boyle, and G. A. Grassl. 2014. 'Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ', *Front Microbiol*, 5: 391.

Gallo, R. L., and L. V. Hooper. 2012. 'Epithelial antimicrobial defence of the skin and intestine', *Nat Rev Immunol*, 12: 503-16.

Gallo, R. L., K. J. Kim, M. Bernfield, C. A. Kozak, M. Zanetti, L. Merluzzi, and R. Gennaro. 1997. 'Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse', *J Biol Chem*, 272: 13088-93.

Ganesh, B. P., R. Klopfleisch, G. Loh, and M. Blaut. 2013. 'Commensal *Akkermansia muciniphila* exacerbates gut inflammation in *Salmonella* Typhimurium-infected gnotobiotic mice', *PLoS One*, 8: e74963.

Garner, C. D., D. A. Antonopoulos, B. Wagner, G. E. Duhamel, I. Keresztes, D. A. Ross, V. B. Young, and C. Altier. 2009. 'Perturbation of the small intestine microbial ecology by streptomycin alters pathology

in a *Salmonella enterica* serovar Typhimurium murine model of infection', *Infect Immun*, 77: 2691-702.
Godinez, I., T. Haneda, M. Raffatellu, M. D. George, T. A. Paixao, H. G. Rolan, R. L. Santos, S. Dandekar, R. M. Tsolis, and A. J. Baumler. 2008. 'T cells help to amplify inflammatory responses induced by *Salmonella enterica* serotype Typhimurium in the intestinal mucosa', *Infect Immun*, 76: 2008-17.
Godinez, I., M. Raffatellu, H. Chu, T. A. Paixao, T. Haneda, R. L. Santos, C. L. Bevins, R. M. Tsolis, and A. J. Baumler. 2009. 'Interleukin-23 orchestrates mucosal responses to *Salmonella enterica* serotype Typhimurium in the intestine', *Infect Immun*, 77: 387-98.
Goodpaster, A. M., L. E. Romick-Rosendale, and M. A. Kennedy. 2010. 'Statistical significance analysis of nuclear magnetic resonance-based metabonomics data', *Anal Biochem*, 401: 134-43.
Gu, S., D. Chen, J. N. Zhang, X. Lv, K. Wang, L. P. Duan, Y. Nie, and X. L. Wu. 2013. 'Bacterial community mapping of the mouse gastrointestinal tract', *PLoS One*, 8: e74957.
Gudmundsson, G. H., B. Agerberth, J. Odeberg, T. Bergman, B. Olsson, and R. Salcedo. 1996. 'The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes', *Eur J Biochem*, 238: 325-32.
Hasegawa, M., N. Kamada, Y. Jiao, M. Z. Liu, G. Nunez, and N. Inohara. 2012. 'Protective role of commensals against *Clostridium difficile* infection via an IL-1beta-mediated positive-feedback loop', *J Immunol*, 189: 3085-91.
Hohmann, A. W., G. Schmidt, and D. Rowley. 1978. 'Intestinal colonization and virulence of *Salmonella* in mice', *Infect Immun*, 22: 763-70.
Holani, R., C. Shah, Q. Haji, G. D. Inglis, R. R. E. Uwiera, and E. R. Cobo. 2016. 'Proline-arginine rich (PR-39) cathelicidin: structure, expression and functional implication in intestinal health', *Comp Immunol Microbiol Infect Dis*, 49: 95-101.
Iimura, M., R. L. Gallo, K. Hase, Y. Miyamoto, L. Eckmann, and M. F. Kagnoff. 2005. 'Cathelicidin Mediates Innate Intestinal Defense against Colonization with Epithelial Adherent Bacterial Pathogens', *J Immunol*, 174: 4901-07.
Jana, B., and D. Salomon. 2019. 'Type VI secretion system: a modular toolkit for bacterial dominance', *Future Microbiol*, 14: 1451-63.
Kaiser, P., M. Diard, B. Stecher, and W. D. Hardt. 2012. 'The streptomycin mouse model for *Salmonella* diarrhea: functional analysis of the microbiota, the pathogen's virulence factors, and the host's mucosal immune response', *Immunol Rev*, 245: 56-83.
Koczulla, R., G. von Degenfeld, C. Kupatt, F. Krotz, S. Zahler, T. Gloe, K. Issbrucker, P. Unterberger, M. Zaiou, C. Lebherz, A. Karl, P. Raake, A. Pfosser, P. Boekstegers, U. Welsch, P. S. Hiemstra, C. Vogelmeier, R. L. Gallo, M. Clauss, and R. Bals. 2003. 'An angiogenic role for the human peptide antibiotic LL-37/hCAP-18', *J Clin Invest*, 111: 1665-72.
Koelink, P. J., M. E. Wildenberg, L. W. Stitt, B. G. Feagan, M. Koldijk, A. B. van 't Wout, R. Atreya, M. Vieth, J. F. Brandse, S. Duijst, A. A. Te Velde, Gram D'Haens, B. G. Levesque, and G. R. van den Brink. 2018. 'Development of Reliable, Valid and Responsive Scoring Systems for Endoscopy and Histology in Animal Models for Inflammatory Bowel Disease', *J Crohns Colitis*, 12: 794-803.
Kohler, H., S. P. Rodrigues, A. T. Maurelli, and B. A. McCormick. 2002. 'Inhibition of *Salmonella* Typhimurium enteropathogenicity by piperidine, a metabolite of the polyamine cadaverine', *J Infect Dis*, 186: 1122-30.
Koon, H. W., D. Q. Shih, J. Chen, K. Bakirtzi, T. C. Hing, I. Law, S. Ho, R. Ichikawa, D. Zhao, H. Xu, R. Gallo, P. Dempsey, G. Cheng, S. R. Targan, and C. Pothoulakis. 2011. 'Cathelicidin signaling via the Toll-like receptor protects against colitis in mice', *Gastroenterology*, 141: 1852-63 e1-3.
Kress, E., J. Merres, L. J. Albrecht, S. Hammerschmidt, T. Pufe, S. C. Tauber, and L. O. Brandenburg. 2017. 'CRAMP deficiency leads to a pro-inflammatory phenotype and impaired phagocytosis after exposure to bacterial meningitis pathogens', *Cell Commun Signal*, 15: 32.

Kumar, R., P. K. Surendran, and N. & Thampuran. 2010. 'Rapid quantification of *Salmonella* in seafood by real-time PCR assay.', *J Microbiol Biotechnol*, N.20: 569-73.
Kurosaka, K., Q. Chen, F. Yarovinsky, J. J. Oppenheim, and D. Yang. 2005. 'Mouse cathelin-related antimicrobial peptide chemoattracts leukocytes using formyl peptide receptor-like 1/mouse formyl peptide receptor-like 2 as the receptor and acts as an immune adjuvant', *J Immunol*, 174: 6257-65.
Larrick, J. W., J. G. Morgan, I. Palings, M. Hirata, and M. H. Yen. 1991. 'Complementary DNA sequence of rabbit CAP18--a unique lipopolysaccharide binding protein', *Biochem Biophys Res Commun*, 179: 170-5.
Lawley, T. D., and A. W. Walker. 2013. 'Intestinal colonization resistance', *Immunology*, 138: 1-11.
Litvak, Y., M. X. Byndloss, R. M. Tsolis, and A. J. Baumler. 2017. 'Dysbiotic *Proteobacteria* expansion: a microbial signature of epithelial dysfunction', *Curr Opin Microbiol*, 39: 1-6.
Lupp, C., M. L. Robertson, M. E. Wickham, I. Sekirov, O. L. Champion, E. C. Gaynor, and B. B. Finlay. 2007. 'Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of *Enterobacteriaceae*', *Cell Host Microbe*, 2: 119-29.
Martinez, F. A., E. M. Balciunas, A. Converti, P. D. Cotter, and R. P. de Souza Oliveira. 2013. 'Bacteriocin production by *Bifidobacterium* spp. A review', *Biotechnol Adv*, 31: 482-8.
Miki, T., R. Goto, M. Fujimoto, N. Okada, and W. D. Hardt. 2017. 'The Bactericidal Lectin RegIIIbeta Prolongs Gut Colonization and Enteropathy in the Streptomycin Mouse Model for *Salmonella* Diarrhea', *Cell Host Microbe*, 21: 195-207.
Mochizuki, T., H. Satsu, T. Nakano, and M. Shimizu. 2004. 'Regulation of the human taurine transporter by TNF-alpha and an anti-inflammatory function of taurine in human intestinal Caco-2 cells', *Biofactors*, 21: 141-4.
Momose, Y., K. Hirayama, and K. Itoh. 2008. 'Competition for proline between indigenous *Escherichia coli* and *E. coli* O157:H7 in gnotobiotic mice associated with infant intestinal microbiota and its contribution to the colonization resistance against *E. coli* O157:H7', *ANTON LEEUW INT J G*, 94: 165-71.
Morton, J. T., J. Sanders, R. A. Quinn, D. McDonald, A. Gonzalez, Y. Vazquez-Baeza, J. A. Navas-Molina, S. J. Song, J. L. Metcalf, E. R. Hyde, M. Lladser, P. C. Dorrestein, and R. Knight. 2017. 'Balance Trees Reveal Microbial Niche Differentiation', *mSystems*, 2.
Mukherjee, S., and L. V. Hooper. 2015. 'Antimicrobial defense of the intestine', *Immunity*, 42: 28-39.
Mukherjee, S., S. Vaishnava, and L. V. Hooper. 2008. 'Multi-layered regulation of intestinal antimicrobial defense', *Cell Mol Life Sci*, 65: 3019-27.
Nakoneczna, I., & Hsu, H. S. (1980). 'The comparative histopathology of primary and secondary lesions in murine salmonellosis.', *Br J Exp Pathol*, 61: 76.
Nizet, V., T. Ohtake, X. Lauth, J. Trowbridge, J. Rudisill, R. A. Dorschner, V. Pestonjamasp, J. Piraino, K. Huttner, and R. L. Gallo. 2001. 'Innate antimicrobial peptide protects the skin from invasive bacterial infection', *Nature*, 414: 454-7.
Nunes, J. S., S. D. Lawhon, C. A. Rossetti, S. Khare, J. F. Figueiredo, T. Gull, R. C. Burghardt, A. J. Baumler, R. M. Tsolis, H. L. Andrews-Polymenis, and L. G. Adams. 2010. 'Morphologic and cytokine profile characterization of *Salmonella enterica* serovar typhimurium infection in calves with bovine leukocyte adhesion deficiency', *Vet Pathol*, 47: 322-33.
Paxman, E. J., N. S. Boora, D. Kiss, D. P. Laplante, S. King, T. Montina, and G. A. S. Metz. 2018. 'Prenatal Maternal Stress from a Natural Disaster Alters Urinary Metabolomic Profiles in Project Ice Storm Participants', *Sci Rep*, 8: 12932.
Pestonjamasp, V. K., K. H. Huttner, and R. L. Gallo. 2001. 'Processing site and gene structure for the murine antimicrobial peptide CRAMP', *Peptides*, 22: 1643-50.
Que, J. U., and D. J. & Hentges. 1985. 'Effect of streptomycin administration on colonization resistance to *Salmonella* Typhimurium in mice.', *Infect Immun*, 48(1): 169-74.

Raffatellu, M., R. L. Santos, D. E. Verhoeven, M. D. George, R. P. Wilson, S. E. Winter, I. Godinez, S. Sankaran, T. A. Paixao, M. A. Gordon, J. K. Kolls, S. Dandekar, and A. J. Baumler. 2008. 'Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes *Salmonella* dissemination from the gut', *Nat Med*, 14: 421-8.

Rakoff-Nahoum, S., J. Paglino, F. Eslami-Varzaneh, S. Edberg, and R. Medzhitov. 2004. 'Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis', *Cell*, 118: 229-41.

Raupach, B. , S. K. Peuschel, D. M. Monack, and A. Zychlinsky. 2006. 'Caspase-1-mediated activation of interleukin-1beta (IL-1beta) and IL-18 contributes to innate immune defenses against *Salmonella enterica* serovar Typhimurium infection', *Infect Immun*, 74: 4922-6.

Reuter, S. E., and A. M. Evans. 2012. 'Carnitine and acylcarnitines: pharmacokinetic, pharmacological and clinical aspects', *Clin Pharmacokinet*, 51: 553-72.

Rigottier-Gois, L. 2013. 'Dysbiosis in inflammatory bowel diseases: the oxygen hypothesis', *ISME J*, 7: 1256-61.

Rios-Covian, D., P. Ruas-Madiedo, A. Margolles, M. Gueimonde, C. G. de Los Reyes-Gavilan, and N. Salazar. 2016. 'Intestinal short chain fatty acids and their link with diet and human health', *Front Microbiol*, 7: 185.

Rosenberger CM, Gallo RL, and Finlay BB. 2004 Feb 24. 'Interplay between antibacterial effectors: a macrophage antimicrobial peptide impairs intracellular *Salmonella* replication.', *Proc Natl Acad Sci U S A*, 101(8):: 2422-7.

Salzman, N. H., M. A. Underwood, and C. L. Bevins. 2007. 'Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa', *Semin Immunol*, 19: 70-83.

Santos RL, Zhang S, Tsolis RM, Kingsley RA, Adams LG, and Bäumler AJ. 2001. 'Animal models of *Salmonella* infections: enteritis versus typhoid fever.', *Microb Infect*, 3(14): 1335-44.

Sassone-Corsi, M., and M. Raffatellu. 2015. 'No vacancy: how beneficial microbes cooperate with immunity to provide colonization resistance to pathogens', *J Immunol*, 194: 4081-7.

Schuller-Levis, G. B., and E. Park. 2004. 'Taurine and its chloramine: modulators of immunity', *Neurochem Res*, 29: 117-26.

Spees, A. M. , D. D. Kingsbury, T. Wangdi, M. N. Xavier, R. M. Tsolis, and A. J. Baumler. 2014. 'Neutrophils are a source of gamma interferon during acute *Salmonella enterica* serovar Typhimurium colitis', *Infect Immun*, 82: 1692-7.

Stecher, B. , R. Robbiani, A. W. Walker, A. M. Westendorf, M. Barthel, M. Kremer, S. Chaffron, A. J. Macpherson, J. Buer, J. Parkhill, G. Dougan, C. von Mering, and W. D. Hardt. 2007. '*Salmonella enterica* serovar Typhimurium exploits inflammation to compete with the intestinal microbiota', *PLoS Biol*, 5: 2177-89.

Stecher, B., A. J. Macpherson, S. Hapfelmeier, M. Kremer, T. Stallmach, and W. D. Hardt. 2005. 'Comparison of *Salmonella enterica* serovar Typhimurium colitis in germfree mice and mice pretreated with streptomycin', *Infect Immun*, 73: 3228-41.

Sukhotnik, I., I. Aranovich, Y. Ben Shahar, N. Bitterman, Y. Pollak, D. Berkowitz, D. Chepurov, A. G. Coran, and A. Bitterman. 2016. 'Effect of taurine on intestinal recovery following intestinal ischemia-reperfusion injury in a rat', *Pediatr Surg Int*, 32: 161-8.

Travis, S. M., N. N. Anderson, W. R. Forsyth, C. Espiritu, B. D. Conway, E. P. Greenberg, P. B. McCray, Jr., R. I. Lehrer, M. J. Welsh, and B. F. Tack. 2000. 'Bactericidal activity of mammalian cathelicidin-derived peptides', *Infect Immun*, 68: 2748-55.

Trivedi, R. N., P. Agarwal, M. Kumawat, P. K. Pesingi, V. K. Gupta, T. K. Goswami, and M. Mahawar. 2015. 'Methionine sulfoxide reductase A (MsrA) contributes to *Salmonella* Typhimurium survival against oxidative attack of neutrophils', *Immunobiology*, 220: 1322-7.

