Salmonella

The genus *Salmonella* was named after Daniel Salmon, an American veterinary pathologist

TAXONOMY AND CLASSIFICATION

KINGDOM: Bacteria

PHYLUM: Proteobacteria

CLASS: Gammaproteobacteria

ORDER: Enterobacteriales

FAMILY: Enterobacteriaceae

GENUS: *Salmonella*

SPECIES: *Salmonella enterica*

SUBSPECIES: S. enterica subsp. enterica (I), S. enterica subsp. salamae (II), S. enterica subsp. arizonae (IIIa), S. enterica subsp. diarizonae (IIIb), S. enterica subsp. houtenae (IV), S. enterica subsp. indica (VI)

Serotypes: ~2600

One of the principal causes of food poisoning in several countries in the last 100 years

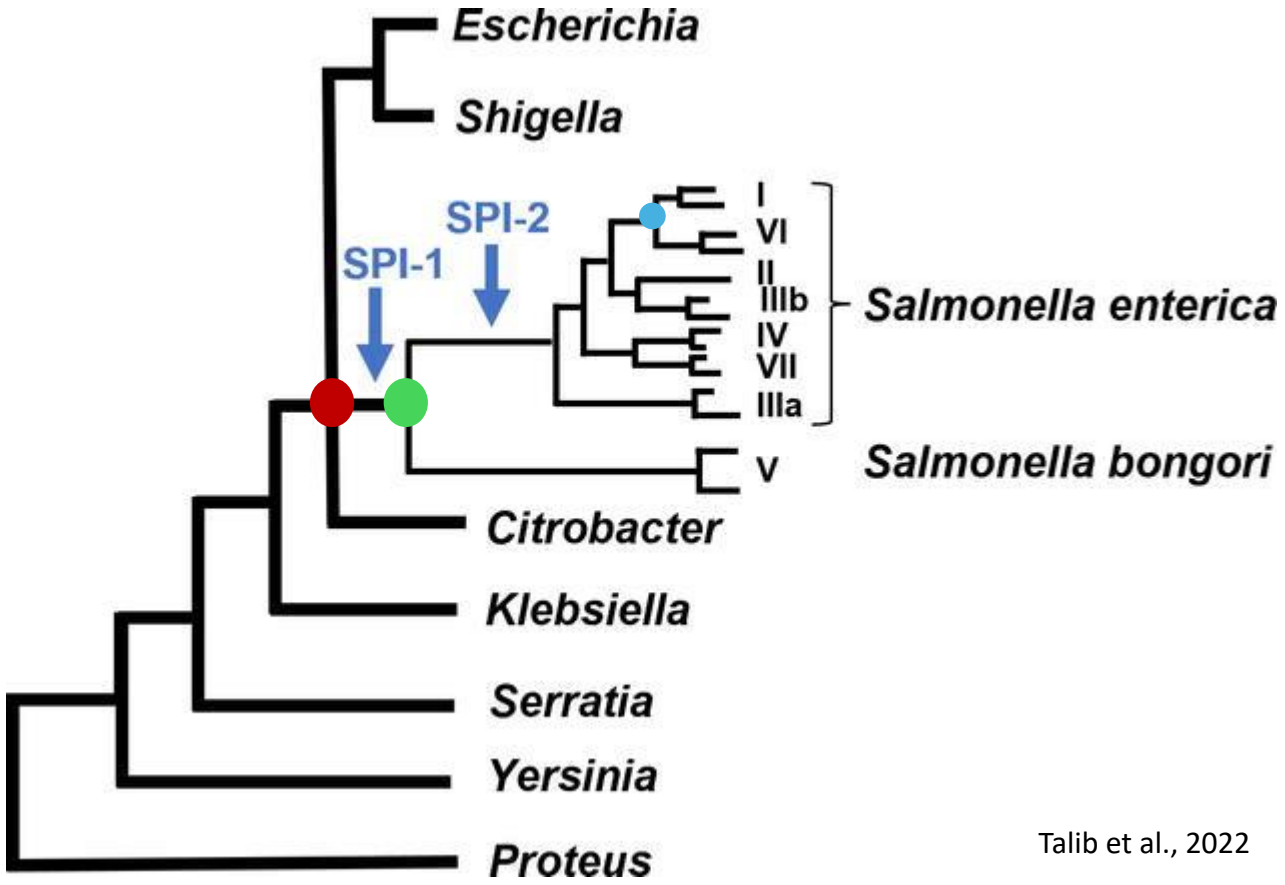
≈ 16 million cases of typhoid fever annually

1.3 billion cases of gastroenteritis

and 3 million deaths involving this bacterium annually

Zoonotic pathogen of substantial concern to global human and animal health. It is a leading cause of morbidity and mortality in people worldwide

General features



Talib et al., 2022

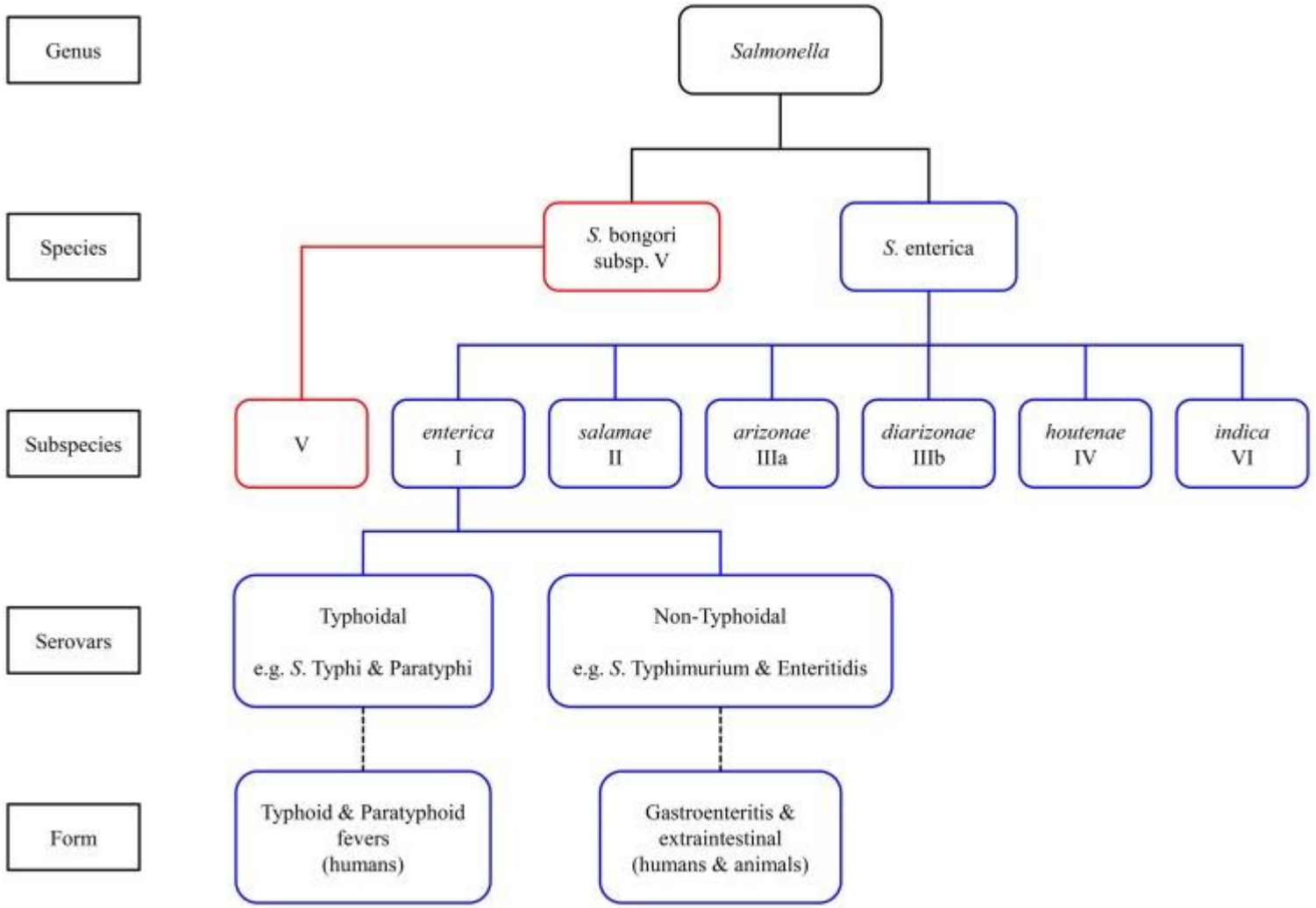
- Enterobacteriaceae
- Gram-negative
- Rod-shaped bacilli
- Facultative anaerobe
- Motile
- Numerous fimbiae

Acquisition of genes necessary for colonization and invasion of intestinal epithelium → *Salmonella* pathogenicity island 1 (**SPI1**)

Acquisition of genes necessary for survival within phagocytic cells such as macrophage and for systemic infection → *Salmonella* pathogenicity island 2 (**SPI2**)

Third evolutionary event → expansion of the host spectrum to warm-blooded animals (humans, sheep, poultry, cattle, pigs, rodents, horses)

Classification



The **genus** *Salmonella* comprises

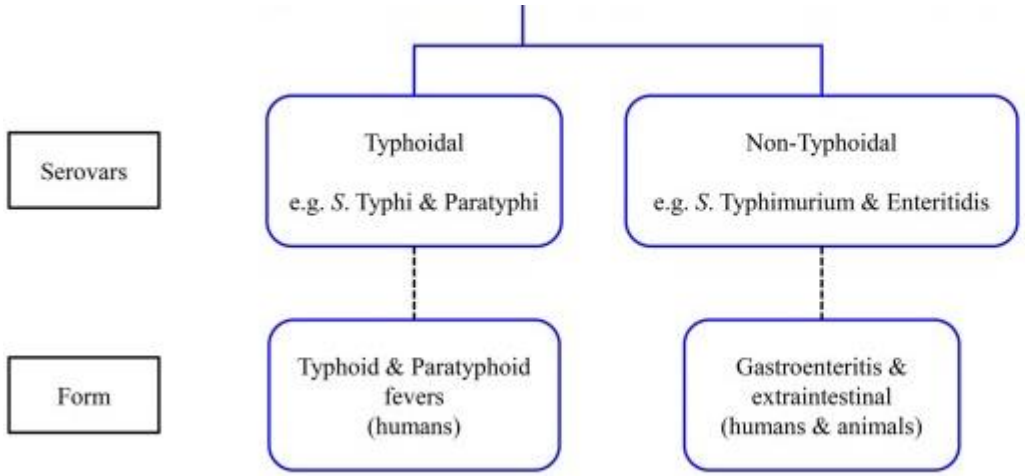
two **species**, *S. enterica* and *S. bongori*