Uzzau, Sergio, Derek J. Brown, T. Wallis, Salvatore Rubino, Guido Leori, Serge Bernard, Josep Casadesús, David J. Platt, and and John Elmerdahl Olsen. 2000. 'Host adapted serotypes of *Salmonella enterica*', *Epidemiol Infect*, 125: 229-55.

van Harten, R. M., E. van Woudenbergh, A. van Dijk, and H. P. Haagsman. 2018. 'Cathelicidins: Immunomodulatory Antimicrobials', *Vaccines (Basel)*, 6.

Vente, J. P., M. F. von Meyenfeldt, H. M. van Eijk, C. L. van Berlo, D. J. Gouma, C. J. van der Linden, and P. B. Soeters. 1989. 'Plasma-amino acid profiles in sepsis and stress', *Ann Surg*, 209: 57-62.

Veselkov, Kirill A., John C. Lindon, Timothy MD Ebbels, Derek Crockford, Vladimir V. Volynkin, Elaine Holmes, David B. Davies, and and Jeremy K. Nicholson. 2009. '"Recursive segment-wise peak alignment of biological 1H NMR spectra for improved metabolic biomarker recovery." ', *Anal Chem*, 1 56-66.

Wadolkowski, E. A., D. C. Laux, and P. S. Cohen. 1988. 'Colonization of the streptomycin-treated mouse large intestine by a human fecal *Escherichia coli* strain: role of growth in mucus', *Infect Immun*, 56: 1030-5.

Wannemacher, R. W., Jr. 1977. 'Key role of various individual amino acids in host response to infection', *Am J Clin Nutr*, 30: 1269-80.

Wannemacher, R. W., Jr., A. S. Klainer, R. E. Dinterman, and W. R. Beisel. 1976. 'The significance and mechanism of an increased serum phenylalanine-tyrosine ratio during infection', *Am J Clin Nutr*, 29: 997-1006.

Wannemacher, R. W., Jr., M. C. Powanda, R. S. Pekarek, and W. R. Beisel. 1971. 'Tissue amino acid flux after exposure of rats to *Diplococcus pneumoniae*', *Infect Immun*, 4: 556-62.

Winter, S. E., P. Thiennimitr, M. G. Winter, B. P. Butler, D. L. Huseby, R. W. Crawford, J. M. Russell, C. L. Bevins, L. G. Adams, R. M. Tsolis, J. R. Roth, and A. J. Baumler. 2010. 'Gut inflammation provides a respiratory electron acceptor for *Salmonella*', *Nature*, 467: 426-9.

Winterbourn, C. C., A. J. Kettle, and M. B. Hampton. 2016. 'Reactive oxygen species and neutrophil function', *Annu Rev Biochem*, 85: 765-92.

Wu, W. K., C. C. Wong, Z. J. Li, L. Zhang, S. X. Ren, and C. H. Cho. 2010. 'Cathelicidins in inflammation and tissue repair: Potential therapeutic applications for gastrointestinal disorders', *Acta Pharmacol Sin*, 31: 1118-22.

Yin, F., A. Farzan, Q. C. Wang, H. Yu, Y. Yin, Y. Hou, R. Friendship, and J. Gong. 2014. 'Reduction of *Salmonella enterica* serovar Typhimurium DT104 infection in experimentally challenged weaned pigs fed a lactobacillus-fermented feed', *Foodborne Pathog Dis*, 11: 628-34.

Yoshimura, T., M. H. McLean, A. K. Dzutsev, X. Yao, K. Chen, J. Huang, W. Gong, J. Zhou, Y. Xiang, H. Badger J, C. O'HUigin, V. Thovarai, L. Tessarollo, S. K. Durum, G. Trinchieri, X. W. Bian, and J. M. Wang. 2018. 'The Antimicrobial Peptide CRAMP Is Essential for Colon Homeostasis by Maintaining Microbiota Balance', *J Immunol*, 200: 2174-85.

Zachar, Z., and D. C. Savage. 1979. 'Microbial interference and colonization of the murine gastrointestinal tract by *Listeria monocytogenes*', *Infect Immun*, 23: 168-74.

Zanetti, M., G. Del Sal, P. Storici, C. Schneider, and D. Romeo. 1993. 'The cDNA of the neutrophil antibiotic Bac5 predicts a pro-sequence homologous to a cysteine proteinase inhibitor that is common to other neutrophil antibiotics', *J Biol Chem*, 268: 522-6.

Zhang, Y., Y. Jiang, C. Sun, Q. Wang, Z. Yang, X. Pan, M. Zhu, and W. Xiao. 2014. 'The human cathelicidin LL-37 enhances airway mucus production in chronic obstructive pulmonary disease', *Biochem Biophys Res Commun*, 443: 103-9.

# Chapter 4: General conclusions and future research

## 4.1 General conclusions

*Salmonella enterica* serovar Typhimurium infection in human beings is a primary cause of enterocolitis. *Salmonella* Typhimurium is a zoonotic pathogen with a wide host range, including avian and mammalian livestock in addition to people. The contamination of animal food products with this bacterium is of great importance to public health, and pork is a main source of this pathogen infecting human beings (De Freitas Neto *et al.* 2010). Annual exports of pork from Canada represents 8.5% of the total pork exported globally (Brisson *et al.* 2014). The exponential increase in numbers of people has dramatically expanded the local and international demand for pork products, which constitutes challenges for sustainable and environmentally responsible production of pork. Since *S.* Typhimurium is also able to incite disease in pigs, these animals serve as a major non-human reservoir of the pathogen. At present, treatment of *Salmonella* enterocolitis in swine is restricted to medical care including fluid therapy and antibiotics, which is expensive and not always effective (Boyen *et al.* 2008). The use of antibiotics at non-therapeutic levels as growth promoters, therapeutic and metaphylactic treatments has been historically carried out (Brown *et al.* 2017), and as a result, has become a major factor in the development of antimicrobial resistance by pathogens such as *Salmonella* (Haley *et al.* 2012). Preventing contamination of pork in the food production continuum by this pathogen, without the use of antimicrobials, is one of the main challenges that the swine industry faces. On farm strategies (e.g. improving hygiene, reducing animal densities, establishing effect pest control programs, testing *Salmonella* in incoming animals) to mitigate contamination have been extensively investigated (Berends *et al.* 1996). However, to date, no strategy has been demonstrated to completely eliminate the pathogen from farms, and rates of salmonellosis in people has remained relatively constant since the 1950's (Parmley *et al.* 2013).

Surveillance programs are carried out in Canada to detect the presence of *S. enterica* on swine carcasses and pork (Parmley *et al.* 2013), and have been used to differentiate the bacterium into phage types associated with human disease, and to determine the origin of subtypes. Moreover, phage typing has allowed the detection of certain multi-drug resistance phage types and their incidence in human salmonellosis. In this regard, *S. enterica* Typhimurium phage type DT104 has been associated with high morbidity and mortality of human beings worldwide, due at least in part to its resistance to multiple antibiotics (Helms *et al.* 2005). Although salmonellosis has been broadly studied in swine, a more detailed characterization of the disease that includes assessment of pathogen-host-microbiota interactions is still lacking. The presence of a complex microbiota in enteric diseases plays an essential role in protection of the host. The mechanisms of protection conferred to the host by the microbiota

from invading pathogens are referred to as colonization resistance (CR) (Lawley *et al.* 2013). Thus, evaluation of the structure of the microbiota and alterations caused by *S.* Typhimurium are required. Although the mechanisms of CR have been extensively studied, many aspects remain enigmatic and further elucidation is warranted. It has been proposed that the commensal microbiota works in conjunction with the host response to avoid pathogen colonization (Lawley *et al.* 2013). Thus, stimulation of the production of natural antimicrobial peptides (i.e. host defense peptides; HDPs) by the host has been demonstrated to be enhanced by autochthonous enteric microorganisms (Salzman *et al.* 2003; Salzman *et al.* 2007). Cathelicidin is the group of HDPs that has not been extensively studied *in vivo*. However, the *in vitro* antimicrobial activity of cathelicidins against several pathogens, including *Salmonella* Typhimurium (Rosenberger CM *et al.* 2004), has indicated the possibility of using these peptides as non-antibiotic strategy to mitigate enteric infections. However, *in vivo* studies are necessary to elucidate the biological function of these molecules against *S.* Typhimurium, and the interactions occurring within the host and the microbiota.

The two studies presented in this thesis evaluated the impact of enteric infection by *S.* Typhimurium DT104 on: (1) piglets; and (2) mice. In both models of salmonellosis, the host immune response was measured temporally and spatially, with emphasis on determining the localized and systemic impacts of disease. In addition, the comparative characterization of enteric bacterial communities in infected and non-infected animals was completed. To more fully elucidate the role of cathelicidin in salmonellosis, a knock-out murine model was employed, and the impact of antibiotic administration (i.e. streptomycin sulfate) to create a dysbiosis in the enteric autochthonous bacteria community was also examined.

**4.2 Salient outcomes of porcine model of salmonellosis**

a) Establishment of a model of salmonellosis in piglets

b) Temporal (2, 6, and 10 days post-inoculation [dpi]) and spatial (small to large intestine) characterization of the host response and microbiota changes were established under *S.* Typhimurium infection in swine

c) Development of acute, subacute and chronic stages of the disease correspond to 2, 6, and 10 dpi, respectively

d) Piglets at 2 dpi presented higher body temperatures, body weight loss, and suppression of feed consumption

e) The cecum of infected piglets at 2 dpi was the most affected intestinal site presenting higher histopathologic scores (e.g. epithelial injury, inflammatory infiltrate, fibrosis and villus fusion) and upregulation of several proinflammatory cytokines (e.g. *TNFα*, *IFNγ*, *IL17, IL1β, IL8, iNOS*)

f) Upregulation of host defense peptides, defensins (*βD2*), C-type lectins (*REGIIIγ*) and cathelicidins (*PR39*) was documented after *S.* Typhimurium infection

g) The composition of the microbiota as determined by culture-based and culture independent methods differed

h) The structure of the microbiota as determined by next-generation sequence analysis showed that the main changes happened at 2 dpi in the cecum of infected piglets

i) Lower Shannon diversity, an increase in relative abundance of *Proteobacteria*, a decrease in the relative abundance of *Clostridiaceae* and *Lachnospiraceae* were observed in the cecum of infected piglets at 2 dpi

j) Culture-based methods identified an association of certain species (e.g. *Streptococcus gallolyticus* and *Bacteroides uniformis*) with inflamed tissues

k) Infected piglets recovered from the disease at 10 dpi observed as lower histopathologic scores, no changes in gene expression and recovery of microbiota composition

l) Key outcomes of the first study included: (i) temporal (2, 6, 10 dpi) and spatial (small to large intestine) characterization of salmonellosis in pigs was achieved; (ii) reproduction of acute, subacute and chronic stages of the disease corresponded to time points selected; (iii) acute (2dpi) stage of salmonellosis was demonstrated to have the highest impact in host response and microbiota; (iv) the cecum was described as the most affected intestinal site; (v) specific bacteria species (*S. gallolyticus* and *B.uniformis*) were highly associated with inflamed tissues; (vi) upregulation of HDPs (*PR39*) was demonstrated following infection with *S.* Typhimurium

m) The porcine salmonellosis model mimicked self-limiting salmonellosis observed in human beings

**4.3 Salient outcomes of murine cathelicidin knockout model of *Salmonella* Typhimurium infection**

a) Cathelicidin-related antimicrobial peptide (mCRAMP) knockout (KO) mice were more susceptible to local and systemic infection by *S.* Typhimurium

b) Temporal evaluation of salmonellosis in mCRAMP$^{-/-}$ mice showed more severe disease at 48 hpi

c) The cecum was the most affected intestinal site with higher histopathologic damage (e.g. epithelial injury, neutrophil infiltration, goblet cell loss, crypt loss, epithelial hyperplasia, cryptitis and irregular crypt) observed in mCRAMP$^{-/-}$ with or without the administration of streptomycin

d) Proinflammatory genes (*Ifnγ, Kc, Inos, RegIIIγ, Il1β*) were upregulated in infected mCRAMP$^{-/-}$ independent of streptomycin administration

e) Upregulation of mCRAMP occurred in the cecum of infected mCRAMP$^{+/+}$ mice at 48 hpi

f) Higher concentrations of KC and MPO were observed in ileum of infected mCRAMP$^{-/-}$ regardless of streptomycin treatment

g) Higher susceptibility to systemic salmonellosis was observed in infected mCRAMP$^{-/-}$ mice, as indicated by changes in the liver metabolome (e.g. taurine, cadaverine, leucine, valine)

h) The greatest impacts on the liver metabolome were observed at 48 hpi (e.g. phenylalanine, taurine, cadaverine and carnitine) in mCRAMP$^{+/+}$ mice treated with streptomycin, and in mCRAMP$^{-/-}$ mice not administered the antibiotic.

i) The administration of streptomycin sulfate resulted in significant alterations to the composition and diversity of mouse microbiota

j) Non-infected mCRAMP$^{-/-}$ mice not administered streptomycin had a complex microbiota that could harbour mechanisms of CR against the pathogen

k) Conspicuous changes in the composition (e.g. *Proteobacteria* and *Akkermansiaceae*) and diversity (lower Shannon index) of the microbiota were observed in mCRAMP$^{-/-}$ infected mice

l) Key outcomes of the second study included: (i) absence of mCRAMP increased susceptibility to local and systemic salmonellosis; (ii) at 48 hpi infected mice developed a more severe disease; (iii) cecum was the most affected intestinal site; (iv) mCRAMP gene was upregulated in infected mCRAMP$^{+/+}$ at 48 hpi; (v) liver metabolome was more affected at 48 hpi and in mCRAMP null mice; (vi) mCRAMP$^{-/-}$ infected mice showed conspicuous alterations in the microbiota composition

Several studies have been conducted to characterize the pathophysiology of salmonellosis in pigs (Arguello *et al.* 2018; Boyen *et al.* 2008; Chirullo *et al.* 2015). The characterization of the GIT microbiota of piglets has been previously carried out using culture-independent and culture-dependent methods (Arguello *et al.* 2018; Chirullo *et al.* 2015; Drumo *et al.* 2015; Fenske *et al.* 2020). However, to my knowledge, no studies have characterized the temporal changes associated with swine salmonellosis at the histopathologic, immune, and microbiota level. Additionally, the combination of culture-based and culture-independent methods to evaluate the swine microbiota under infection has not previously been described. The results of the study conducted herein provide a better understanding of the progression of this disease in time and space. Importantly, inoculating piglets with *S.* Typhimurium DT104 allowed for the description of clinical signs observed in piglets at the different stages of the disease (acute, subacute, and chronic). Additionally, this study also indicated that animals shed higher loads of pathogen in acute stages of the disease, which progressively decreasing over time. Identifying clinical signs and *Salmonella* shedding patterns in different stages of the disease provides valuable information for the industry. For example, early on farm detection of *S.* Typhimurium infection can reduce

contamination of barns and the food chain (Berends *et al.* 1996). Evaluation of the temporal progression of salmonellosis also showed that although infected piglets recovered fully at 10 dpi, *S.* Typhimurium was still detected in their feces. This is consistent with previous studies that described piglets as reservoirs and intermittent shedders of the pathogen (Scherer *et al.* 2008). The results of the study presented in this thesis also provides a better understanding of the progression of the disease along the GIT. Although other studies have reported infection of the distal small intestine and large intestine (Wilcock *et al.* 1976; Chirullo *et al.* 2015), these studies did not conduct detailed evaluations of mucosal alterations and immune responses. In this regard, my evaluation of the immune response and histopathologic alterations identified the cecum at 2 dpi as the most affected site. Upregulation of proinflammatory genes with the consequent infiltration of neutrophils and damage of the mucosal layer has been previously described as the mechanism responsible for watery diarrhea observed in salmonellosis (Bals *et al.* 2001).

It is thought that *S.* Typhimurium induces inflammation to reduce competition from autochthonous bacteria, and thereby facilitate access to the host enteric mucosa for the pathogen (Chirullo *et al.* 2015). In the current study, the microbiota was characterized using culture-based and culture-independent methods in concert with assessments of the immune responses triggered at different intestinal sites in an effort to elucidate the mechanisms involved. The current emphasis of NGS to characterize the intestinal microbiota presents several limitations including poor taxonomic resolution, poor sensitivity, and an inability to differentiate live from dead bacteria (Inglis *et al.* 2012). In order to overcome these limitations, culturomics was also employed. The composition of the microbiota was observed to differ conspicuously between the two strategies, and the structure of the microbiota was likely best represented by NGS. However, the application of culturomics identified certain taxa that were intimately associated with inflamed tissues, including *S. gallolyticus* and *B. uniformis*. Importantly, culture-based methods allowed me to acquire these bacteria for further *in vitro* and *in vivo* evaluations (i.e. in future studies). *Streptococcus gallolyticus* has previously been associated with colon cancer in humans (Kumar *et al.* 2017). Thus, the isolation of *S. gallolyticus* from inflamed tissues could be intimately related to the ability of this species to survive under hazardous conditions.

Another salient finding of my research was that the cathelicidin, PR-39 was upregulated in the cecum of piglets with salmonellosis. Previous studies have reported that cathelicidins interfere with colonization by pathogens in pigs (Gao *et al.* 2014; Linde *et al.* 2001). In this regard, the second experiment was conducted in mCRAMP KO mice to ascertain the role of cathelicidins in *S.* Typhimurium infection. Within the cathelicidin family, the murine cathelicidin-related antimicrobial peptide

(mCRAMP) has previously been shown to impair colonization of invasive pathogens (Nizet *et al.* 2001; Chromek *et al.* 2006; Iimura *et al.* 2005). However, the role that this peptide plays in *S.* Typhimurium infection has only previously been studied *in vitro* (Gallo *et al.* 1997; Rosenberger CM *et al.* 2004). Additionally, mCRAMP has been proposed to modulate the colonic microbiota maintaining homeostasis (Yoshimura *et al.* 2018). Thus, I aimed to elucidate the temporal role that cathelicidin plays in the modulation of the immune response, the liver metabolome, and the microbiota in mice infected with *S.* Typhimurium. It is noteworthy that mice inoculated with *S.* Typhimurium without previous administration of a broad spectrum antibiotic do not develop enterocolitis; instead they develop a typhoid-like fever (Gal-Mor *et al.* 2014). The administration of streptomycin sulfate to disrupt the microbiota resulting in *Salmonella* enterocolitis has been used to mimic human salmonellosis (Barthel *et al.* 2003). However, the disruption of the microbiota with the consequent elimination of CR is a salient limitation of this dysbiosis model. A primary goal of my research was to comparatively characterize the composition of the microbiota of infected and non-infected mCRAMP$^{+/+}$ and mCRAMP$^{-/-}$ that were and were not administered the antibiotic. I also aimed to evaluate the possibility that infected mCRAMP$^{-/-}$ could develop enteric salmonellosis without the necessity of disrupting their microbiota with an antibiotic. The experiment that I conducted showed that deficiency of mCRAMP conspicuously increased the susceptibility of mice to local and systemic infection by *S.* Typhimurium. Evaluating immune responses at the gene, protein, and cellular levels showed a more severe proinflammatory response triggered in mCRAMP$^{-/-}$ compared to wild type mice that developed both locally and systemically. Additionally, the higher degree of injury to mucosal observed in histopathologic analysis (e.g. epithelial injury, neutrophil infiltration, goblet cell loss, etc.) in mCRAMP$^{-/-}$ mice are consistent with a higher degree of predisposition to *Salmonella* damage in these animals.

Mice infected with *S.* Typhimurium and pretreated with streptomycin can also develop septicemia (Barthel *et al.* 2003), but no previous study to my knowledge has evaluated the effect of *Salmonella* infection on the metabolome of the liver; the liver is a primary organ in which septicemia effects are observed (Carter *et al.* 1974). My research provides a detailed evaluation of the liver metabolome over time. Significant modifications were observed when mice were inoculated with the pathogen. Additionally, these alterations were more severe in mCRAMP deficient mice increasing over time. These findings indicated that absence of mCRAMP predisposes to salmonellosis locally, as well as systemically. Since mCRAMP has been implicated in modulating the microbiota (Yoshimura *et al.* 2018), my research also characterizes the impact of this peptide or lack thereof on the structure of the enteric microbiota ± salmonellosis. Only subtle differences were observed in the composition of the microbiota between

mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ that were not infected with *S*. Typhimurium, which was attributed in the required rearing segregation of these two genotypes. However, the structure of the microbiota was the same in mCRAMP$^{-/-}$ and mCRAMP$^{+/+}$ mice. Significantly, alterations in the microbiota of mCRAMP$^{-/-}$ mice were mainly associated with *Salmonella* infection, exhibited by lower Shannon diversity and increment of *Proteobacteria* and *Akkermansiaceae* observed under infection. In conclusion, my findings showed that mice devoid of cathelicidin were more susceptible to localized and systemic salmonellosis via a combination of mechanisms.

**4.4 Knowledge gaps and future research**

The evaluation of *S.* Typhimurium infection in mice and pigs allowed me to collect valuable information that can be used in future experiments. For example, the experiment that I conducted in piglets with salmonellosis permitted me to identify bacterial species (e.g. *S. gallolyticus* and *B. uniformis*) that were associated with inflamed tissues. The isolation of these species by culture-based methods and their accession into the Intestinal Bacterial Collection (IBaC) at Agriculture and Agri-Food Canada could be utilized for future research. In this regard, these bacteria could be used to determine why they are able to survive in inflamed tissues and what mechanisms they employ to outgrow competing bacteria. Gnotobiotic mice models of inflammation could be employed to ascertain the propensity of these taxa to colonize inflamed tissue. Additionally, the determination of competitive colonization mechanisms in *in vivo* models could be evaluated, including the characterization of the niches occupied by these taxa that allow them to persist under such hostile conditions.

The second experiment that I conducted using mCRAMP KO mice, delivered very valuable information that could be used for future investigations. My research showed that the used of mCRAMP KO mice resulted in enterocolitis without the requirement of administering streptomycin, precluding the confounding impacts of the antibiotic and host-microbiota interaction. Utilization of mCRAMP KO mice as a model of *Salmonella* enterocolitis can be applied to study specific aspects of the host-pathogen-microbiota interaction toward the development of effective mitigation strategies. This model of enterocolitis could be used to study the propensity of bacteria to colonize inflamed tissue incited by the pathogen (see above). As well, this model could be used to further test the differential killing hypothesis associated with *S.* Typhimurium infection. In this regard, determination of SCFA, evaluation of oxygen levels, and the identification of intestinal metabolites could be combined with transcriptomics to elucidate function *in vivo.* One of the potential drawbacks of the mCRAMP KO experiment was the subtle differences observed in composition of the microbiota of KO mice compared to wild type mice. Although the differences observed were subtle, future studies could be conducted in mice in which the

homogeneousness of the microbiota is addressed. For example, germ-free (GF) mCRAMP KO could be generated using recognized methods (e.g. aseptic birth by caesarean section, followed by transfer to lactating GF mothers) and subsequently colonized with a define microbiota or undefined microbiota from mCRAMP$^{+/+}$ mice. A recent study was able to establish CR against *S.* Typhimurim in gnotobiotic mice colonized with a mix of 12 different bacteria from the phyla, *Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia,* and *Actinobacteria* (Brugiroux *et al.* 2016). Thus, future studies could study the mechanisms of CR using GF mCRAMP$^{-/-}$ and GF mCRAMP$^{+/+}$ mice colonized with these taxa followed by inoculation with *S.* Typhimurium. The higher abundance of bacteria within the family, *Akkermansiaceae* that I observed in infected mCRAMP$^{-/-}$ mice could be a direct effect of mCRAMP absence thereby resulting in the predisposition of these animals to salmonellosis. A previous study demonstrated the association of *Akkermansiaceae* family with *S.* Typhimurium infection (Ganesh *et al.* 2013). However, the results that I obtained may indicate that this alteration in the composition of the microbiota could be a consequence of mCRAMP deficiency. Thus, future research could be conducted using gnotobiotic mCRAMP$^{-/-}$ mice to ascertain the role (cause or consequence) of bacteria within the family, *Akkermansiaceae*, including *Akkermansia* species in salmonellosis, and in particular, on enterocolitis.

The development of novel, effective, and non-antibiotic strategies to treat salmonellosis in people and livestock is currently a research priority. The necessity to develop alternatives to antibiotics is highlighted by the increasing development of antimicrobial resistance in pathogens such as *S.* Typhimurium (Helms *et al.* 2005), which limits the effectiveness of medically-important antibiotics. Based on my findings, it is possible that cathelicidins may be an effective and non-antibiotic therapeutic treatment. Experiments could be conducted to deliver mCRAMP as a therapeutic against *Salmonella* enterocolitis using mCRAMP KO mice with validation in pigs. These experiments could provide mechanistic and practical outcomes including enhancement of health condition and shedding reduction. A previous study conducted in mice showed that delivery of mCRAMP by enema mitigated colonic inflammation triggered by dextran sodium sulfate (DSS) (Wu *et al.* 2010). Delivery of cathelicidin by enema does not allow targeted delivery (e.g. to the proximal colon and cecum) nor determination of the therapeutic doses needed to effectively attenuate inflammation. A major obstacle to the development of cathelicidin as a therapeutic treatment is deactivation of the peptide in the proximal alimentary canal (e.g. stomach and small intestine). The formulation of cathelicidin to prevent deactivation in transit to the large intestine would thus be required. In this regard, my research team is helping develop a delivery system that releases bioactive molecules to the distal intestine (i.e. at the site of inflammation), and my

research has led to the inclusion of cathelicidin in the development of this technology. In addition to application as a therapeutic agent, cathelicidins may also have potential as a non-antibiotic alternative to antimicrobial growth promoters (AGPs). In this regard, the intestine is under a state of constant controlled inflammation, which is catabolically costly to the host (Brown *et al.* 2017). Research has shown that some AGPs may function as immunomodulatory agents, thereby damping enteric inflammation allowing increased metabolic energy to be targeted to muscle development (i.e. opposed to mounting an inflammatory response) (Costa *et al.* 2011). This is referred to as the immunomodulation hypothesis of AGP action (Brown *et al.* 2017). It is possible that the delivery of cathelicidin could modulate immune responses within the intestine serving as an effective AGP alternative. This would benefit animal health as well and enhancing economical production of livestock (e.g. by improving feed efficiency), and this possibility warrants investigation using models and metrics developed/facilitated by my research.

### 4.5 References


Arguello, H., J. Estelle, S. Zaldivar-Lopez, A. Jimenez-Marin, A. Carvajal, M. A. Lopez-Bascon, F. Crispie, O. O'Sullivan, P. D. Cotter, F. Priego-Capote, L. Morera, and J. J. Garrido. 2018. 'Early *Salmonella* Typhimurium infection in pigs disrupts microbiome composition and functionality principally at the ileum mucosa', *Sci Rep*, 8: 7788.

Bals, R., C. Lang, D. J. Weiner, C. Vogelmeier, U. Welsch, and J. M. Wilson. 2001. 'Rhesus monkey (Macaca mulatta) mucosal antimicrobial peptides are close homologues of human molecules', *Clin Diagn Lab Immunol*, 8: 370-5.

Barthel, M. , S. Hapfelmeier, L. Quintanilla-Martinez, M. Kremer, M. Rohde, M. Hogardt, K. Pfeffer, H. Russmann, and W. D. Hardt. 2003. 'Pretreatment of Mice with Streptomycin Provides a *Salmonella enterica* Serovar Typhimurium Colitis Model That Allows Analysis of Both Pathogen and Host', *Infect Immun*, 71: 2839-58.

Berends, B. R. , H. A. Urlings, J. M. Snijders, and F. Van Knapen. 1996. 'Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs', *Int J Food Microbiol*, 30: 37-53.

Boyen, F. Haesebrouck, F. Maes, D. Van Immerseel, F. Ducatelle, and F. R. Pasmans. 2008. 'Non-typhoidal *Salmonella* infections in pigs: a closer look at epidemiology, pathogenesis and control', *Vet Microbiol*, 130: 1-19.

Brisson, Yan. 2014. 'The changing face of the Canadian hog industry. Available at: https://www.statcan.gc.ca/'.

Brown, K., R. R. E. Uwiera, M. L. Kalmokoff, S. P. J. Brooks, and G. D. Inglis. 2017. 'Antimicrobial growth promoter use in livestock: a requirement to understand their modes of action to develop effective alternatives', *Int J Antimicrob Agents*, 49: 12-24.

Brugiroux, S., M. Beutler, C. Pfann, D. Garzetti, H. J. Ruscheweyh, D. Ring, M. Diehl, S. Herp, Y. Lotscher, S. Hussain, B. Bunk, R. Pukall, D. H. Huson, P. C. Munch, A. C. McHardy, K. D. McCoy, A. J. Macpherson, A. Loy, T. Clavel, D. Berry, and B. Stecher. 2016. 'Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium', *Nat Microbiol*, 2: 16215.

Carter, P. B., and F. M. Collins. 1974. 'The route of enteric infection in normal mice', *J Exp Med*, 139: 1189-203.

Chirullo, B. , M. Pesciaroli, R. Drumo, J. Ruggeri, E. Razzuoli, C. Pistoia, P. Petrucci, N. Martinelli, L. Cucco, L. Moscati, M. Amadori, C. F. Magistrali, G. L. Alborali, and P. Pasquali. 2015. '*Salmonella* Typhimurium exploits inflammation to its own advantage in piglets', *Front Microbiol*, 6: 985.

Chromek, M., Z. Slamova, P. Bergman, L. Kovacs, L. Podracka, I. Ehren, T. Hokfelt, G. H. Gudmundsson, R. L. Gallo, B. Agerberth, and A. Brauner. 2006. 'The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection', *Nat Med*, 12: 636-41.

Costa, E., R. R. Uwiera, J. P. Kastelic, L. B. Selinger, and G. D. Inglis. 2011. 'Non-therapeutic administration of a model antimicrobial growth promoter modulates intestinal immune responses', *Gut pathogens*, 3: 14.

De Freitas Neto, O. C., Penha Filho, R. A. C., P. Barrow, and A. & Berchieri Junior. 2010. 'Sources of human non-typhoid salmonellosis: a review', *Revista Brasileira de Ciência Avícola*, 12(1): 01-11.

Drumo, R., M. Pesciaroli, J. Ruggeri, M. Tarantino, B. Chirullo, C. Pistoia, P. Petrucci, N. Martinelli, L. Moscati, E. Manuali, S. Pavone, M. Picciolini, S. Ammendola, G. Gabai, A. Battistoni, G. Pezzotti, G. L. Alborali, V. Napolioni, P. Pasquali, and C. F. Magistrali. 2015. '*Salmonella enterica* serovar typhimurium exploits inflammation to modify swine intestinal microbiota', *Front Cell Infect Microbiol*, 5: 106.

Fenske, G. J., S. Ghimire, L. Antony, J. Christopher-Hennings, and J. Scaria. 2020. 'Integration of culture-dependent and independent methods provides a more coherent picture of the pig gut microbiome', *FEMS Microbiol Ecol*, 96.

Gal-Mor, O., E. C. Boyle, and G. A. Grassl. 2014. 'Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ', *Front Microbiol*, 5: 391.

Gallo, R. L., K. J. Kim, M. Bernfield, C. A. Kozak, M. Zanetti, L. Merluzzi, and R. Gennaro. 1997. 'Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse', *J Biol Chem*, 272: 13088-93.