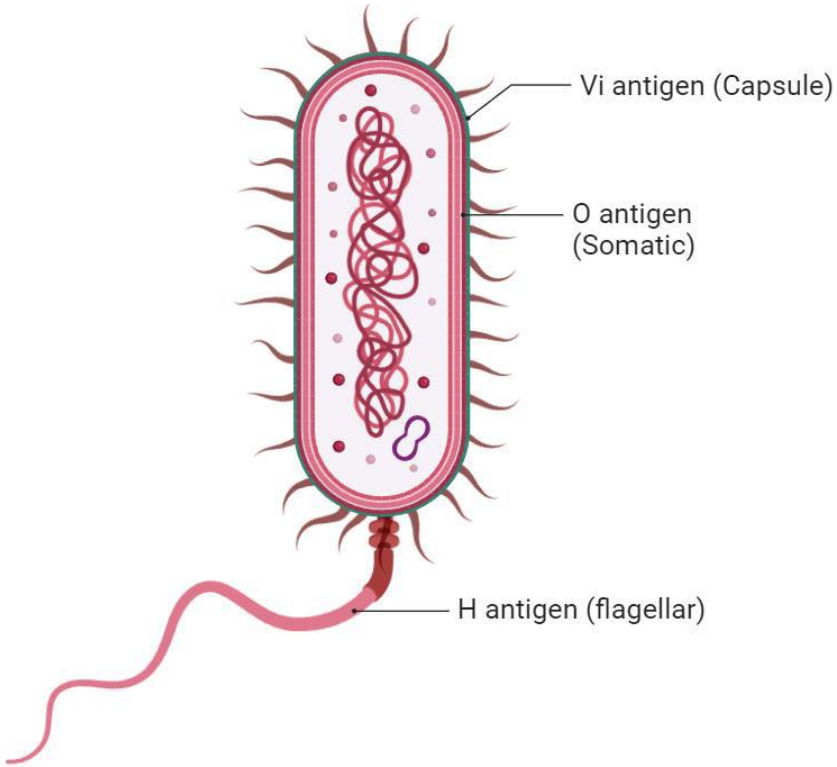
Further divided into six **subspecies** which are

biochemically differentiated into **serovars** based on the composition of their carbohydrate, flagellar, and lipopolysaccharide (LPS) structures

Classification

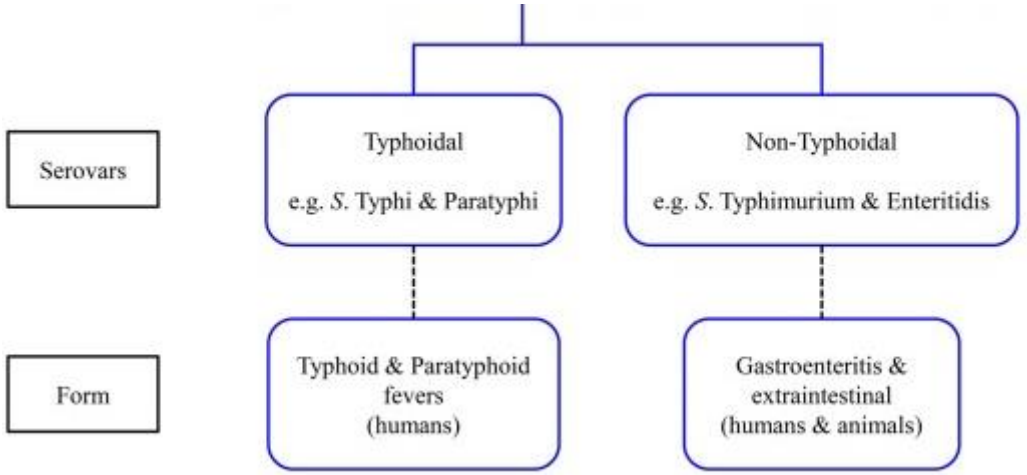
Serotypes or **serovars** can be designated by an antigenic formula based on somatic (O) and flagellar (H) antigens in addition to capsular (Vi) antigens

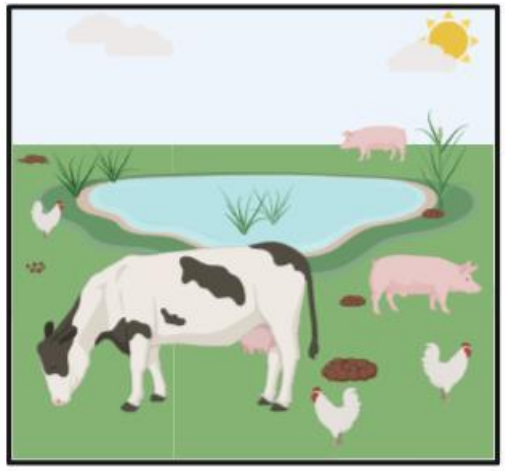




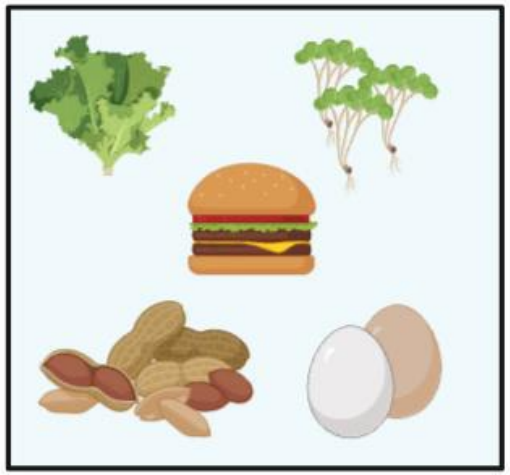
Salmonellosis

- Food-borne infection caused by *Salmonella* bacteria
- Transmission: oral – fecal route
- Sources: contaminated, improperly stored or handled food; contaminated water; household pets; environmental factors

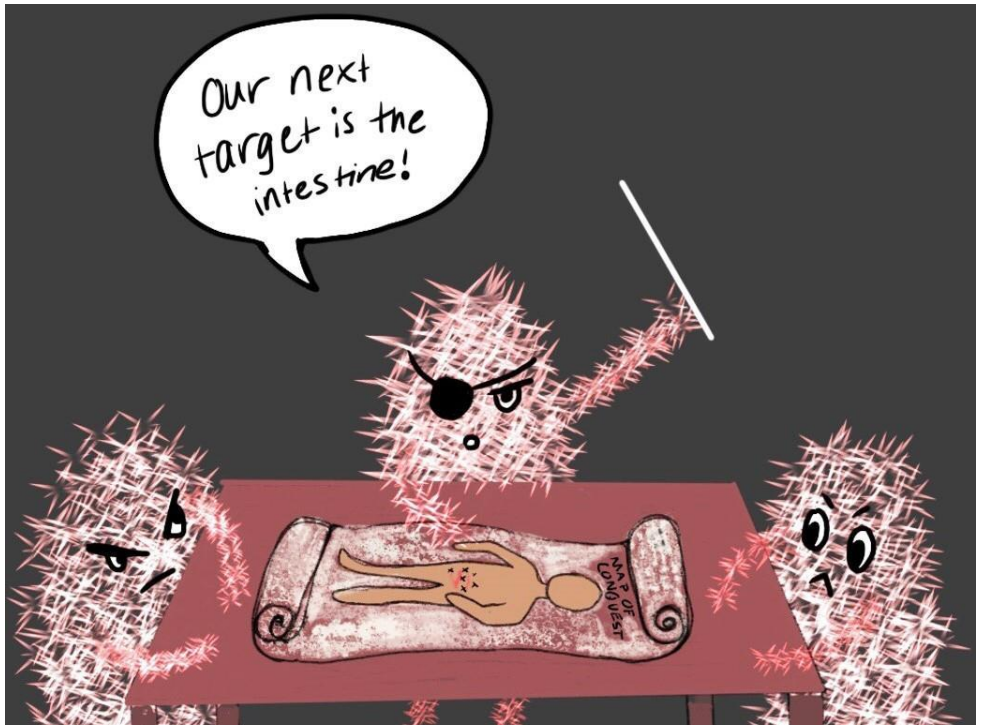




Environment

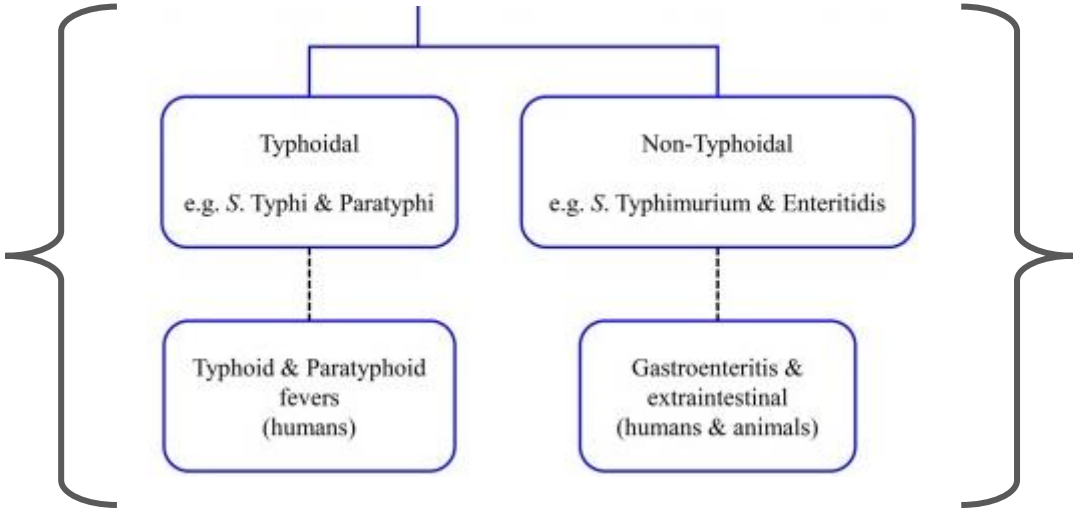


Food



Salmonellosis

- **Typhoid fever** is a global problem, with more than 27 million cases worldwide each year resulting in an estimated 217,000 deaths
- **Mortality** usually results from intestinal perforation and peritonitis or from a severe toxic encephalopathy associated with myocarditis and hemodynamic shock
- *S. Typhi* is an exclusively **human pathogen** causing a bacteremic disease



The variations in the clinical features of infection with this intracellular pathogen relate to differences in the interaction between different *Salmonella* serovars and the host.