Ganesh, B. P., R. Klopfleisch, G. Loh, and M. Blaut. 2013. 'Commensal *Akkermansia muciniphila* exacerbates gut inflammation in *Salmonella* Typhimurium-infected gnotobiotic mice', *PLoS One*, 8: e74963.

Gao, Y., Y. Rong, Y. Wang, H. Xiong, X. Huang, F. Han, J. Feng, and Y. Wang. 2014. 'Expression pattern of porcine antimicrobial peptide PR-39 and its induction by enterotoxigenic Escherichia coli (ETEC) F4ac', *Vet Immunol Immunopathol*, 160: 260-5.

Haley, C. A. , D. A. Dargatz, E. J. Bush, M. M. Erdman, and P. J. Fedorka-Cray. 2012. '*Salmonella* prevalence and antimicrobial susceptibility from the National Animal Health Monitoring System Swine 2000 and 2006 studies', *J Food Prot*, 75: 428-36.

Helms, M., S. Ethelberg, K. Molbak, and D. T. Study Group. 2005. 'International *Salmonella* Typhimurium DT104 infections, 1992-2001', *Emerg Infect Dis*, 11: 859-67.

Iimura, M., R. L. Gallo, K. Hase, Y. Miyamoto, L. Eckmann, and M. F. Kagnoff. 2005. 'Cathelicidin Mediates Innate Intestinal Defense against Colonization with Epithelial Adherent Bacterial Pathogens', *J Immunol*, 174: 4901-07.

Inglis, G. D., M. C. Thomas, D. K. Thomas, M L. Kalmokoff, S. P. J. Brooks, and L. B. Selinger. 2012. 'Methods to measure intestinal bacteria: a review', *J AOAC Int*, 95: 5-23.

Kumar, R., J. L. Herold, D. Schady, J. Davis, S. Kopetz, M. Martinez-Moczygemba, B. E. Murray, F. Han, Y. Li, E. Callaway, R. S. Chapkin, W. M. Dashwood, R. H. Dashwood, T. Berry, C. Mackenzie, and Y. Xu. 2017. 'Streptococcus gallolyticus subsp. gallolyticus promotes colorectal tumor development', *PLoS Pathog*, 13: e1006440.

Lawley, T. D., and A. W. Walker. 2013. 'Intestinal colonization resistance', *Immunology*, 138: 1-11.

Linde, C. M., S. E. Hoffner, E. Refai, and M. Andersson. 2001. 'In vitro activity of PR-39, a proline-arginine-rich peptide, against susceptible and multi-drug-resistant *Mycobacterium tuberculosis*', *J Antimicrob Chemother*, 47: 575-80.

Nizet, V., T. Ohtake, X. Lauth, J. Trowbridge, J. Rudisill, R. A. Dorschner, V. Pestonjamasp, J. Piraino, K. Huttner, and R. L. Gallo. 2001. 'Innate antimicrobial peptide protects the skin from invasive bacterial infection', *Nature*, 414: 454-7.

Parmley, E. J. , K. Pintar, S. Majowicz, B. Avery, A. Cook, C. Jokinen, V. Gannon, D. R. Lapen, E. Topp, T. A. Edge, M. Gilmour, F. Pollari, R. Reid-Smith, and R. Irwin. 2013. 'A Canadian application of one health: integration of *Salmonella* data from various Canadian surveillance programs (2005-2010)', *Foodborne Pathog Dis*, 10: 747-56.

Rosenberger CM, Gallo RL, and Finlay BB. 2004 Feb 24. 'Interplay between antibacterial effectors: a macrophage antimicrobial peptide impairs intracellular *Salmonella* replication.', *Proc Natl Acad Sci U S A*, 101(8):: 2422-7.

Salzman, N. H., D. Ghosh, K. M. Huttner, Y. Paterson, and C. L. Bevins. 2003. 'Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin', *Nature*, 422: 522-6.

Salzman, N. H., M. A. Underwood, and C. L. Bevins. 2007. 'Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa', *Semin Immunol*, 19: 70-83.

Scherer, K., I. Szabo, U. Rosler, B. Appel, A. Hensel, and K. Nockler. 2008. 'Time course of infection with *Salmonella* Typhimurium and its influence on fecal shedding, distribution in inner organs, and antibody response in fattening pigs', *J Food Prot*, 71: 699-705.

Wilcock, B. P., C. H. Armstrong, and H. J. & Olander. 1976. 'The significance of the serotype in the clinical and pathological features of naturally occurring porcine salmonellosis.', *Can J Comp Med*, 40: 80.

Wu, W. K., C. C. Wong, Z. J. Li, L. Zhang, S. X. Ren, and C. H. Cho. 2010. 'Cathelicidins in inflammation and tissue repair: Potential therapeutic applications for gastrointestinal disorders', *Acta Pharmacol Sin*, 31: 1118-22.

Yoshimura, T., M. H. McLean, A. K. Dzutsev, X. Yao, K. Chen, J. Huang, W. Gong, J. Zhou, Y. Xiang, H. Badger J, C. O'HUigin, V. Thovarai, L. Tessarollo, S. K. Durum, G. Trinchieri, X. W. Bian, and J. M. Wang. 2018. 'The Antimicrobial Peptide CRAMP Is Essential for Colon Homeostasis by Maintaining Microbiota Balance', *J Immunol*, 200: 2174-85.

## Comprehensive reference list


Agbor, T. A., and B. A. McCormick. 2011. '*Salmonella* effectors: important players modulating host cell function during infection', *Cell Microbiol*, 13: 1858-69.

Al-Jashamy, K., A. Murad, M. Zeehaida, M. Rohaini, and J. Hasnan. 2010. 'Prevalence of colorectal cancer associated with *Streptococcus bovis* among inflammatory bowel and chronic gastrointestinal tract disease patients', *Asian Pac J Cancer Prev*, 11: 1765-8.

Alain, B. Pajarillo E., J. P. Chae, M. P. Balolong, H. Bum Kim, and D. K. Kang. 2014. 'Assessment of fecal bacterial diversity among healthy piglets during the weaning transition', *J Gen Appl Microbiol*, 60: 140-6.

Ali, M. M., D. L. Newsom, J. F. Gonzalez, A. Sabag-Daigle, C. Stahl, B. Steidley, J. Dubena, J. L. Dyszel, J. N. Smith, Y. Dieye, R. Arsenescu, P. N. Boyaka, S. Krakowka, T. Romeo, E. J. Behrman, P. White, and B. M. Ahmer. 2014. 'Fructose-asparagine is a primary nutrient during growth of *Salmonella* in the inflamed intestine', *PLoS Pathog*, 10: e1004209.

Allen, H. K., T. Looft, D. O. Bayles, S. Humphrey, U. Y. Levine, D. Alt, and T. B. Stanton. 2011. 'Antibiotics in feed induce prophages in swine fecal microbiomes', *MBio*, 2.

Althouse, C. , S. Patterson, P. Fedorka-Cray, and R. E. Isaacson. 2003. 'Type 1 fimbriae of *Salmonella enterica* serovar Typhimurium bind to enterocytes and contribute to colonization of swine in vivo', *Infect Immun*, 71: 6446-52.

Anderson, Paul E., Deirdre A. Mahle, Travis E. Doom, Nicholas V. Reo, Nicholas J. DelRaso, and Michael L. Raymer. 2010. 'Dynamic adaptive binning: an improved quantification technique for NMR spectroscopic data', *Metabolomics*, 7: 179-90.

Arguello, H., J. Estelle, S. Zaldivar-Lopez, A. Jimenez-Marin, A. Carvajal, M. A. Lopez-Bascon, F. Crispie, O. O'Sullivan, P. D. Cotter, F. Priego-Capote, L. Morera, and J. J. Garrido. 2018. 'Early *Salmonella* Typhimurium infection in pigs disrupts microbiome composition and functionality principally at the ileum mucosa', *Sci Rep*, 8: 7788.

Arpaia, N., J. Godec, L. Lau, K. E. Sivick, L. M. McLaughlin, M. B. Jones, T. Dracheva, S. N. Peterson, D. M. Monack, and G. M. Barton. 2011. 'TLR signaling is required for *Salmonella* Typhimurium virulence', *Cell*, 144: 675-88.

Artis, D. 2008. 'Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut', *Nat Rev Immunol*, 8: 411-20.

Baggesen, D. L., G. Sorensen, E. M. Nielsen, and H. C. Wegener. 2010. 'Phage typing of *Salmonella* Typhimurium - is it still a useful tool for surveillance and outbreak investigation?', *Euro Surveill*, 15: 19471.

Balaji, R., K. J. Wright, C. M. Hill, S. S. Dritz, E. L. Knoppel, and J. E. Minton. 2000. 'Acute phase responses of pigs challenged orally with *Salmonella* Typhimurium', *J Anim Sci*, 78: 1885-91.

Bals, R., C. Lang, D. J. Weiner, C. Vogelmeier, U. Welsch, and J. M. Wilson. 2001. 'Rhesus monkey (Macaca mulatta) mucosal antimicrobial peptides are close homologues of human molecules', *Clin Diagn Lab Immunol*, 8: 370-5.

Bals, R., and J. M. Wilson. 2003. 'Cathelicidins a family of multifunctional antimicrobial peptides', *Cell Mol Life Sci*, 60: 711-20.

Barnes, D. M., Z. Song, K. C. Klasing, and W. Bottje. 2002. 'Protein metabolism during an acute phase response in chickens', *Amino Acids*, 22: 15-26.

Barthel, M. , S. Hapfelmeier, L. Quintanilla-Martinez, M. Kremer, M. Rohde, M. Hogardt, K. Pfeffer, H. Russmann, and W. D. Hardt. 2003. 'Pretreatment of Mice with Streptomycin Provides a *Salmonella enterica* Serovar Typhimurium Colitis Model That Allows Analysis of Both Pathogen and Host', *Infect Immun*, 71: 2839-58.

Bearson, S. M., H. K. Allen, B. L. Bearson, T. Looft, B. W. Brunelle, J. D. Kich, C. K. Tuggle, D. O. Bayles, D. Alt, U. Y. Levine, and T. B. Stanton. 2013. 'Profiling the gastrointestinal microbiota in response to *Salmonella*: low versus high *Salmonella* shedding in the natural porcine host', *Infect Genet Evol*, 16: 330-40.

Berdy, B., A. L. Spoering, L. L. Ling, and S. S. Epstein. 2017. 'In situ cultivation of previously uncultivable microorganisms using the ichip', *Nat Protoc*, 12: 2232-42.

Berends, B. R. , H. A. Urlings, J. M. Snijders, and F. Van Knapen. 1996. 'Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs', *Int J Food Microbiol*, 30: 37-53.

Bohnhoff, M. , B. L. Drake, and C. P. Miller. 1954. 'Effect of streptomycin on susceptibility of intestinal tract to experimental *Salmonella* infection', *Proc Soc Exp Biol Med*, 86: 132-7.

Boleij, A., and H. Tjalsma. 2013. 'The itinerary of *Streptococcus gallolyticus* infection in patients with colonic malignant disease', *Lancet Infect Dis*, 13: 719-24.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodriguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. B. Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y. X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, 2nd, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vazquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. 'Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2', *Nat Biotechnol*, 37: 852-57.

Boyen, F. , F. Pasmans, F. Van Immerseel, E. Morgan, C. Adriaensen, J. P. Hernalsteens, A. Decostere, R. Ducatelle, and F. Haesebrouck. 2006. '*Salmonella* Typhimurium SPI-1 genes promote intestinal but not tonsillar colonization in pigs', *Microbes Infect*, 8: 2899-907.

Boyen, F. Haesebrouck, F. Maes, D. Van Immerseel, F. Ducatelle, and F. R. Pasmans. 2008. 'Non-typhoidal *Salmonella* infections in pigs: a closer look at epidemiology, pathogenesis and control', *Vet Microbiol*, 130: 1-19.

Boyen, F., F. Haesebrouck, A. Vanparys, J. Volf, M. Mahu, F. Van Immerseel, I. Rychlik, J. Dewulf, R. Ducatelle, and F. Pasmans. 2008. 'Coated fatty acids alter virulence properties of *Salmonella* Typhimurium and decrease intestinal colonization of pigs', *Vet Microbiol*, 132: 319-27.

Boyer, P. E., S. D'Costa, L. L. Edwards, M. Milloway, E. Susick, L. B. Borst, S. Thakur, J. M. Campbell, J. D. Crenshaw, J. Polo, and A. J. Moeser. 2015. 'Early-life dietary spray-dried plasma influences immunological and intestinal injury responses to later-life *Salmonella* Typhimurium challenge', *Br J Nutr*, 113: 783-93.

Boyle, E. C. , N. F. Brown, and B. B. Finlay. 2006. '*Salmonella enterica* serovar Typhimurium effectors SopB, SopE, SopE2 and SipA disrupt tight junction structure and function', *Cell Microbiol*, 8: 1946-57.

Brisson, Yan. 2014. 'The changing face of the Canadian hog industry. Available at: https://www.statcan.gc.ca/'.

Brogden, K. A. 2005. 'Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?', *Nat Rev Microbiol*, 3: 238-50.

Brooks SPJ, Green-Johnson J, Inglis GD, Uwiera RRE, Kalmokoff M:. 2011. *Gut microbiology - relatively unexplored domain* (Comprehensive Biotechnology).

Brown, K., R. R. E. Uwiera, M. L. Kalmokoff, S. P. J. Brooks, and G. D. Inglis. 2017. 'Antimicrobial growth promoter use in livestock: a requirement to understand their modes of action to develop effective alternatives', *Int J Antimicrob Agents*, 49: 12-24.

Browne, H. P., S. C. Forster, B. O. Anonye, N. Kumar, B. A. Neville, M. D. Stares, D. Goulding, and T. D. Lawley. 2016. 'Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation', *Nature*, 533: 543-6.

Broz, P., M. B. Ohlson, and D. M. Monack. 2012. 'Innate immune response to *Salmonella* Typhimurium, a model enteric pathogen', *Gut Microbes*, 3: 62-70.

Brugiroux, S., M. Beutler, C. Pfann, D. Garzetti, H. J. Ruscheweyh, D. Ring, M. Diehl, S. Herp, Y. Lotscher, S. Hussain, B. Bunk, R. Pukall, D. H. Huson, P. C. Munch, A. C. McHardy, K. D. McCoy, A. J. Macpherson, A. Loy, T. Clavel, D. Berry, and B. Stecher. 2016. 'Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium', *Nat Microbiol*, 2: 16215.

Bui, T. P., J. Ritari, S. Boeren, P. de Waard, C. M. Plugge, and W. M. de Vos. 2015. 'Production of butyrate from lysine and the Amadori product fructoselysine by a human gut commensal', *Nat Commun*, 6: 10062.

Burgos-Ramos, E., J. A. Chowen, E. Arilla-Ferreiro, S. Canelles, J. Argente, and V. Barrios. 2011. 'Chronic central leptin infusion modifies the response to acute central insulin injection by reducing the interaction of the insulin receptor with IRS2 and increasing its association with SOCS3', *J Neurochem*, 117: 175-85.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. Johnson, and S. P. Holmes. 2016. 'DADA2: High-resolution sample inference from Illumina amplicon data', *Nat Methods*, 13: 581-3.

Canibe, N. , O. Hojberg, S. Hojsgaard, and B. B. Jensen. 2005. 'Feed physical form and formic acid addition to the feed affect the gastrointestinal ecology and growth performance of growing pigs', *J Anim Sci*, 83: 1287-302.

Carter, P. B., and F. M. Collins. 1974. 'The route of enteric infection in normal mice', *J Exp Med*, 139: 1189-203.

Casey, P. G. , G. E. Gardiner, G. Casey, B. Bradshaw, P. G. Lawlor, P. B. Lynch, F. C. Leonard, C. Stanton, R. P. Ross, G. F. Fitzgerald, and C. Hill. 2007. 'A five-strain probiotic combination reduces pathogen shedding and alleviates disease signs in pigs challenged with *Salmonella enterica* Serovar Typhimurium', *Appl Environ Microbiol*, 73: 1858-63.