- Infections with **non-typhoidal *Salmonella*** (NTS) serovars, also cause a significant disease burden, with an estimated 93.8 million cases worldwide and 155,000 deaths each year
- NTS serovars usually cause **self-limiting** diarrhea with secondary bacteremia occurring in less than 10% of patients
- The **host range** is broad, including poultry and cattle

Typhoid Fever

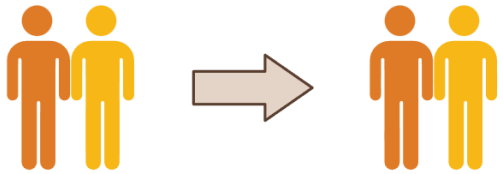
(Enteric fever)

Symptoms

Fever, headache, lethargy, and anorexia, with only one-third of individuals experiencing intestinal symptoms. Antimicrobial therapy is required for successful resolution of infection.

Caused by typhoidal serotypes: *S*. Typhi and *S*. Paratyphi

- Enlarged liver and spleen
- High mortality rate, especially if untreated (20%)
- Chronic asymptomatic human carriers can spread the disease (Mary Mallon)

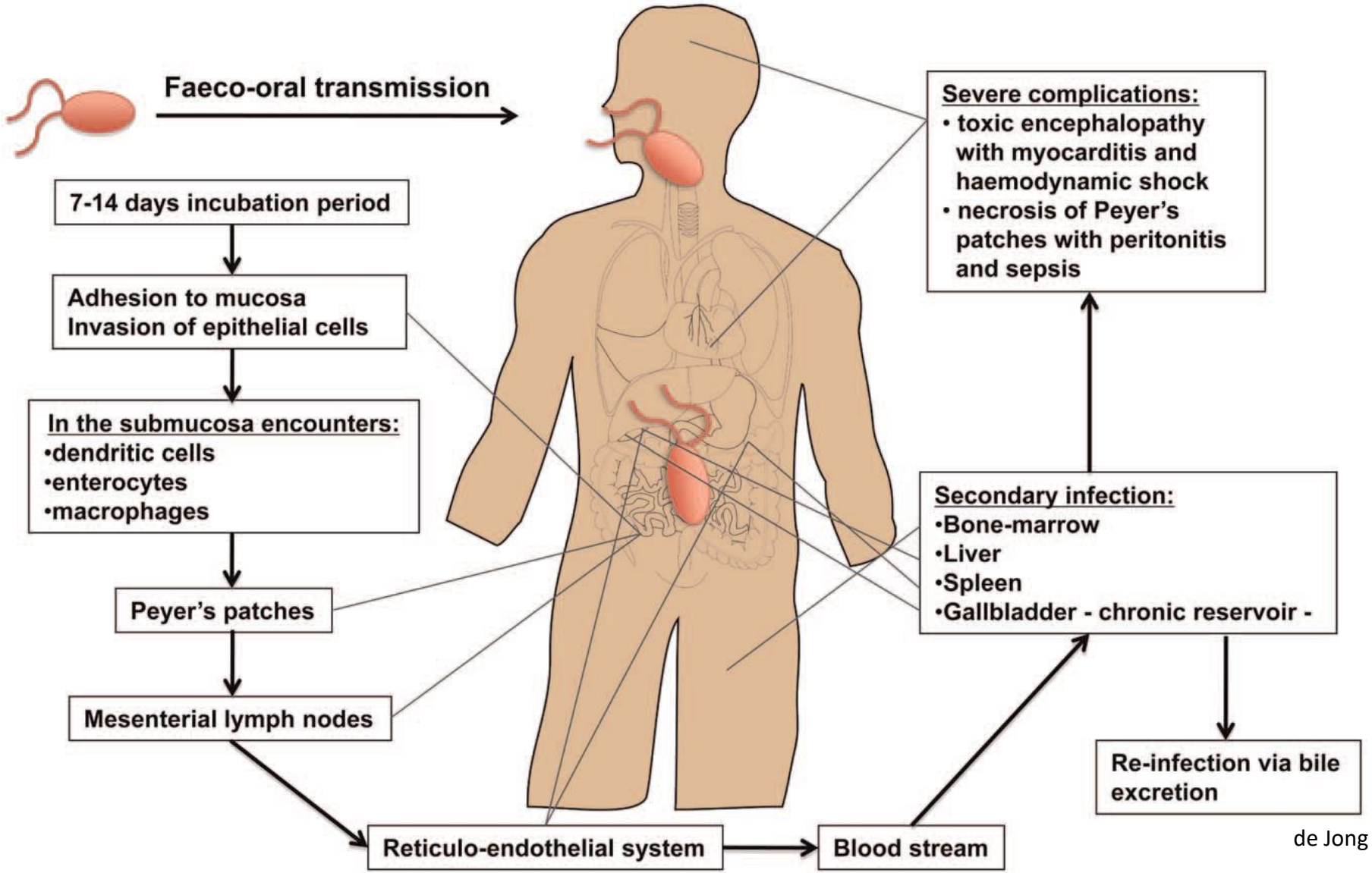


Exclusively
human pathogen

- most common among children, especially in areas of Asia and Africa that lack clean water and adequate sanitation, and is also an important travel-associated disease.

Typhoid Fever

(Enteric fever)



de Jong et al., 2012

Gastroenteritis

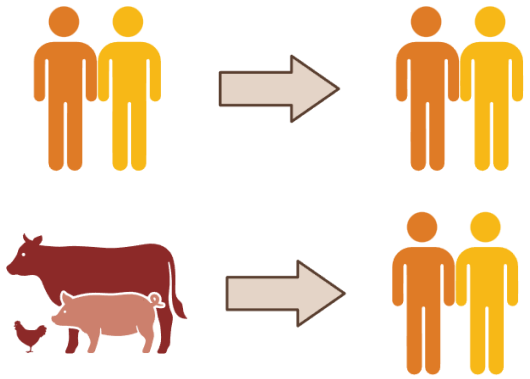
NTS salmonellosis

Symptoms

Fever, abdominal pain, vomiting, and diarrhea. Children under the age of 5 years, the elderly, and immunosuppressed adults are at risk of systemic dissemination of the pathogen and require antimicrobial therapy to treat the infection.

Tipically uncomplicated condition caused by non-typhoidal serotypes: **S. Typhimurium**, **S. Enteritidis**

- self-limiting, resolve without antibiotics
- serious complications may occur in immunocompromised patients, young children and ederly (appendicitis, pancreatitis..)



- Host range is broad, including poultry and cattle, and NTS infection is commonly due to food poisoning in developed countries.

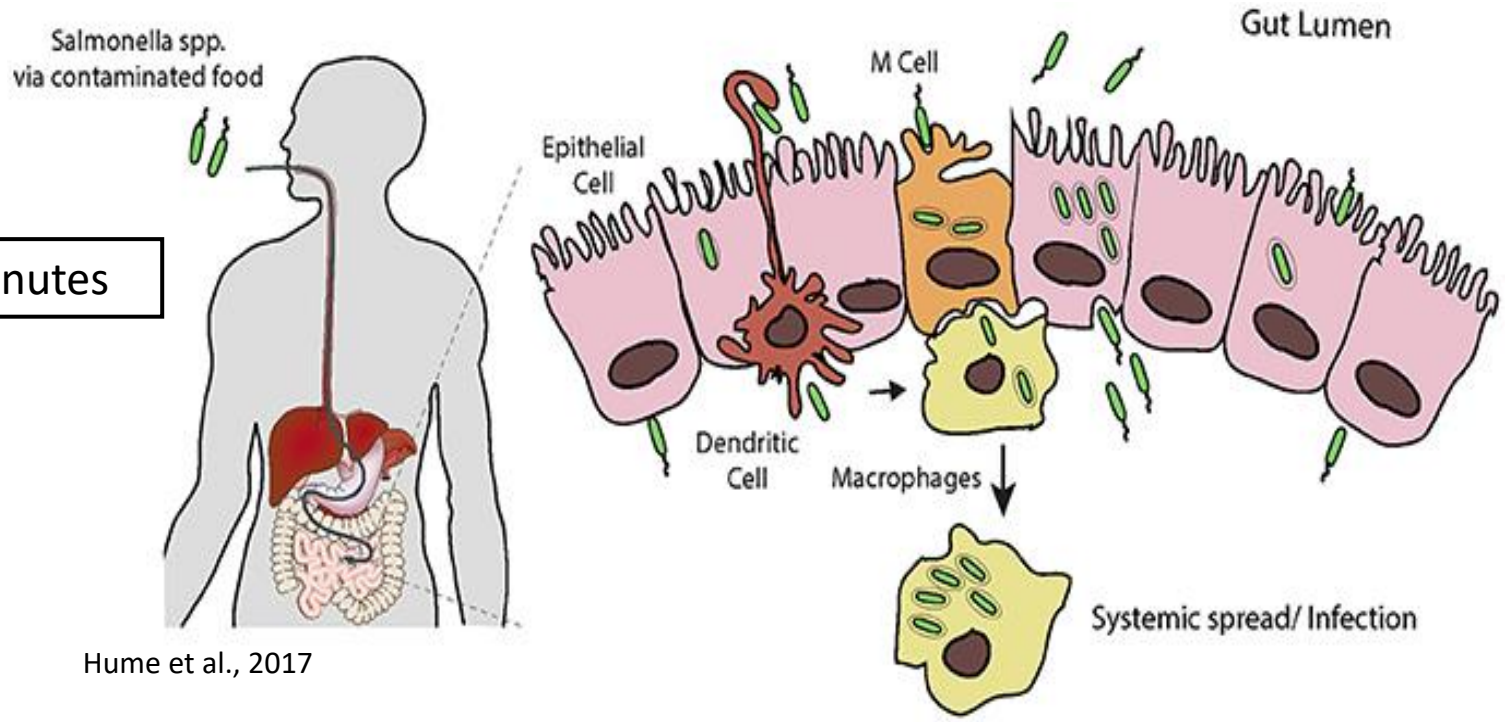
- Worldwide disease → most common form of Salmonellosis

Pathogenetic mechanisms

Common Initial Infection Step

- Microorganism ingested with contaminated food and/or water
- passage through the stomach,
- cross the intestinal epithelium to colonize the host.