Castillo, M., S. M. Martin-Orue, E. G. Manzanilla, I. Badiola, M. Martin, and J. Gasa. 2006. 'Quantification of total bacteria, enterobacteria and lactobacilli populations in pig digesta by real-time PCR', *Vet Microbiol*, 114: 165-70.

Cherayil, B. J., and D. Antos. 2001. 'Inducible nitric oxide synthase and *Salmonella* infection', *Microbes Infect*, 3: 771-6.

Chirullo, B. , M. Pesciaroli, R. Drumo, J. Ruggeri, E. Razzuoli, C. Pistoia, P. Petrucci, N. Martinelli, L. Cucco, L. Moscati, M. Amadori, C. F. Magistrali, G. L. Alborali, and P. Pasquali. 2015. '*Salmonella* Typhimurium exploits inflammation to its own advantage in piglets', *Front Microbiol*, 6: 985.

Chong, J., M. Yamamoto, and J. Xia. 2019. 'MetaboAnalystR 2.0: From Raw Spectra to Biological Insights', *Metabolites*, 9.

Chromek, M., Z. Slamova, P. Bergman, L. Kovacs, L. Podracka, I. Ehren, T. Hokfelt, G. H. Gudmundsson, R. L. Gallo, B. Agerberth, and A. Brauner. 2006. 'The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection', *Nat Med*, 12: 636-41.

Clark, M. A., M. A. Jepson, N. L. Simmons, and B. H. Hirst. 1994. 'Preferential interaction of *Salmonella* Typhimurium with mouse Peyer's patch M cells', *Res Microbiol*, 145: 543-52.

Coburn, B. , G. A. Grassl, and B. B. Finlay. 2007. '*Salmonella*, the host and disease: a brief review', *Immunol Cell Biol*, 85: 112-8.

Cole, J. R., Q. Wang, J. A. Fish, B. Chai, D. M. McGarrell, Y. Sun, C. T. Brown, A. Porras-Alfaro, C. R. Kuske, and J. M. Tiedje. 2014. 'Ribosomal Database Project: data and tools for high throughput rRNA analysis', *Nucleic Acids Res*, 42: D633-42.

Collado-Romero, M. , C. Arce, M. Ramirez-Boo, A. Carvajal, and J. J. Garrido. 2010. 'Quantitative analysis of the immune response upon *Salmonella* Typhimurium infection along the porcine intestinal gut', *Vet Res*, 41: 23.

Costa, E., N. J. Puhl, L. B. Selinger, and G. D. Inglis. 2009. 'Characterization of mucosa-associated bacterial communities of the mouse intestine by terminal restriction fragment length polymorphism: utility of sampling strategies and methods to reduce single-stranded DNA artifacts', *J Microbiol Methods*, 78: 175-80.

Costa, E., R. R. Uwiera, J. P. Kastelic, L. B. Selinger, and G. D. Inglis. 2011. 'Non-therapeutic administration of a model antimicrobial growth promoter modulates intestinal immune responses', *Gut pathogens*, 3: 14.

Creus, E. , J. F. Perez, B. Peralta, F. Baucells, and E. Mateu. 2007. 'Effect of acidified feed on the prevalence of *Salmonella* in market-age pigs', *Zoonoses Public Hlth*, 54: 314-9.

De Freitas Neto, O. C., Penha Filho, R. A. C., P. Barrow, and A. & Berchieri Junior. 2010. 'Sources of human non-typhoid salmonellosis: a review', *Rev Bras Cienc Avic*, 12(1): 01-11.

den Besten, G., K. van Eunen, A. K. Groen, K. Venema, D. J. Reijngoud, and B. M. Bakker. 2013. 'The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism', *J Lipid Res*, 54: 2325-40.

Donaldson, G. P., S. M. Lee, and S. K. Mazmanian. 2016. 'Gut biogeography of the bacterial microbiota', *Nat Rev Microbiol*, 14: 20-32.

Dou, S., P. Gadonna-Widehem, V. Rome, D. Hamoudi, L. Rhazi, L. Lakhal, T. Larcher, N. Bahi-Jaber, A. Pinon-Quintana, A. Guyonvarch, I. L. Huerou-Luron, and L. Abdennebi-Najar. 2017. 'Characterisation of early-life fecal microbiota in susceptible and healthy pigs to post-weaning diarrhoea', *PLoS One*, 12: e0169851.

Drumo, R., M. Pesciaroli, J. Ruggeri, M. Tarantino, B. Chirullo, C. Pistoia, P. Petrucci, N. Martinelli, L. Moscati, E. Manuali, S. Pavone, M. Picciolini, S. Ammendola, G. Gabai, A. Battistoni, G. Pezzotti, G. L. Alborali, V. Napolioni, P. Pasquali, and C. F. Magistrali. 2015. '*Salmonella enterica* serovar typhimurium exploits inflammation to modify swine intestinal microbiota', *Front Cell Infect Microbiol*, 5: 106.

Dubin, P. J., and J. K. Kolls. 2007. 'IL-23 mediates inflammatory responses to mucoid *Pseudomonas aeruginosa* lung infection in mice', *Am J Physiol Lung Cell Mol Physiol*, 292: L519-28.

Duimstra, J. R., L. L. Myers, J. E. Collins, D. A. Benfield, D. S. Shoop, and W. C. Bradbury. 1991. 'Enterovirulence of enterotoxigenic *Bacteroides fragilis* in gnotobiotic pigs', *Vet Pathol*, 28: 514-8.

Edgar, R. C. 2004. 'MUSCLE: multiple sequence alignment with high accuracy and high throughput', *Nucleic Acids Res*, 32: 1792-7.

Erben, Ulrike, Christoph Loddenkemper, Katja Doerfel, Simone Spieckermann, Dirk Haller, Markus M. Heimesaat, Martin Zeitz, Britta Siegmund, and and Anja A. Kühl. 2014. 'A guide to histomorphological evaluation of intestinal inflammation in mouse models.', *Int J Clin Exp Pathol*, 7: 4557.

Everest, P., J. Ketley, S. Hardy, G. Douce, S. Khan, J. Shea, D. Holden, D. Maskell, and G. Dougan. 1999. 'Evaluation of *Salmonella* Typhimurium mutants in a model of experimental gastroenteritis', *Infect Immun*, 67: 2815-21.

Fan, D., L. A. Coughlin, M. M. Neubauer, J. Kim, M. S. Kim, X. Zhan, T. R. Simms-Waldrip, Y. Xie, L. V. Hooper, and A. Y. Koh. 2015. 'Activation of HIF-1alpha and LL-37 by commensal bacteria inhibits *Candida albicans* colonization', *Nat Med*, 21: 808-14.

Fan, F., Y. Wu, and J. Liu. 2010. 'Expression and purification of two different antimicrobial peptides, PR-39 and Protegrin-1 in *Escherichia coli*', *Protein Expr Purif*, 73: 147-51.

Fattorossi, A., R. Biselli, A. Casciaro, S. Tzantzoglou, and C. de Simone. 1993. 'Regulation of normal human polymorphonuclear leucocytes by carnitine', *Mediators Inflamm*, 2: S37-41.

Fedorka-Cray, P. J. , L. C. Kelley, T. J. Stabel, J. T. Gray, and J. A. Laufer. 1995. 'Alternate routes of invasion may affect pathogenesis of *Salmonella* Typhimurium in swine', *Infect Immun*, 63: 2658-64.

Fenske, G. J., S. Ghimire, L. Antony, J. Christopher-Hennings, and J. Scaria. 2020. 'Integration of culture-dependent and independent methods provides a more coherent picture of the pig gut microbiome', *FEMS Microbiol Ecol*, 96.

Fernandez, I. M., M. Silva, R. Schuch, W. A. Walker, A. M. Siber, A. T. Maurelli, and B. A. McCormick. 2001. 'Cadaverine prevents the escape of *Shigella flexneri* from the phagolysosome: a connection between bacterial dissemination and neutrophil transepithelial signaling', *J Infect Dis*, 184: 743-53.

'FoodNet Canada annual report 2017'. 2018. *Available at: https://www.canada.ca/*.

Freter, R., H. Brickner, M. Botney, D. Cleven, and A. Aranki. 1983. 'Mechanisms that control bacterial populations in continuous-flow culture models of mouse large intestinal flora', *Infect Immun*, 39: 676-85.

Fu, Y., and J. E. A & Galán. 1999. '*Salmonella* protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion.', *Nature,*, 401(6750): 293-97.

Furter, M., M. E. Sellin, G. C. Hansson, and W. D. Hardt. 2019. 'Mucus Architecture and Near-Surface Swimming Affect Distinct *Salmonella* Typhimurium Infection Patterns along the Murine Intestinal Tract', *Cell Rep*, 27: 2665-78 e3.

Fyderek, K., M. Strus, K. Kowalska-Duplaga, T. Gosiewski, A. Wedrychowicz, U. Jedynak-Wasowicz, M. Sladek, S. Pieczarkowski, P. Adamski, P. Kochan, and P. B. Heczko. 2009. 'Mucosal bacterial microflora and mucus layer thickness in adolescents with inflammatory bowel disease', *World J Gastroenterol*, 15: 5287-94.

Gal-Mor, O., E. C. Boyle, and G. A. Grassl. 2014. 'Same species, different diseases: how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ', *Front Microbiol*, 5: 391.

Gallo, R. L., and L. V. Hooper. 2012. 'Epithelial antimicrobial defence of the skin and intestine', *Nat Rev Immunol*, 12: 503-16.

Gallo, R. L., K. J. Kim, M. Bernfield, C. A. Kozak, M. Zanetti, L. Merluzzi, and R. Gennaro. 1997. 'Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse', *J Biol Chem*, 272: 13088-93.

Gallo, R. L., M. Ono, T. Povsic, C. Page, E. Eriksson, M. Klagsbrun, and M. Bernfield. 1994. 'Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich antimicrobial peptide from wounds', *Proc Natl Acad Sci U S A*, 91: 11035-9.

Ganesh, B. P., R. Klopfleisch, G. Loh, and M. Blaut. 2013. 'Commensal *Akkermansia muciniphila* exacerbates gut inflammation in *Salmonella* Typhimurium-infected gnotobiotic mice', *PLoS One*, 8: e74963.

Gantois, I., R. Ducatelle, F. Pasmans, F. Haesebrouck, I. Hautefort, A. Thompson, J. C. Hinton, and F. Van Immerseel. 2006. 'Butyrate specifically down-regulates salmonella pathogenicity island 1 gene expression', *Appl Environ Microbiol*, 72: 946-9.

Gao, Y., Y. Rong, Y. Wang, H. Xiong, X. Huang, F. Han, J. Feng, and Y. Wang. 2014. 'Expression pattern of porcine antimicrobial peptide PR-39 and its induction by enterotoxigenic Escherichia coli (ETEC) F4ac', *Vet Immunol Immunopathol*, 160: 260-5.

Garner, C. D., D. A. Antonopoulos, B. Wagner, G. E. Duhamel, I. Keresztes, D. A. Ross, V. B. Young, and C. Altier. 2009. 'Perturbation of the small intestine microbial ecology by streptomycin alters pathology in a *Salmonella enterica* serovar Typhimurium murine model of infection', *Infect Immun*, 77: 2691-702.

Gewirtz, A. T. , T. A. Navas, S. Lyons, P. J. Godowski, and J. L. Madara. 2001. 'Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression', *J Immunol*, 167: 1882-5.

Gewirtz, A. T. , P. O. Simon, C. K. Jr. Schmitt, L. J. Taylor, C. H. Hagedorn, A. D. O'Brien, A. S. Neish, and J. L. Madara. 2001. '*Salmonella* Typhimurium translocates flagellin across intestinal epithelia, inducing a proinflammatory response', *J Clin Invest*, 107: 99-109.

Giannella, R. A., S. B. Formal, G. J. Dammin, and H. & Collins. 1973. 'Pathogenesis of salmonellosis Studies of fluid secretion, mucosal invasion, and morphologic reaction in the rabbit ileum.', *J Clin Invest*, 52(2): 441.

Glynn, J. R. , and S. R. Palmer. 1992. 'Incubation period, severity of disease, and infecting dose: evidence from a *Salmonella* outbreak', *Am J Epidemiol*, 136: 1369-77.

Godinez, I., T. Haneda, M. Raffatellu, M. D. George, T. A. Paixao, H. G. Rolan, R. L. Santos, S. Dandekar, R. M. Tsolis, and A. J. Baumler. 2008. 'T cells help to amplify inflammatory responses induced by *Salmonella enterica* serotype Typhimurium in the intestinal mucosa', *Infect Immun*, 76: 2008-17.

Godinez, I., M. Raffatellu, H. Chu, T. A. Paixao, T. Haneda, R. L. Santos, C. L. Bevins, R. M. Tsolis, and A. J. Baumler. 2009. 'Interleukin-23 orchestrates mucosal responses to *Salmonella enterica* serotype Typhimurium in the intestine', *Infect Immun*, 77: 387-98.

Goodpaster, A. M., L. E. Romick-Rosendale, and M. A. Kennedy. 2010. 'Statistical significance analysis of nuclear magnetic resonance-based metabonomics data', *Anal Biochem*, 401: 134-43.

Gu, S., D. Chen, J. N. Zhang, X. Lv, K. Wang, L. P. Duan, Y. Nie, and X. L. Wu. 2013. 'Bacterial community mapping of the mouse gastrointestinal tract', *PLoS One*, 8: e74957.

Gudmundsson, G. H., B. Agerberth, J. Odeberg, T. Bergman, B. Olsson, and R. Salcedo. 1996. 'The human gene FALL39 and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes', *Eur J Biochem*, 238: 325-32.

Gutzmann, F. , H. Layton, K. Simkins, and H. Jarolmen. 1976. 'Influence of antibiotic-supplemented feed on occurrence and persistence of *Salmonella* typhimurium in experimentally infected swine', *Am J Vet Res*, 37: 649-55.

Haley, C. A. , D. A. Dargatz, E. J. Bush, M. M. Erdman, and P. J. Fedorka-Cray. 2012. '*Salmonella* prevalence and antimicrobial susceptibility from the National Animal Health Monitoring System Swine 2000 and 2006 studies', *J Food Prot*, 75: 428-36.

Hammami, R., B. Fernandez, C. Lacroix, and I. Fliss. 2013. 'Anti-infective properties of bacteriocins: an update', *Cell Mol Life Sci*, 70: 2947-67.

Hapfelmeier, S. , and W. D. Hardt. 2005. 'A mouse model for *S.* typhimurium-induced enterocolitis', *Trends Microbiol*, 13: 497-503.

Hardt, W. D., L. M. Chen, K. E. Schuebel, X. R. Bustelo, and J. E & Galán. 1998. '*S.* typhimurium encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells.', *Cell Host Microbe*, 93(5): 815-26.

Hasegawa, M., N. Kamada, Y. Jiao, M. Z. Liu, G. Nunez, and N. Inohara. 2012. 'Protective role of commensals against *Clostridium difficile* infection via an IL-1beta-mediated positive-feedback loop', *J Immunol*, 189: 3085-91.

He, T., Y. H. Zhu, J. Yu, B. Xia, X. Liu, G. Y. Yang, J. H. Su, L. Guo, M. L. Wang, and J. F. Wang. 2019. '*Lactobacillus johnsonii* L531 reduces pathogen load and helps maintain short-chain fatty acid levels in the intestines of pigs challenged with *Salmonella enterica* Infantis', *Vet Microbiol*, 230: 187-94.