Transit time: 90 minutes

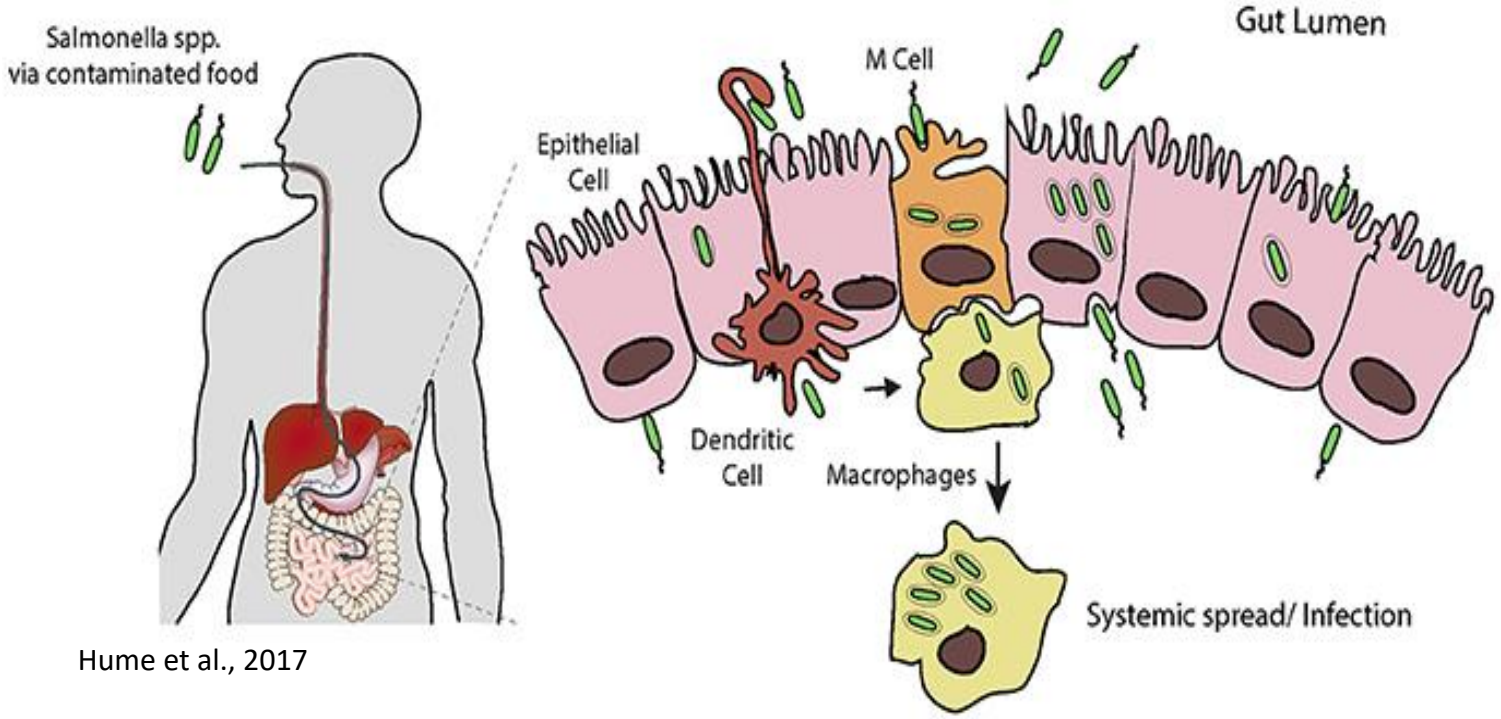


Hume et al., 2017

Pathogenetic mechanisms

S. typhimurium

- Invades phagocytic intestinal cells (antigen-sampling M cells and dendritic cells) and non-phagocytic epithelial cells.
- Once across the epithelium invades further epithelial cells from the basolateral side (survival and replication)
- Usually remain localized to intestinal tissues, where the host's inflammatory response to the invading pathogen is responsible for the symptoms of gastroenteritis



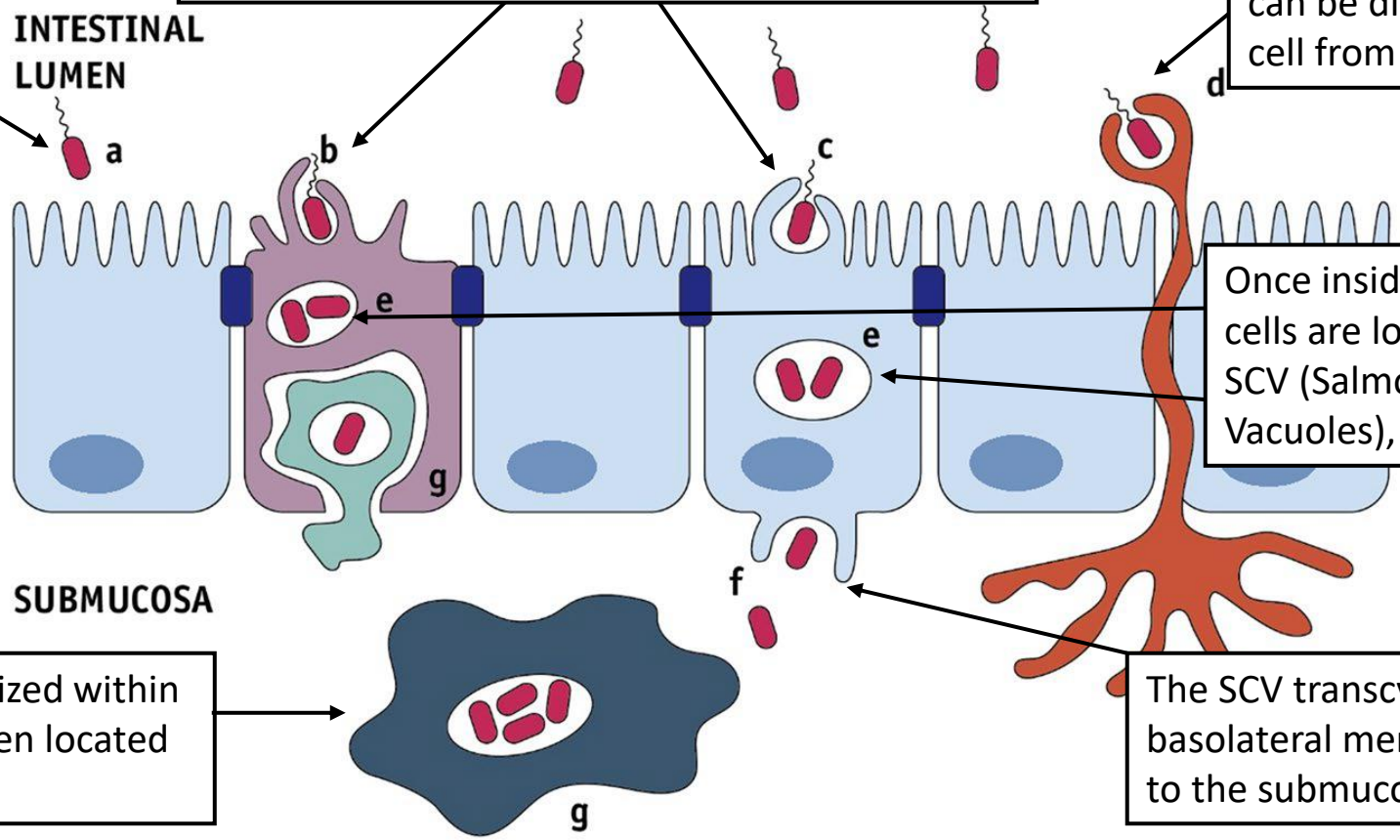
Hume et al., 2017

Overview of the Infection Process caused by ST

Salmonella adheres to the intestinal epithelial and M cells using many of adhesions factors present on its cell surface.

Effector proteins are released into enterocyte causing changes on its cytoskeleton and forming structures in its surface known as ruffles.

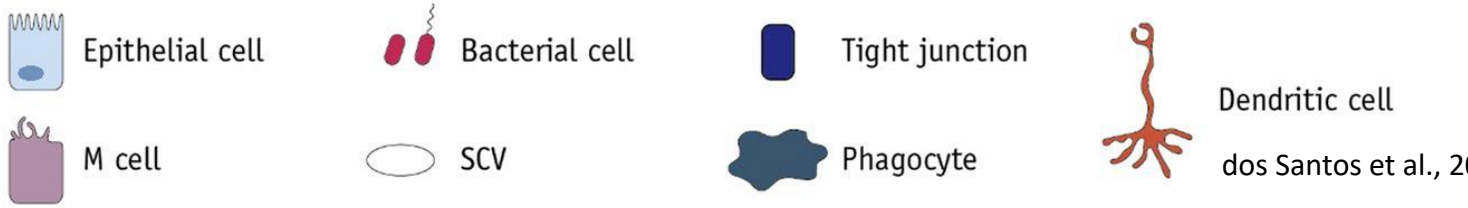
Alternatively, the bacterial cells can be directly taken by dendritic cell from the submucosa



Once inside cytoplasm, *Salmonella* cells are located into SCV (Salmonella Containing Vacuoles), where it multiplies

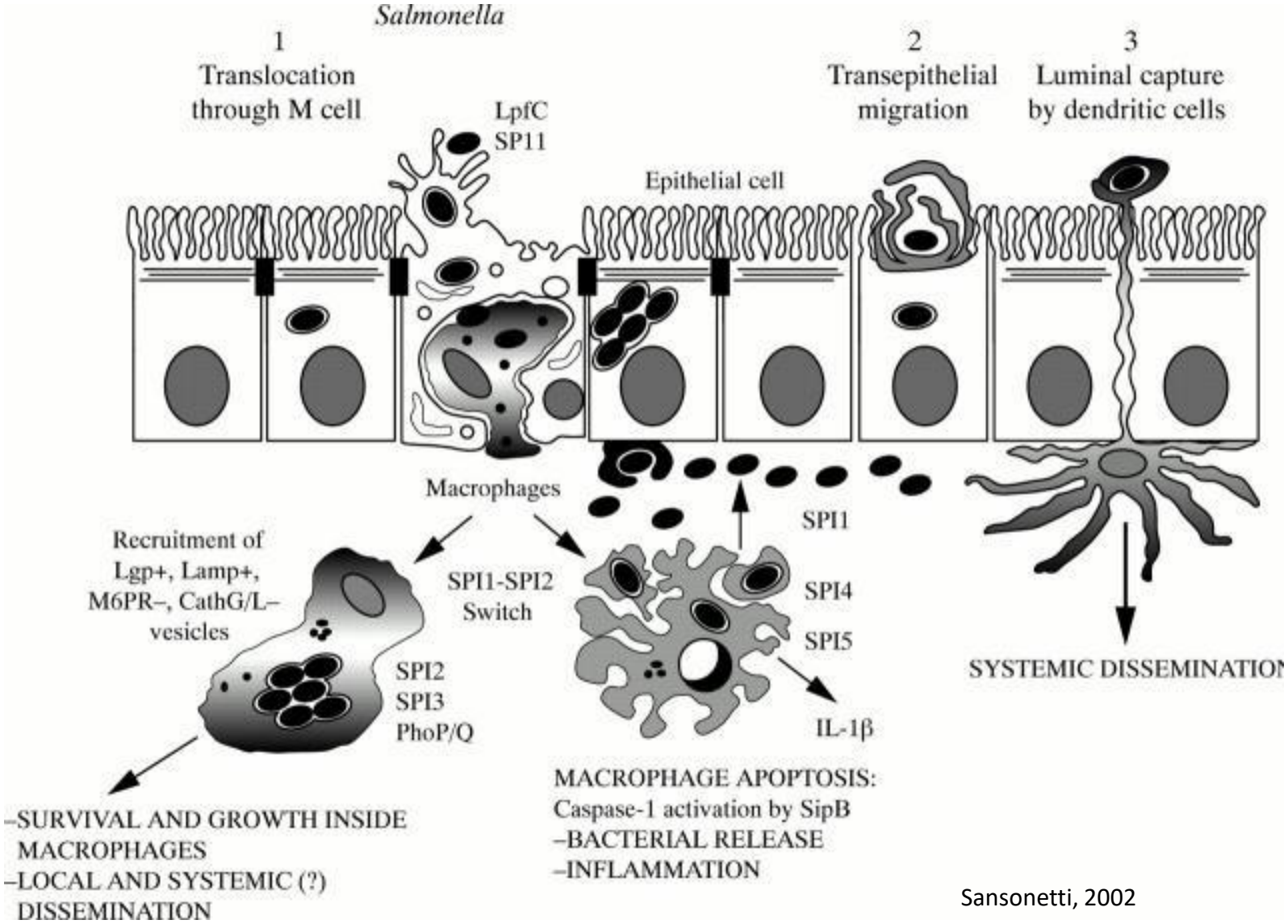
Bacteria is internalized within phagocytes and then located again into SCV