Helms, M., S. Ethelberg, K. Molbak, and D. T. Study Group. 2005. 'International *Salmonella* Typhimurium DT104 infections, 1992-2001', *Emerg Infect Dis*, 11: 859-67.
Hensel, M. 2000. '*Salmonella* pathogenicity island 2', *Mol Microbiol*, 36: 1015-23.
Henzler, D. J. , and H. M. Opitz. 1992. 'The role of mice in the epizootiology of *Salmonella* Enteritidis infection on chicken layer farms', *Avian Dis*, 36: 625-31.
Hobbie, S., L. M. Chen, R. J. Davis, and J. E. & Galan. 1997. 'Involvement of mitogen-activated protein kinase pathways in the nuclear responses and cytokine production induced by *Salmonella* Typhimurium in cultured intestinal epithelial cells.', *J Immunol*, 159(11): 5550-59.
Hohmann, A. W., G. Schmidt, and D. Rowley. 1978. 'Intestinal colonization and virulence of *Salmonella* in mice', *Infect Immun*, 22: 763-70.
Holani, R., C. Shah, Q. Haji, G. D. Inglis, R. R. E. Uwiera, and E. R. Cobo. 2016. 'Proline-arginine rich (PR-39) cathelicidin: structure, expression and functional implication in intestinal health', *Comp Immunol Microbiol Infect Dis*, 49: 95-101.
Hooper, L. V., T. Midtvedt, and J. I. Gordon. 2002. 'How host-microbial interactions shape the nutrient environment of the mammalian intestine', *Annu Rev Nutr*, 22: 283-307.
Iacob, S., D. G. Iacob, and L. M. Luminos. 2018. 'Intestinal Microbiota as a Host Defense Mechanism to Infectious Threats', *Front Microbiol*, 9: 3328.
Iimura, M., R. L. Gallo, K. Hase, Y. Miyamoto, L. Eckmann, and M. F. Kagnoff. 2005. 'Cathelicidin Mediates Innate Intestinal Defense against Colonization with Epithelial Adherent Bacterial Pathogens', *J Immunol*, 174: 4901-07.
Inglis, G. D., M. C. Thomas, D. K. Thomas, M L. Kalmokoff, S. P. J. Brooks, and L. B. Selinger. 2012. 'Methods to measure intestinal bacteria: a review', *J AOAC Int*, 95: 5-23.
Ivanov, II, K. Atarashi, N. Manel, E. L. Brodie, T. Shima, U. Karaoz, D. Wei, K. C. Goldfarb, C. A. Santee, S. V. Lynch, T. Tanoue, A. Imaoka, K. Itoh, K. Takeda, Y. Umesaki, K. Honda, and D. R. Littman. 2009. 'Induction of intestinal Th17 cells by segmented filamentous bacteria', *Cell*, 139: 485-98.
James Kozich, Patrick Schloss, Niel Baxter, Matt Jenior, Charles Koumpouras, Lucas Bishop. 2013. Access at https://github.com/SchlossLab/MiSeq_WetLab_SOP/blob/master/MiSeq_WetLab_SOP.md. "16S rRNA sequencing with the Illumina MiSeq: library generation, QC, & sequencing." In.
Jana, B., and D. Salomon. 2019. 'Type VI secretion system: a modular toolkit for bacterial dominance', *Future Microbiol*, 14: 1451-63.
Jiminez, J. A., T. C. Uwiera, D. W. Abbott, R. R. E. Uwiera, and G. D. Inglis. 2017. 'Butyrate supplementation at high concentrations alters enteric bacterial communities and reduces intestinal inflammation in mice infected with *Citrobacter rodentium*', *mSphere*, 2.
Johnson, B. J., Dritz, S. S., Skjolaas-Wilson, K. A., Burkey, T. E., & Minton, J. E. 2005. 'Interactive responses in gut immunity, and systemic and local changes in the insulin-like growth factor system in nursery pigs in response to *Salmonella enterica* serovar Typhimurium.', *J Ani Sci*, 83: E48-E56.
Jones, B. D., N. Ghori, and S & Falkow. 1994. '*Salmonella* typhimurium initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches.', *J Exp Med*, 180(1): 15-23.
Jung, T. H., J. H. Park, W. M. Jeon, and K. S. Han. 2015. 'Butyrate modulates bacterial adherence on LS174T human colorectal cells by stimulating mucin secretion and MAPK signaling pathway', *Nutr Res Pract*, 9: 343-9.
Kaiser, P., M. Diard, B. Stecher, and W. D. & Hardt. (2012). 'The streptomycin mouse model for *Salmonella* diarrhea: functional analysis of the microbiota, the pathogen's virulence factors, and the host's mucosal immune response', *Immunol Rev*, 245: 56-83.
Kaiser, P., M. Diard, B. Stecher, and W. D. Hardt. 2012. 'The streptomycin mouse model for *Salmonella* diarrhea: functional analysis of the microbiota, the pathogen's virulence factors, and the host's mucosal immune response', *Immunol Rev*, 245: 56-83.

Kelly, J., K. Daly, A. W. Moran, S. Ryan, D. Bravo, and S. P. Shirazi-Beechey. 2017. 'Composition and diversity of mucosa-associated microbiota along the entire length of the pig gastrointestinal tract; dietary influences', *Environ Microbiol*, 19: 1425-38.

Keshav, S. 2006. 'Paneth cells: leukocyte-like mediators of innate immunity in the intestine', *J Leukoc Biol*, 80: 500-8.

Kim, C. H. , D. Kim, Y. Ha, K. D. Cho, B. H. Lee, I. W. Seo, S. H. Kim, and C. Chae. 2009. 'Expression of mucins and trefoil factor family protein-1 in the colon of pigs naturally infected with *Salmonella* Typhimurium', *J Comp Pathol*, 140: 38-42.

Koczulla, R., G. von Degenfeld, C. Kupatt, F. Krotz, S. Zahler, T. Gloe, K. Issbrucker, P. Unterberger, M. Zaiou, C. Lebherz, A. Karl, P. Raake, A. Pfosser, P. Boekstegers, U. Welsch, P. S. Hiemstra, C. Vogelmeier, R. L. Gallo, M. Clauss, and R. Bals. 2003. 'An angiogenic role for the human peptide antibiotic LL-37/hCAP-18', *J Clin Invest*, 111: 1665-72.

Koelink, P. J., M. E. Wildenberg, L. W. Stitt, B. G. Feagan, M. Koldijk, A. B. van 't Wout, R. Atreya, M. Vieth, J. F. Brandse, S. Duijst, A. A. Te Velde, Gram D'Haens, B. G. Levesque, and G. R. van den Brink. 2018. 'Development of Reliable, Valid and Responsive Scoring Systems for Endoscopy and Histology in Animal Models for Inflammatory Bowel Disease', *J Crohns Colitis*, 12: 794-803.

Kohler, H., S. P. Rodrigues, A. T. Maurelli, and B. A. McCormick. 2002. 'Inhibition of *Salmonella* Typhimurium enteropathogenicity by piperidine, a metabolite of the polyamine cadaverine', *J Infect Dis*, 186: 1122-30.

Koon, H. W., D. Q. Shih, J. Chen, K. Bakirtzi, T. C. Hing, I. Law, S. Ho, R. Ichikawa, D. Zhao, H. Xu, R. Gallo, P. Dempsey, G. Cheng, S. R. Targan, and C. Pothoulakis. 2011. 'Cathelicidin signaling via the Toll-like receptor protects against colitis in mice', *Gastroenterology*, 141: 1852-63 e1-3.

Kress, E., J. Merres, L. J. Albrecht, S. Hammerschmidt, T. Pufe, S. C. Tauber, and L. O. Brandenburg. 2017. 'CRAMP deficiency leads to a pro-inflammatory phenotype and impaired phagocytosis after exposure to bacterial meningitis pathogens', *Cell Commun Signal*, 15: 32.

Kumar, R., J. L. Herold, D. Schady, J. Davis, S. Kopetz, M. Martinez-Moczygemba, B. E. Murray, F. Han, Y. Li, E. Callaway, R. S. Chapkin, W. M. Dashwood, R. H. Dashwood, T. Berry, C. Mackenzie, and Y. Xu. 2017. '*Streptococcus gallolyticus subsp. gallolyticus* promotes colorectal tumor development', *PLoS Pathog*, 13: e1006440.

Kumar, R., P. K. Surendran, and N. & Thampuran. 2010. 'Rapid quantification of *Salmonella* in seafood by real-time PCR assay.', *J Microbiol Biotechnol*, N.20: 569-73.

Kumar, S., G. Stecher, M. Li, C. Knyaz, and K. Tamura. 2018. 'MEGA X: molecular evolutionary genetics analysis across computing platforms', *Mol Biol Evol*, 35: 1547-49.

Kurosaka, K., Q. Chen, F. Yarovinsky, J. J. Oppenheim, and D. Yang. 2005. 'Mouse cathelin-related antimicrobial peptide chemoattracts leukocytes using formyl peptide receptor-like 1/mouse formyl peptide receptor-like 2 as the receptor and acts as an immune adjuvant', *J Immunol*, 174: 6257-65.

Kyrova, K. , H. Stepanova, I. Rychlik, O. Polansky, L. Leva, Z. Sekelova, M. Faldyna, and J. Volf. 2014. 'The response of porcine monocyte derived macrophages and dendritic cells to *Salmonella* Typhimurium and lipopolysaccharide', *BMC Vet Res*, 10: 244.

Lagier, J. C., F. Armougom, M. Million, P. Hugon, I. Pagnier, C. Robert, F. Bittar, G. Fournous, G. Gimenez, M. Maraninchi, J. F. Trape, E. V. Koonin, B. La Scola, and D. Raoult. 2012. 'Microbial culturomics: paradigm shift in the human gut microbiome study', *Clin Microbiol Infect*, 18: 1185-93.

Lagier, J. C., S. Khelaifia, M. T. Alou, S. Ndongo, N. Dione, P. Hugon, A. Caputo, F. Cadoret, S. I. Traore, E. H. Seck, G. Dubourg, G. Durand, G. Mourembou, E. Guilhot, A. Togo, S. Bellali, D. Bachar, N. Cassir, F. Bittar, J. Delerce, M. Mailhe, D. Ricaboni, M. Bilen, N. P. Dangui Nieko, N. M. Dia Badiane, C. Valles, D. Mouelhi, K. Diop, M. Million, D. Musso, J. Abrahao, E. I. Azhar, F. Bibi, M. Yasir, A. Diallo, C. Sokhna, F. Djossou, V. Vitton, C. Robert, J. M. Rolain, B. La Scola, P. E. Fournier, A. Levasseur, and D.

Raoult. 2016. 'Culture of previously uncultured members of the human gut microbiota by culturomics', *Nat Microbiol*, 1: 16203.
Larrick, J. W., J. G. Morgan, I. Palings, M. Hirata, and M. H. Yen. 1991. 'Complementary DNA sequence of rabbit CAP18--a unique lipopolysaccharide binding protein', *Biochem Biophys Res Commun*, 179: 170-5.
Larsen, N., F. K. Vogensen, F. W. van den Berg, D. S. Nielsen, A. S. Andreasen, B. K. Pedersen, W. A. Al-Soud, S. J. Sorensen, L. H. Hansen, and M. Jakobsen. 2010. 'Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults', *PLoS One*, 5: e9085.
Lawley, T. D., and A. W. Walker. 2013. 'Intestinal colonization resistance', *Immunology*, 138: 1-11.
Lee, S. M., G. P. Donaldson, Z. Mikulski, S. Boyajian, K. Ley, and S. K. Mazmanian. 2013. 'Bacterial colonization factors control specificity and stability of the gut microbiota', *Nature*, 501: 426-9.
Letellier, A. Messier, S. Pare, J. Menard, J. Quessy, S. 1999. 'Distribution of *Salmonella* in swine herds in Quebec', *Vet Microbiol*, 67: 299-306.
Lhocine, N. , E. T. Arena, P. Bomme, F. Ubelmann, M. C. Prevost, S. Robine, and P. J. Sansonetti. 2015. 'Apical invasion of intestinal epithelial cells by *Salmonella* Typhimurium requires villin to remodel the brush border actin cytoskeleton', *Cell Host Microbe*, 17: 164-77.
Li, H., J. P. Limenitakis, T. Fuhrer, M. B. Geuking, M. A. Lawson, M. Wyss, S. Brugiroux, I. Keller, J. A. Macpherson, S. Rupp, B. Stolp, J. V. Stein, B. Stecher, U. Sauer, K. D. McCoy, and A. J. Macpherson. 2015. 'The outer mucus layer hosts a distinct intestinal microbial niche', *Nat Commun*, 6: 8292.
Li, J., M. Post, R. Volk, Y. Gao, M. Li, C. Metais, K. Sato, J. Tsai, W. Aird, R. D. Rosenberg, T. G. Hampton, F. Sellke, P. Carmeliet, and M. Simons. 2000. 'PR39, a peptide regulator of angiogenesis', *Nat Med*, 6: 49-55.
Linde, C. M., S. E. Hoffner, E. Refai, and M. Andersson. 2001. 'In vitro activity of PR-39, a proline-arginine-rich peptide, against susceptible and multi-drug-resistant *Mycobacterium tuberculosis*', *J Antimicrob Chemother*, 47: 575-80.
Litvak, Y., M. X. Byndloss, R. M. Tsolis, and A. J. Baumler. 2017. 'Dysbiotic *Proteobacteria* expansion: a microbial signature of epithelial dysfunction', *Curr Opin Microbiol*, 39: 1-6.
Liu, H., E. Ivarsson, J. Dicksved, T. Lundh, and J. E. Lindberg. 2012. 'Inclusion of chicory (Cichorium intybus L.) in pigs' diets affects the intestinal microenvironment and the gut microbiota', *Appl Environ Microbiol*, 78: 4102-9.
Liu, J. Z. , S. Jellbauer, A. J. Poe, V. Ton, M. Pesciaroli, T. E. Kehl-Fie, N. A. Restrepo, M. P. Hosking, R. A. Edwards, A. Battistoni, P. Pasquali, T. E. Lane, W. J. Chazin, T. Vogl, J. Roth, E. P. Skaar, and M. Raffatellu. 2012. 'Zinc sequestration by the neutrophil protein calprotectin enhances -*Salmonella* growth in the inflamed gut', *Cell Host Microbe*, 11: 227-39.
Looft, T., T. A. Johnson, H. K. Allen, D. O. Bayles, D. P. Alt, R. D. Stedtfeld, W. J. Sul, T. M. Stedtfeld, B. Chai, J. R. Cole, S. A. Hashsham, J. M. Tiedje, and T. B. Stanton. 2012. 'In-feed antibiotic effects on the swine intestinal microbiome', *Proc Natl Acad Sci U S A*, 109: 1691-6.
Lostroh, C. P. , and C. A. Lee. 2001. 'The *Salmonella* pathogenicity island-1 type III secretion system', *Microbes Infect*, 3: 1281-91.
Lupp, C., M. L. Robertson, M. E. Wickham, I. Sekirov, O. L. Champion, E. C. Gaynor, and B. B. Finlay. 2007. 'Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of *Enterobacteriaceae*', *Cell Host Microbe*, 2: 119-29.
Ly, K. T. , and J. E. Casanova. 2007. 'Mechanisms of *Salmonella* entry into host cells', *Cell Microbiol*, 9: 2103-11.
Lynch, M. D., and J. D. Neufeld. 2015. 'Ecology and exploration of the rare biosphere', *Nat Rev Microbiol*, 13: 217-29.

Malago, J. J., J. F. Koninkx, P. C. Tooten, E. A. van Liere, and J. E. van Dijk. 2005. 'Anti-inflammatory properties of heat shock protein 70 and butyrate on *Salmonella*-induced interleukin-8 secretion in enterocyte-like Caco-2 cells', *Clin Exp Immunol*, 141: 62-71.

Mandal, S., W. Van Treuren, R. A. White, M. Eggesbo, R. Knight, and S. D. Peddada. 2015. 'Analysis of composition of microbiomes: a novel method for studying microbial composition', *Microb Ecol Health Dis*, 26: 27663.