The SCV transcytose to the basolateral membrane and release to the submucosa



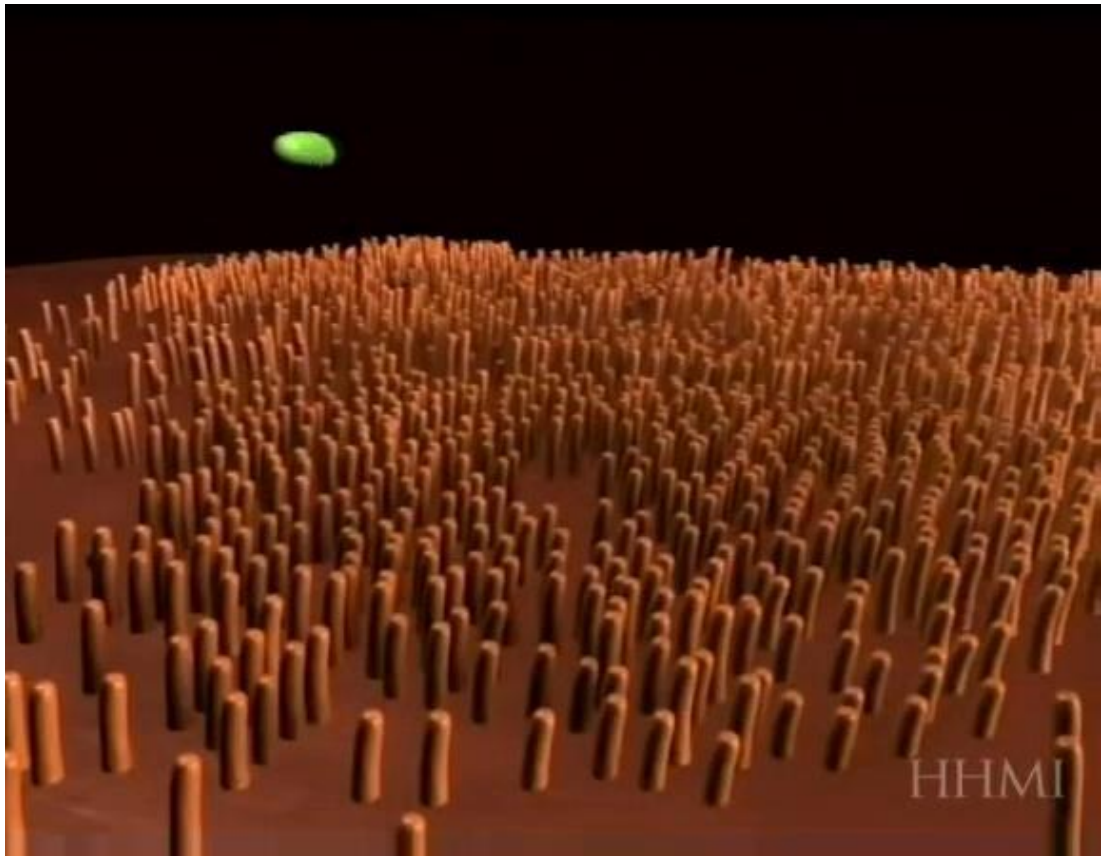
dos Santos et al., 2018

Overview of the Infection Process caused by ST



Sansonetti, 2002

Overview of the Infection Process caused by ST

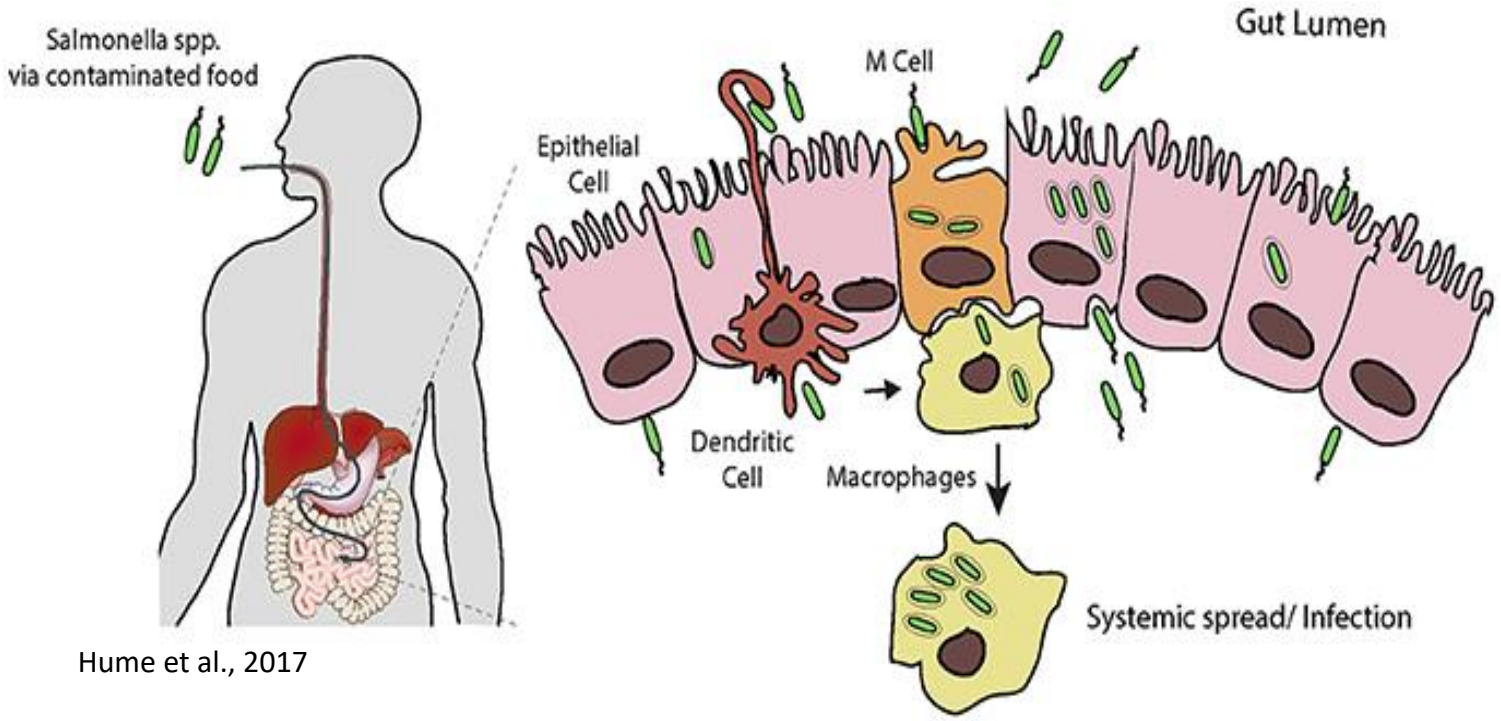


https://youtu.be/q5-sxUbEu5M?feature=shared

Pathogenetic mechanisms

S. typhi

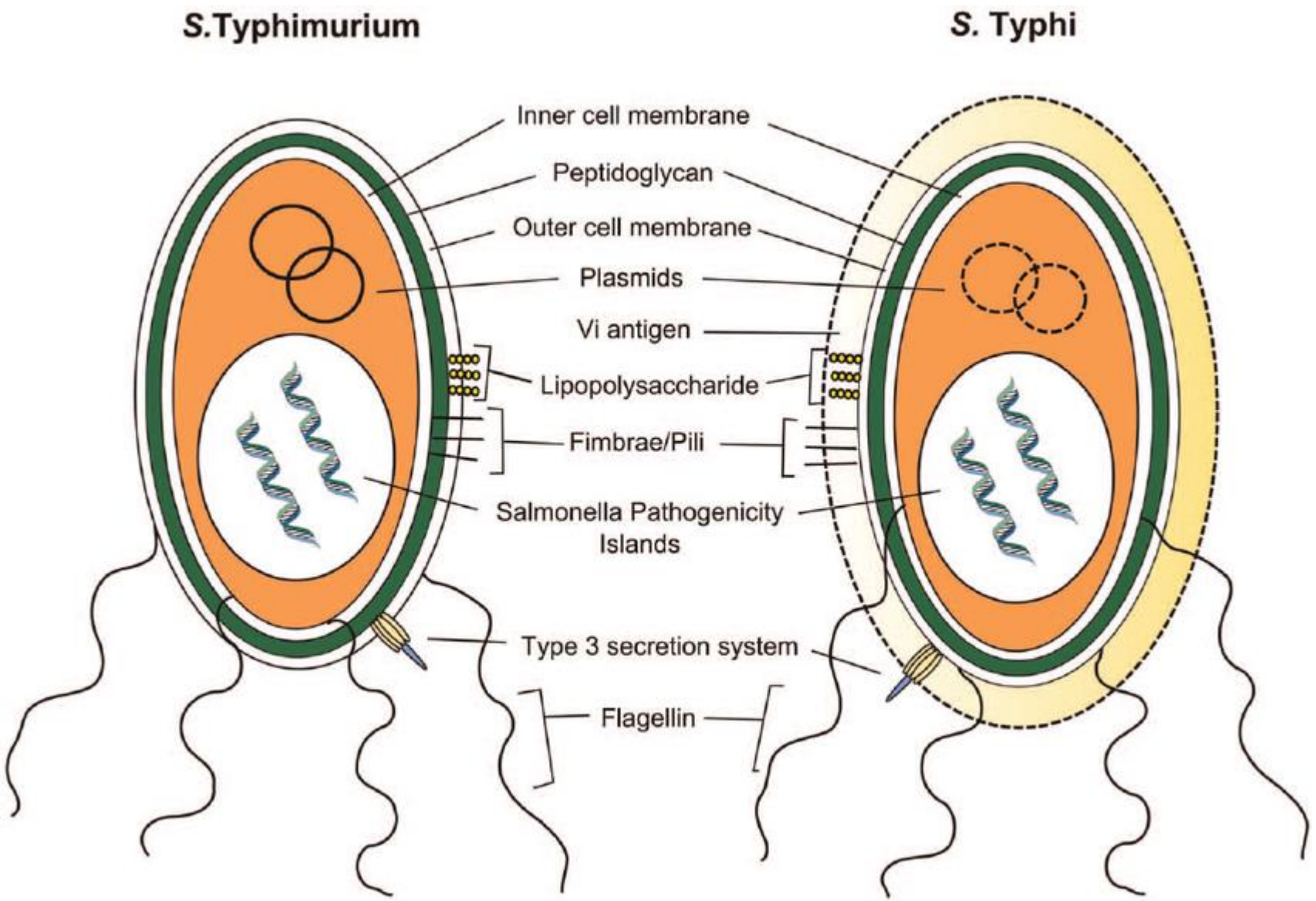
- Enters and crosses the intestinal epithelium in an analogous manner to *S. typhimurium*
- Polysaccharide capsule → no inflammatory response
- The invading pathogen can also survive and replicate in macrophages → systemic spread
- Colonizes host organs such as the spleen, liver, and gallbladder, inducing the symptoms of typhoid fever



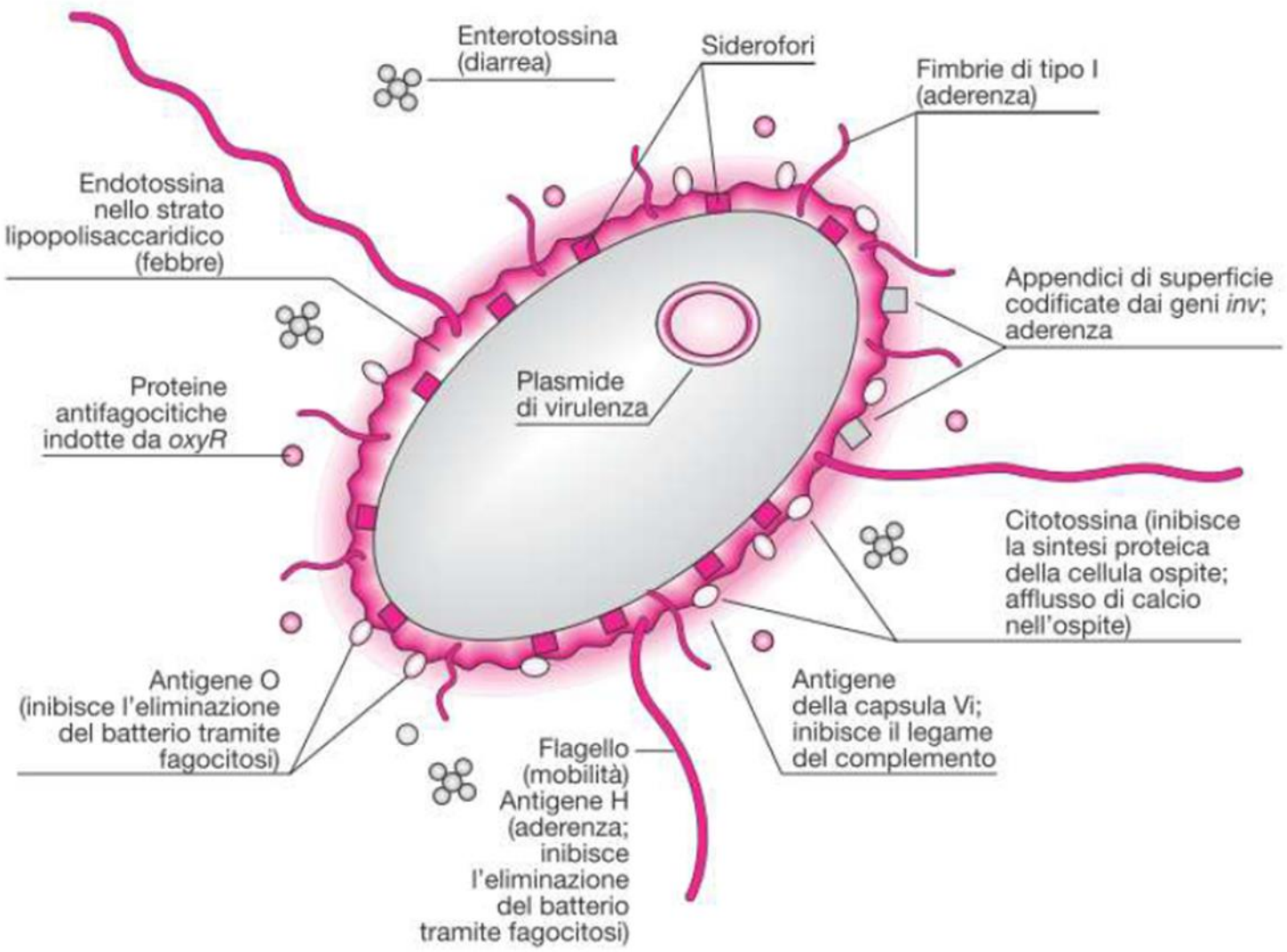
Hume et al., 2017

Virulence of *S.* Typhimurium and *S.* Typhi

About 90% of the genes in ***S.* Typhi** and ***S.* Typhimurium** serovars are identical.
The 10% of genes that differ include virulence factors, which determine their pathogenic potential



Virulence factors



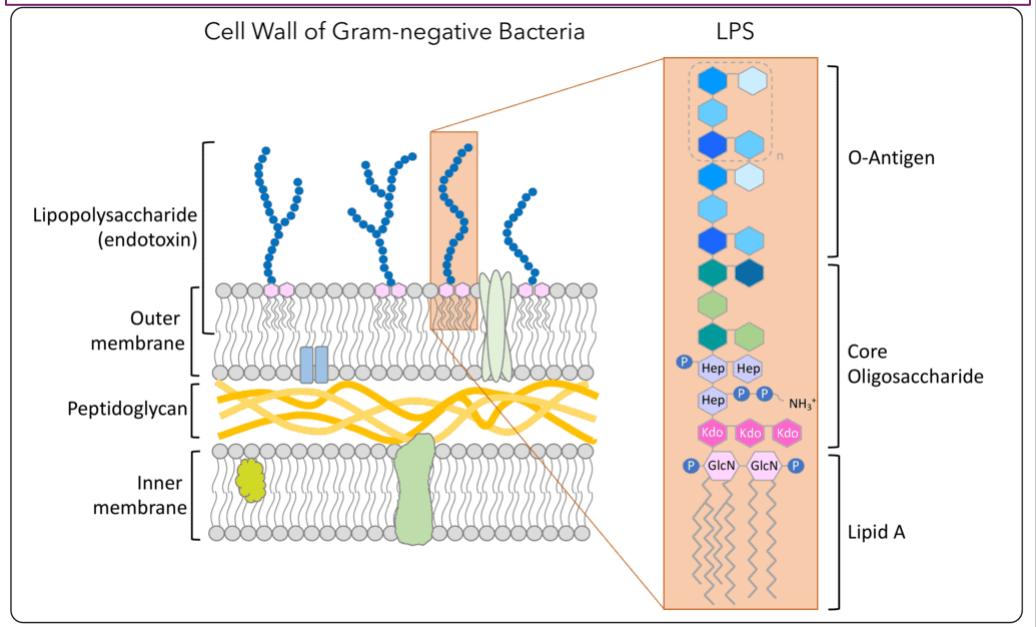
Fattori di virulenza associati alla membrana

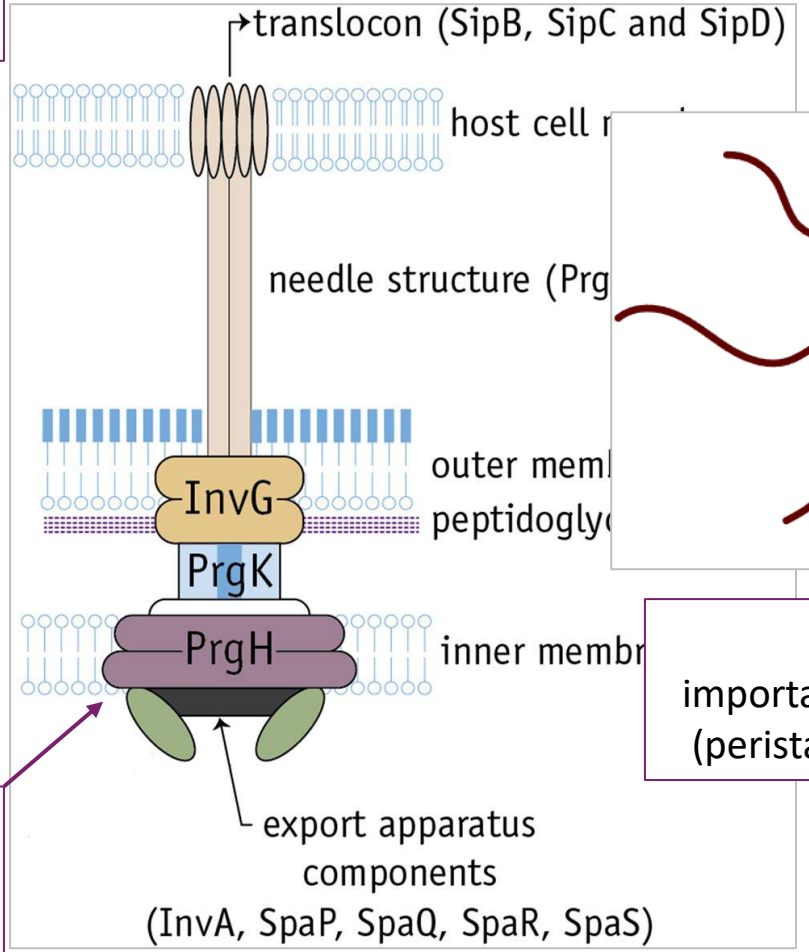
Fattori di virulenza citosolici

Fattori di virulenza secretori

Virulence factors associated with the membrane

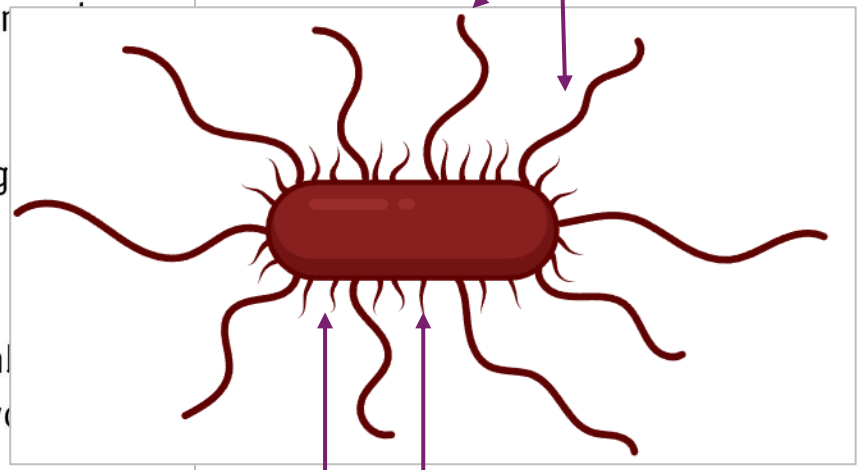
Lipopolysaccharide
provides an effective protective barrier and incite inflammation in tissues





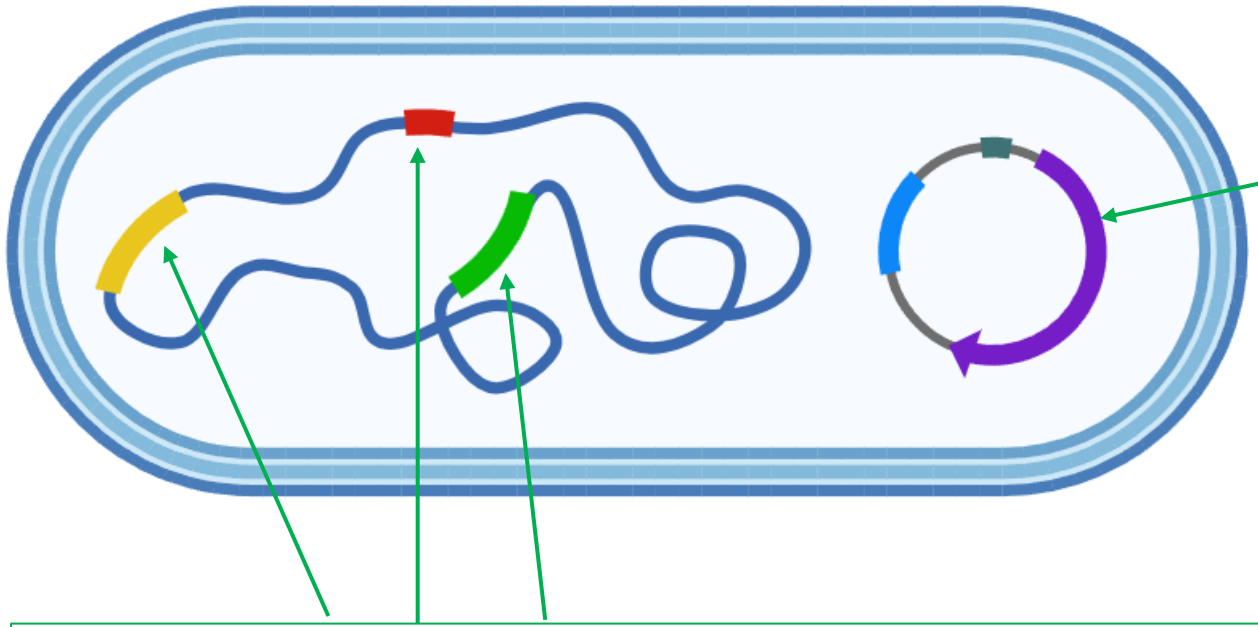
Type III Secretion System (T3SS)
inject effector proteins into the cytosol of host cells and modulate host cells signaling cascades for the benefit of bacteria

Flagella
enable *S.* to reach the epithelial barrier after ingestion



Fimbriae
important for initial adhesion, colonisation (peristaltic movement), biofilm formation

Virulence factors in the cytosol



Plasmids

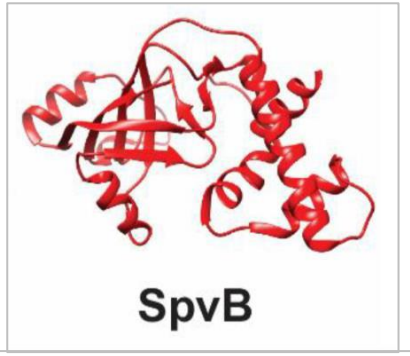
Genes associated with virulence and antimicrobial resistance

- In *S.* Typhimurium LT2: **pSLT** → spv genes encoding **SpvB** toxin
- In *S.* Typhi: **pR(ST98)** → genes involved in drug resistance and induction of apoptosis in macrophages