Martinez, F. A., E. M. Balciunas, A. Converti, P. D. Cotter, and R. P. de Souza Oliveira. 2013. 'Bacteriocin production by *Bifidobacterium* spp. A review', *Biotechnol Adv*, 31: 482-8.

Mathias, A., and B. Corthesy. 2011. 'N-Glycans on secretory component: mediators of the interaction between secretory IgA and gram-positive commensals sustaining intestinal homeostasis', *Gut Microbes*, 2: 287-93.

McCormick, B. A., P. M. Hofman, J. Kim, D. K. Carnes, S. I. Miller, and J. L. Madara. 1995. 'Surface attachment of *Salmonella* Typhimurium to intestinal epithelia imprints the subepithelial matrix with gradients chemotactic for neutrophils', *J Cell Biol*, 131: 1599-608.

Meurens, F. , M. Berri, G. Auray, S. Melo, B. Levast, I. Virlogeux-Payant, C. Chevaleyre, V. Gerdts, and H. Salmon. 2009. 'Early immune response following *Salmonella enterica* subspecies *enterica* serovar Typhimurium infection in porcine jejunal gut loops', *Vet Res*, 40: 5.

Meurens, F. Berri, G. M. Auray, S. Melo, B. Levast, I. Virlogeux-Payant, C. Chevaleyre, V. Gerdts, and H Salmon. 2009. 'Early immune response following *Salmonella enterica* subspecies *enterica* serovar Typhimurium infection in porcine jejunal gut loops', *Vet Res*, 40: 5.

Meyerholz, D. K. , T. J. Stabel, M. R. Ackermann, S. A. Carlson, B. D. Jones, and J. Pohlenz. 2002. 'Early epithelial invasion by *Salmonella enterica* serovar Typhimurium DT104 in the swine ileum', *Vet Pathol*, 39: 712-20.

Miki, T., R. Goto, M. Fujimoto, N. Okada, and W. D. Hardt. 2017. 'The Bactericidal Lectin RegIIIbeta Prolongs Gut Colonization and Enteropathy in the Streptomycin Mouse Model for *Salmonella* Diarrhea', *Cell Host Microbe*, 21: 195-207.

Misselwitz, B. , S. K. Kreibich, S. Rout, B. Stecher, B. Periaswamy, and W. D. Hardt. 2011. '*Salmonella enterica* serovar Typhimurium binds to HeLa cells via Fim-mediated reversible adhesion and irreversible type three secretion system 1-mediated docking', *Infect Immun*, 79: 330-41.

Mochizuki, T., H. Satsu, T. Nakano, and M. Shimizu. 2004. 'Regulation of the human taurine transporter by TNF-alpha and an anti-inflammatory function of taurine in human intestinal Caco-2 cells', *Biofactors*, 21: 141-4.

Molla, B., A. Sterman, J. Mathews, V. Artuso-Ponte, M. Abley, W. Farmer, P. Rajala-Schultz, W. E. Morrow, and W. A. Gebreyes. 2010. '*Salmonella enterica* in commercial swine feed and subsequent isolation of phenotypically and genotypically related strains from fecal samples', *Appl Environ Microbiol*, 76: 7188-93.

Momose, Y., K. Hirayama, and K. Itoh. 2008. 'Competition for proline between indigenous *Escherichia coli* and *E. coli* O157:H7 in gnotobiotic mice associated with infant intestinal microbiota and its contribution to the colonization resistance against *E. coli* O157:H7', *Anton Leeuw Int J G*, 94: 165-71.

Mon, K. K., P. Saelao, M. M. Halstead, G. Chanthavixay, H. C. Chang, L. Garas, E. A. Maga, and H. Zhou. 2015. '*Salmonella enterica* serovars Enteritidis infection alters the indigenous microbiota diversity in young layer chicks', *Front Vet Sci*, 2: 61.

Mookherjee, N., L. M. Rehaume, and R. E. Hancock. 2007. 'Cathelicidins and functional analogues as antisepsis molecules', *Expert Opin Ther Targets*, 11: 993-1004.

Moote, P. E., S. J. M. Zaytsoff, R. Ortega Polo, D. W. Abbott, R. R. E. Uwiera, and G. D. Inglis. 2020. 'Application of culturomics to characterize diverse anaerobic bacteria from the gastrointestinal tract of broiler chickens in relation to environmental reservoirs', *Can J Microbiol,* 1-15.

Morton, J. T., J. Sanders, R. A. Quinn, D. McDonald, A. Gonzalez, Y. Vazquez-Baeza, J. A. Navas-Molina, S. J. Song, J. L. Metcalf, E. R. Hyde, M. Lladser, P. C. Dorrestein, and R. Knight. 2017. 'Balance Trees Reveal Microbial Niche Differentiation', *mSystems*, 2.

Mukherjee, S., and L. V. Hooper. 2015. 'Antimicrobial defense of the intestine', *Immunity*, 42: 28-39.

Mukherjee, S., S. Vaishnava, and L. V. Hooper. 2008. 'Multi-layered regulation of intestinal antimicrobial defense', *Cell Mol Life Sci*, 65: 3019-27.

Nakoneczna, I., & Hsu, H. S. (1980). 'The comparative histopathology of primary and secondary lesions in murine salmonellosis.', *Br J Exp Pathol*, 61: 76.

Neeloffer Mookherjee, Kelly L. Brown and Robert E.W. Hancock. 2013. 'Cathelicidins.' in, Handbook of biologically active peptides (Academic press).

Nizet, V., T. Ohtake, X. Lauth, J. Trowbridge, J. Rudisill, R. A. Dorschner, V. Pestonjamasp, J. Piraino, K. Huttner, and R. L. Gallo. 2001. 'Innate antimicrobial peptide protects the skin from invasive bacterial infection', *Nature*, 414: 454-7.

Nunes, J. S., S. D. Lawhon, C. A. Rossetti, S. Khare, J. F. Figueiredo, T. Gull, R. C. Burghardt, A. J. Baumler, R. M. Tsolis, H. L. Andrews-Polymenis, and L. G. Adams. 2010. 'Morphologic and cytokine profile characterization of *Salmonella enterica* serovar typhimurium infection in calves with bovine leukocyte adhesion deficiency', *Vet Pathol*, 47: 322-33.

O'Donnell, H., O. H. Pham, L. X. Li, S. M. Atif, S. J. Lee, M. M. Ravesloot, J. L. Stolfi, S. P. Nuccio, P. Broz, D. M. Monack, A. J. Baumler, and S. J. McSorley. 2014. 'Toll-like receptor and inflammasome signals converge to amplify the innate bactericidal capacity of T helper 1 cells', *Immunity*, 40: 213-24.

Ogawa, H., K. Fukushima, H. Naito, Y. Funayama, M. Unno, K. Takahashi, T. Kitayama, S. Matsuno, H. Ohtani, S. Takasawa, H. Okamoto, and I. Sasaki. 2003. 'Increased expression of HIP/PAP and regenerating gene III in human inflammatory bowel disease and a murine bacterial reconstitution model', *Inflamm Bowel Dis*, 9: 162-70.

Parmley, E. J. , K. Pintar, S. Majowicz, B. Avery, A. Cook, C. Jokinen, V. Gannon, D. R. Lapen, E. Topp, T. A. Edge, M. Gilmour, F. Pollari, R. Reid-Smith, and R. Irwin. 2013. 'A Canadian application of one health: integration of *Salmonella* data from various Canadian surveillance programs (2005-2010)', *Foodborne Pathog Dis*, 10: 747-56.

Patel, J. C., and J. E. Galan. 2006. 'Differential activation and function of Rho GTPases during *Salmonella*-host cell interactions', *J Cell Biol*, 175: 453-63.

Paxman, E. J., N. S. Boora, D. Kiss, D. P. Laplante, S. King, T. Montina, and G. A. S. Metz. 2018. 'Prenatal Maternal Stress from a Natural Disaster Alters Urinary Metabolomic Profiles in Project Ice Storm Participants', *Sci Rep*, 8: 12932.

Pestonjamasp, V. K., K. H. Huttner, and R. L. Gallo. 2001. 'Processing site and gene structure for the murine antimicrobial peptide CRAMP', *Peptides*, 22: 1643-50.

Petersson, J., O. Schreiber, G. C. Hansson, S. J. Gendler, A. Velcich, J. O. Lundberg, S. Roos, L. Holm, and M. Phillipson. 2011. 'Importance and regulation of the colonic mucus barrier in a mouse model of colitis', *Am J Physiol Gastrointest Liver Physiol*, 300: G327-33.

Pohlert, T. . 2018. 'PMCMR:Calculate pairwise multiple comparisons of mean rank sums. Available from https://CRAN.R-project.org/package=PMCMR'.

Prouty, A. M., and J. S. Gunn. 2000. '*Salmonella enterica* serovar Typhimurium invasion is repressed in the presence of bile', *Infect Immun*, 68: 6763-9.

Quan, J., G. Cai, J. Ye, M. Yang, R. Ding, X. Wang, E. Zheng, D. Fu, S. Li, S. Zhou, D. Liu, J. Yang, and Z. Wu. 2018. 'A global comparison of the microbiome compositions of three gut locations in commercial pigs with extreme feed conversion ratios', *Sci Rep*, 8: 4536.

Que, J. U., and D. J. & Hentges. 1985. 'Effect of streptomycin administration on colonization resistance to *Salmonella* Typhimurium in mice.', *Infect Immun*, 48(1): 169-74.

Rabsch, W. 2007. '*Salmonella* typhimurium phage typing for pathogens', *Methods Mol Biol*, 394: 177-211.
Raffatellu, M., R. L. Santos, D. E. Verhoeven, M. D. George, R. P. Wilson, S. E. Winter, I. Godinez, S. Sankaran, T. A. Paixao, M. A. Gordon, J. K. Kolls, S. Dandekar, and A. J. Baumler. 2008. 'Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes *Salmonella* dissemination from the gut', *Nat Med*, 14: 421-8.
Rakoff-Nahoum, S., J. Paglino, F. Eslami-Varzaneh, S. Edberg, and R. Medzhitov. 2004. 'Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis', *Cell*, 118: 229-41.
Rambaut, A. . 2012. 'FigTree v1. 4'.
Ramirez-Farias, C., K. Slezak, Z. Fuller, A. Duncan, G. Holtrop, and P. Louis. 2009. 'Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*', *Br J Nutr*, 101: 541-50.
Rashidan, M., M. Azimirad, M. Alebouyeh, M. Ghobakhlou, H. Asadzadeh Aghdaei, and M. R. Zali. 2018. 'Detection of B. fragilis group and diversity of bft enterotoxin and antibiotic resistance markers cepA, cfiA and nim among intestinal *Bacteroides fragilis* strains in patients with inflammatory bowel disease', *Anaerobe*, 50: 93-100.
Rastall, R. A. 2004. 'Bacteria in the gut: friends and foes and how to alter the balance', *J Nutr*, 134: 2022S-26S.
Raupach, B. , S. K. Peuschel, D. M. Monack, and A. Zychlinsky. 2006. 'Caspase-1-mediated activation of interleukin-1beta (IL-1beta) and IL-18 contributes to innate immune defenses against *Salmonella enterica* serovar Typhimurium infection', *Infect Immun*, 74: 4922-6.
Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J. P. Kraehenbuhl, and P. Ricciardi-Castagnoli. 2001. 'Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria', *Nat Immunol*, 2: 361-7.
'Responsible use of Medically Important Antimicrobials in Animals'. 2018.
Reuter, S. E., and A. M. Evans. 2012. 'Carnitine and acylcarnitines: pharmacokinetic, pharmacological and clinical aspects', *Clin Pharmacokinet*, 51: 553-72.
Rigottier-Gois, L. 2013. 'Dysbiosis in inflammatory bowel diseases: the oxygen hypothesis', *ISME J*, 7: 1256-61.
Rios-Covian, D., P. Ruas-Madiedo, A. Margolles, M. Gueimonde, C. G. de Los Reyes-Gavilan, and N. Salazar. 2016. 'Intestinal short chain fatty acids and their link with diet and human health', *Front Microbiol*, 7: 185.
Rosenberger CM, Gallo RL, and Finlay BB. 2004 Feb 24. 'Interplay between antibacterial effectors: a macrophage antimicrobial peptide impairs intracellular *Salmonella* replication.', *Proc Natl Acad Sci U S A*, 101(8):: 2422-7.
Roy, M. F., and D. Malo. 2002. 'Genetic regulation of host responses to *Salmonella* infection in mice', *Genes Immun*, 3: 381-93.
Russell, E. G. 1979. 'Types and distribution of anaerobic bacteria in the large intestine of pigs', *Appl Environ Microbiol*, 37: 187-93.
Salzman, N. H., D. Ghosh, K. M. Huttner, Y. Paterson, and C. L. Bevins. 2003. 'Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin', *Nature*, 422: 522-6.
Salzman, N. H., M. A. Underwood, and C. L. Bevins. 2007. 'Paneth cells, defensins, and the commensal microbiota: a hypothesis on intimate interplay at the intestinal mucosa', *Semin Immunol*, 19: 70-83.
Sansonetti, P. J. 2004. 'War and peace at mucosal surfaces', *Nat Rev Immunol*, 4: 953-64.
Santos RL, Zhang S, Tsolis RM, Kingsley RA, Adams LG, and Bäumler AJ. 2001. 'Animal models of *Salmonella* infections: enteritis versus typhoid fever.', *Microb Infect*, 3(14): 1335-44.
Sassone-Corsi, M., and M. Raffatellu. 2015. 'No vacancy: how beneficial microbes cooperate with immunity to provide colonization resistance to pathogens', *J Immunol*, 194: 4081-7.

Scherer, K., I. Szabo, U. Rosler, B. Appel, A. Hensel, and K. Nockler. 2008. 'Time course of infection with *Salmonella* Typhimurium and its influence on fecal shedding, distribution in inner organs, and antibody response in fattening pigs', *J Food Prot*, 71: 699-705.

Schlee, M., J. Wehkamp, A. Altenhoefer, T. A. Oelschlaeger, E. F. Stange, and K. Fellermann. 2007. 'Induction of human beta-defensin 2 by the probiotic *Escherichia coli* Nissle 1917 is mediated through flagellin', *Infect Immun*, 75: 2399-407.

Schuller-Levis, G. B., and E. Park. 2004. 'Taurine and its chloramine: modulators of immunity', *Neurochem Res*, 29: 117-26.

Sears, C. L., S. Islam, A. Saha, M. Arjumand, N. H. Alam, A. S. Faruque, M. A. Salam, J. Shin, D. Hecht, A. Weintraub, R. B. Sack, and F. Qadri. 2008. 'Association of enterotoxigenic *Bacteroides fragilis* infection with inflammatory diarrhea', *Clin Infect Dis*, 47: 797-803.

Sicard, J. F., G. Le Bihan, P. Vogeleer, M. Jacques, and J. Harel. 2017. 'Interactions of Intestinal Bacteria with Components of the Intestinal Mucus', *Front Cell Infect Microbiol*, 7: 387.

Soler, L., I. Miller, K. Nobauer, S. Carpentier, and T. Niewold. 2015. 'Identification of the major regenerative III protein (RegIII) in the porcine intestinal mucosa as RegIIIgamma, not RegIIIalpha', *Vet Immunol Immunopathol*, 167: 51-6.

Song, Y., C. Liu, and S. M. Finegold. 2004. 'Real-time PCR quantitation of clostridia in feces of autistic children', *Appl Environ Microbiol*, 70: 6459-65.

Spees, A. M. , D. D. Kingsbury, T. Wangdi, M. N. Xavier, R. M. Tsolis, and A. J. Baumler. 2014. 'Neutrophils are a source of gamma interferon during acute *Salmonella enterica* serovar Typhimurium colitis', *Infect Immun*, 82: 1692-7.

Srikanth, C. V. , R. Mercado-Lubo, K. Hallstrom, and B. A. McCormick. 2011. '*Salmonella* effector proteins and host-cell responses', *Cell Mol Life Sci*, 68: 3687-97.

Stecher, B. , R. Robbiani, A. W. Walker, A. M. Westendorf, M. Barthel, M. Kremer, S. Chaffron, A. J. Macpherson, J. Buer, J. Parkhill, G. Dougan, C. von Mering, and W. D. Hardt. 2007. '*Salmonella enterica* serovar Typhimurium exploits inflammation to compete with the intestinal microbiota', *PLoS Biol*, 5: 2177-89.

Stecher, B., S. Chaffron, R. Kappeli, S. Hapfelmeier, S. Freedrich, T. C. Weber, J. Kirundi, M. Suar, K. D. McCoy, C. von Mering, A. J. Macpherson, and W. D. Hardt. 2010. 'Like will to like: abundances of closely related species can predict susceptibility to intestinal colonization by pathogenic and commensal bacteria', *PLoS Pathog*, 6: e1000711.

Stecher, B., and W. D. Hardt. 2008. 'The role of microbiota in infectious disease', *Trends Microbiol*, 16: 107-14.

Stecher, B., A. J. Macpherson, S. Hapfelmeier, M. Kremer, T. Stallmach, and W. D. Hardt. 2005. 'Comparison of *Salmonella enterica* serovar Typhimurium colitis in germfree mice and mice pretreated with streptomycin', *Infect Immun*, 73: 3228-41.

Steele-Mortimer, O., S. Méresse, J. P. Gorvel, B. H. Toh, and B. B. & Finlay. 1999. 'Biogenesis of *Salmonella* typhimurium-containing vacuoles in epithelial cells involves interactions with the early endocytic pathway', *Cell Microbiol*, 1(1): 33-49.

Sukhotnik, I., I. Aranovich, Y. Ben Shahar, N. Bitterman, Y. Pollak, D. Berkowitz, D. Chepurov, A. G. Coran, and A. Bitterman. 2016. 'Effect of taurine on intestinal recovery following intestinal ischemia-reperfusion injury in a rat', *Pediatr Surg Int*, 32: 161-8.

Tamura, K., M. Nei, and S. Kumar. 2004. 'Prospects for inferring very large phylogenies by using the neighbor-joining method', *Proc Natl Acad Sci U S A*, 101: 11030-5.

Thiennimitr, Parameth, Sebastian E. Winter, Maria G. Winter, Mariana N. Xavier, Vladimir Tolstikov, Douglas L. Huseby, Torsten Sterzenbach, Renée M. Tsolis, John R. Roth, and and Andreas J. Bäumler. 2011. 'Intestinal inflammation allows *Salmonella* to use ethanolamine to compete with the microbiota.', *Proc Natl Acad Sci U S A*, 108: 17480-85.

Toms, C. , and F. Powrie. 2001. 'Control of intestinal inflammation by regulatory T cells', *Microbes Infect*, 3: 929-35.

Travis, S. M., N. N. Anderson, W. R. Forsyth, C. Espiritu, B. D. Conway, E. P. Greenberg, P. B. McCray, Jr., R. I. Lehrer, M. J. Welsh, and B. F. Tack. 2000. 'Bactericidal activity of mammalian cathelicidin-derived peptides', *Infect Immun*, 68: 2748-55.

Trivedi, R. N., P. Agarwal, M. Kumawat, P. K. Pesingi, V. K. Gupta, T. K. Goswami, and M. Mahawar. 2015. 'Methionine sulfoxide reductase A (MsrA) contributes to *Salmonella* Typhimurium survival against oxidative attack of neutrophils', *Immunobiology*, 220: 1322-7.

Tsolis, R. M., R. A. Kingsley, S. M. Townsend, T. A. Ficht, L. G. Adams, and A. J. & Bäumler. (1999). Of mice, calves, and men. *In Mechanisms in the Pathogenesis of Enteric Diseases 2* (Springer, Boston, MA.).

Uthe, J. J., A. Royaee, J. K. Lunney, T. J. Stabel, S. H. Zhao, C. K. Tuggle, and S. M. Bearson. 2007. 'Porcine differential gene expression in response to *Salmonella enterica* serovars Choleraesuis and Typhimurium', *Mol Immunol*, 44: 2900-14.

Uzzau, Sergio, Derek J. Brown, T. Wallis, Salvatore Rubino, Guido Leori, Serge Bernard, Josep Casadesús, David J. Platt, and and John Elmerdahl Olsen. 2000. 'Host adapted serotypes of *Salmonella enterica*', *Epidemiol. Infect*, 125: 229-55.

Vaishnava, S., M. Yamamoto, K. M. Severson, K. A. Ruhn, X. Yu, O. Koren, R. Ley, E. K. Wakeland, and L. V. Hooper. 2011. 'The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine', *Science*, 334: 255-8.

Van der Wolf, P. J. , F. W. Van Schie, A. R. Elbers, B. Engel, H. M. Van der Heijden, W. A. Hunneman, and M. J. Tielen. 2001. 'Administration of acidified drinking water to finishing pigs in order to prevent *Salmonella* infections', *Vet Q*, 23: 121-5.

van Harten, R. M., E. van Woudenbergh, A. van Dijk, and H. P. Haagsman. 2018. 'Cathelicidins: Immunomodulatory Antimicrobials', *Vaccines (Basel)*, 6.

Vandesompele, J., K. De Preter, F. Pattyn, B. Poppe, N. Van Roy, A. De Paepe, and F. Speleman. 2002. 'Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes', *Genome Biol*, 3: RESEARCH0034.

Vazquez-Torres, A., J. Jones-Carson, A. J. Baumler, S. Falkow, R. Valdivia, W. Brown, M. Le, R. Berggren, W. T. Parks, and F. C. Fang. 1999. 'Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes', *Nature*, 401: 804-8.

Vazquez-Torres, A., J. Jones-Carson, A. J. Bäumler, S. Falkow, R. Valdivia, W. Brown, Mysan Le, W. Ruth Berggren, Tony Parks, and and Ferric C. Fang. 1999. 'Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes', *Nature reviews. Immunology*, 401: 804.

Veldhuizen, E. J., M. Rijnders, E. A. Claassen, A. van Dijk, and H. P. Haagsman. 2008. 'Porcine beta-defensin 2 displays broad antimicrobial activity against pathogenic intestinal bacteria', *Mol Immunol*, 45: 386-94.

Veldhuizen, E. J., V. A. Schneider, H. Agustiandari, A. van Dijk, J. L. Tjeerdsma-van Bokhoven, F. J. Bikker, and H. P. Haagsman. 2014. 'Antimicrobial and immunomodulatory activities of PR-39 derived peptides', *PLoS One*, 9: e95939.

Veldhuizen, E. J., A. van Dijk, M. H. Tersteeg, S. I. Kalkhove, J. van der Meulen, T. A. Niewold, and H. P. Haagsman. 2007. 'Expression of beta-defensins pBD-1 and pBD-2 along the small intestinal tract of the pig: lack of upregulation in vivo upon *Salmonella* Typhimurium infection', *Mol Immunol*, 44: 276-83.

Vente, J. P., M. F. von Meyenfeldt, H. M. van Eijk, C. L. van Berlo, D. J. Gouma, C. J. van der Linden, and P. B. Soeters. 1989. 'Plasma-amino acid profiles in sepsis and stress', *Ann Surg*, 209: 57-62.

Verbrugghe, E. , A. Van Parys, B. Leyman, F. Boyen, F. Haesebrouck, and F. Pasmans. 2015. 'HtpG contributes to *Salmonella* Typhimurium intestinal persistence in pigs', *Vet Res*, 46: 118.

Veselkov, Kirill A., John C. Lindon, Timothy MD Ebbels, Derek Crockford, Vladimir V. Volynkin, Elaine Holmes, David B. Davies, and and Jeremy K. Nicholson. 2009. '"Recursive segment-wise peak alignment of biological 1H NMR spectra for improved metabolic biomarker recovery." ', *Anal Chem*, 1 56-66.

Wadolkowski, E. A., D. C. Laux, and P. S. Cohen. 1988. 'Colonization of the streptomycin-treated mouse large intestine by a human fecal *Escherichia coli* strain: role of growth in mucus', *Infect Immun*, 56: 1030-5.

Wall, D. M. , W. J. Nadeau, M. A. Pazos, H. N. Shi, E. E. Galyov, and B. A. McCormick. 2007. 'Identification of the *Salmonella enterica* serotype typhimurium SipA domain responsible for inducing neutrophil recruitment across the intestinal epithelium', *Cell Microbiol*, 9: 2299-313.

Wang, G. 2014. 'Human antimicrobial peptides and proteins', *Pharmaceuticals (Basel)*, 7: 545-94.

Wang, W., H. Hu, R. T. Zijlstra, J. Zheng, and M. G. Ganzle. 2019. 'Metagenomic reconstructions of gut microbial metabolism in weanling pigs', *Microbiome*, 7: 48.

Wang, Y., L. Qu, J. J. Uthe, S. M. Bearson, D. Kuhar, J. K. Lunney, O. P. Couture, D. Nettleton, J. C. Dekkers, and C. K. Tuggle. 2007. 'Global transcriptional response of porcine mesenteric lymph nodes to *Salmonella enterica* serovar Typhimurium', *Genomics*, 90: 72-84.

Wannemacher, R. W., Jr. 1977. 'Key role of various individual amino acids in host response to infection', *Am J Clin Nutr*, 30: 1269-80.

Wannemacher, R. W., Jr., A. S. Klainer, R. E. Dinterman, and W. R. Beisel. 1976. 'The significance and mechanism of an increased serum phenylalanine-tyrosine ratio during infection', *Am J Clin Nutr*, 29: 997-1006.

Wannemacher, R. W., Jr., M. C. Powanda, R. S. Pekarek, and W. R. Beisel. 1971. 'Tissue amino acid flux after exposure of rats to *Diplococcus pneumoniae*', *Infect Immun*, 4: 556-62.

Warnes, G. R., B. Bolker, L. Bonebakker, R. Gentleman, W. H. A. Liaw, T. Lumley, M. Maechler, A. Magnusson, S. Moeller, M. Schwartz, and B. Venables. 2015. 'gplots: various R programming tools for plotting data', *https://www.scienceopen.com/document?vid=0e5d8e31-1fe4-492f-a3d8-8cd71b2b8ad9*.

Wexler, H. M. 2007. '*Bacteroides*: the good, the bad, and the nitty-gritty', *Clin Microbiol Rev*, 20: 593-621.

Wilcock, B. P., C. H. Armstrong, and H. J. & Olander. 1976. 'The significance of the serotype in the clinical and pathological features of naturally occurring porcine salmonellosis.', *Can J Comp Med*, 40: 80.

Wilkins, W. , A. Rajic, C. Waldner, M. McFall, E. Chow, A. Muckle, and L. Rosengren. 2010. 'Distribution of *Salmonella* serovars in breeding, nursery, and grow-to-finish pigs, and risk factors for shedding in ten farrow-to-finish swine farms in Alberta and Saskatchewan', *Can J Vet Res*, 74: 81-90.

Winter, S. E., P. Thiennimitr, M. G. Winter, B. P. Butler, D. L. Huseby, R. W. Crawford, J. M. Russell, C. L. Bevins, L. G. Adams, R. M. Tsolis, J. R. Roth, and A. J. Baumler. 2010. 'Gut inflammation provides a respiratory electron acceptor for *Salmonella*', *Nature*, 467: 426-9.

Winterbourn, C. C., A. J. Kettle, and M. B. Hampton. 2016. 'Reactive oxygen species and neutrophil function', *Annu Rev Biochem*, 85: 765-92.

Wood, R. L. , A. Pospischil, and R. Rose. 1989. 'Distribution of persistent *Salmonella* typhimurium infection in internal organs of swine', *Am J Vet Res*, 50: 1015-21.

Wu, W. K., C. C. Wong, Z. J. Li, L. Zhang, S. X. Ren, and C. H. Cho. 2010. 'Cathelicidins in inflammation and tissue repair: Potential therapeutic applications for gastrointestinal disorders', *Acta Pharmacol Sin*, 31: 1118-22.

Xu, J. Gordon, J. I. 2003. 'Honor thy symbionts', *Proc Natl Acad Sci U S A*, 100: 10452-9.

Xu, Jian, Magnus K. Bjursell, Jason Himrod, Su Deng, Lynn K. Carmichael, Herbert C. Chiang, Lora V. Hooper, and and Jeffrey I. Gordon. (2003). 'A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis', *Science*, 299: 2074-76.

Yang, H., X. Huang, S. Fang, W. Xin, L. Huang, and C. Chen. 2016. 'Uncovering the composition of microbial community structure and metagenomics among three gut locations in pigs with distinct fatness', *Sci Rep*, 6: 27427.

Yin, F., A. Farzan, Q. C. Wang, H. Yu, Y. Yin, Y. Hou, R. Friendship, and J. Gong. 2014. 'Reduction of *Salmonella enterica* serovar Typhimurium DT104 infection in experimentally challenged weaned pigs fed a lactobacillus-fermented feed', *Foodborne Pathog Dis*, 11: 628-34.

Yoshimura, T., M. H. McLean, A. K. Dzutsev, X. Yao, K. Chen, J. Huang, W. Gong, J. Zhou, Y. Xiang, H. Badger J, C. O'HUigin, V. Thovarai, L. Tessarollo, S. K. Durum, G. Trinchieri, X. W. Bian, and J. M. Wang. 2018. 'The Antimicrobial Peptide CRAMP Is Essential for Colon Homeostasis by Maintaining Microbiota Balance', *J Immunol*, 200: 2174-85.

Zachar, Z., and D. C. Savage. 1979. 'Microbial interference and colonization of the murine gastrointestinal tract by *Listeria monocytogenes*', *Infect Immun*, 23: 168-74.

Zaiou, M., and R. L. Gallo. 2002. 'Cathelicidins, essential gene-encoded mammalian antibiotics', *J Mol Med (Berl)*, 80: 549-61.

Zanetti, M., G. Del Sal, P. Storici, C. Schneider, and D. Romeo. 1993. 'The cDNA of the neutrophil antibiotic Bac5 predicts a pro-sequence homologous to a cysteine proteinase inhibitor that is common to other neutrophil antibiotics', *J Biol Chem*, 268: 522-6.

Zeng, M. Y., N. Inohara, and G. Nunez. 2017. 'Mechanisms of inflammation-driven bacterial dysbiosis in the gut', *Mucosal Immunol*, 10: 18-26.

Zhang, L., W. Wu, Y. K. Lee, J. Xie, and H. Zhang. 2018. 'Spatial heterogeneity and co-occurrence of mucosal and luminal microbiome across swine intestinal tract', *Front Microbiol*, 9: 48.

Zhang, S. , R. A. Kingsley, R. L. Santos, H. Andrews-Polymenis, M. Raffatellu, J. Figueiredo, J. Nunes, R. M. Tsolis, L. G. Adams, and A. J. Baumler. 2003. 'Molecular Pathogenesis of *Salmonella enterica* Serotype Typhimurium-Induced Diarrhea', *Infect. Immun*, 71: 1-12.

Zhang, Y., Y. Jiang, C. Sun, Q. Wang, Z. Yang, X. Pan, M. Zhu, and W. Xiao. 2014. 'The human cathelicidin LL-37 enhances airway mucus production in chronic obstructive pulmonary disease', *Biochem Biophys Res Commun*, 443: 103-9.

Zhao, W., Y. Wang, S. Liu, J. Huang, Z. Zhai, C. He, J. Ding, J. Wang, H. Wang, W. Fan, J. Zhao, and H. Meng. 2015. 'The dynamic distribution of porcine microbiota across different ages and gastrointestinal tract segments', *PLoS One*, 10: e0117441.