***Salmonella* Pathogenicity Islands (SPI)**

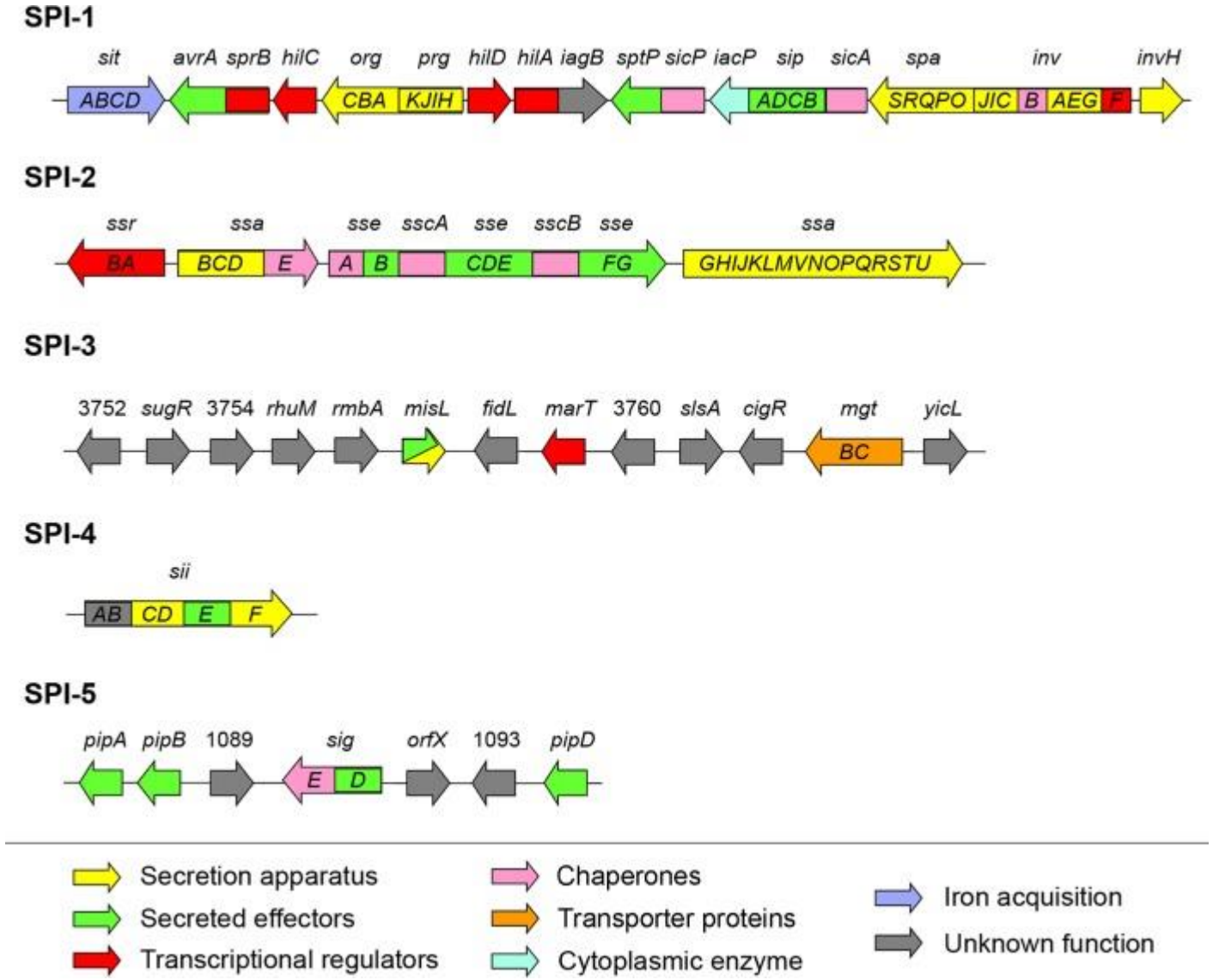
Genomic islands coding for virulence factors or adhesion and invasion proteins infected host

- Acquired by horizontal gene transfer (HGT) → flanked by repeated sequences (IS elements), different G+C content (37-47%)
- Gene expression coordinated by environmental stimuli (T, pH, osmotic pressure)



SpvB (ADP-ribosylating toxin) → secreted by T3SS SPI-2 into the cytoplasm where it causes host cytotoxicity = actin depolymerisation

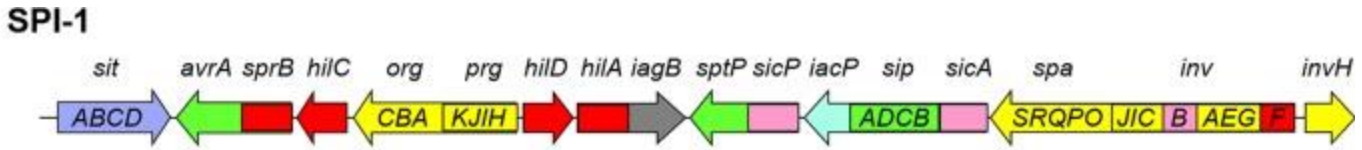
Salmonella Pathogenicity Islands (SPIs)



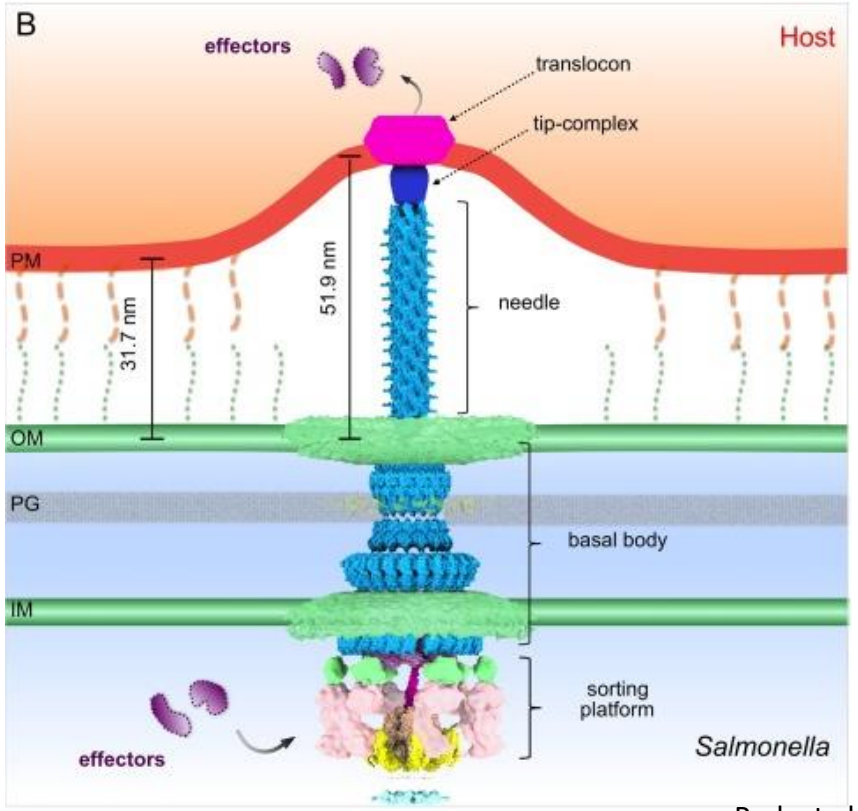
- Variable dimensions (10-40 kb)
- Generally located on bacterial chromosomes (or plasmids)
- 23 SPIs identified (to date)
- Only 5 present in all serotypes and relevant for virulence of the bacterium

Fàbrega and Vila, 2013

SPI-1



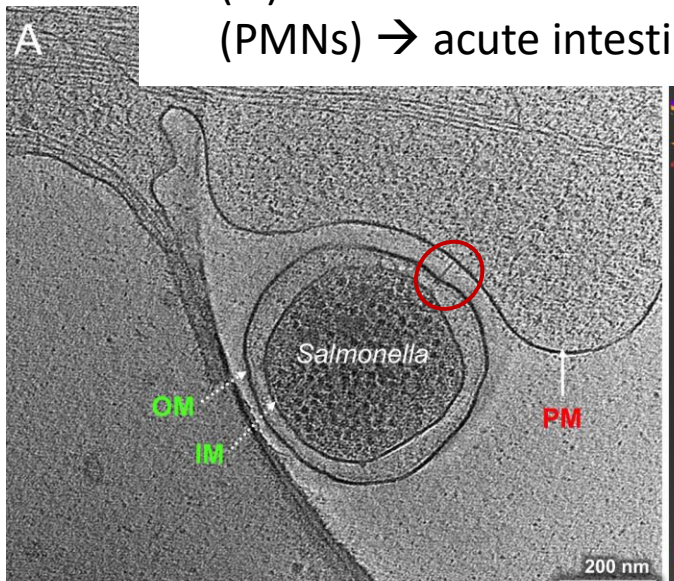
SPI-1 encodes effector proteins that, via the T3SS-1, induce epithelial cell invasion by rearranging the actin cytoskeleton and promoting bacterial internalization.

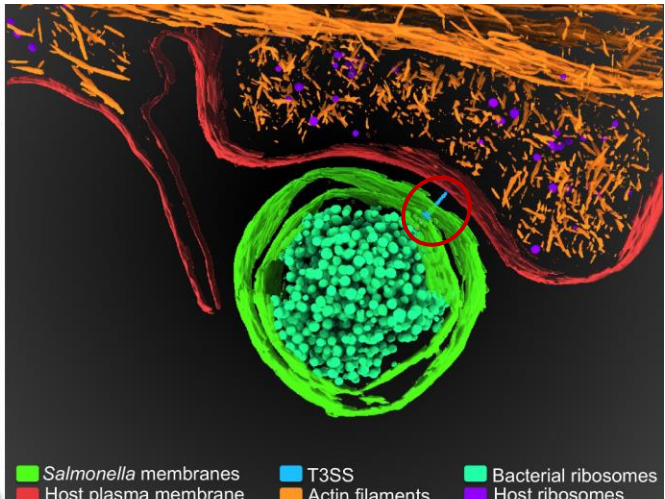


Park et al., 2018

T3SS-1 (Type III Secretion System):

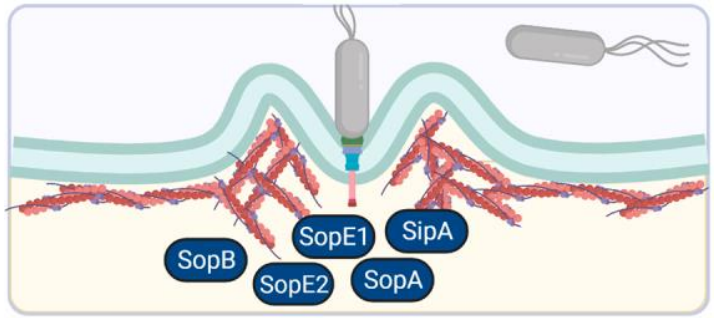
- Sophisticated nanoinjection multi-protein system (20-30 proteins)
- 3 structures (needle complex, export apparatus, sorting platform)
- Contact-dependent release of effector proteins into the host cell cytoplasm
- SPI-1 translocated effectors drive the cell invasion process
- activation of mitogen-activated protein kinase (MAPK) pathways → production of proinflammatory cytokines (IL)-8 → recruitment of polymorphonuclear leukocytes (PMNs) → acute intestinal inflammation



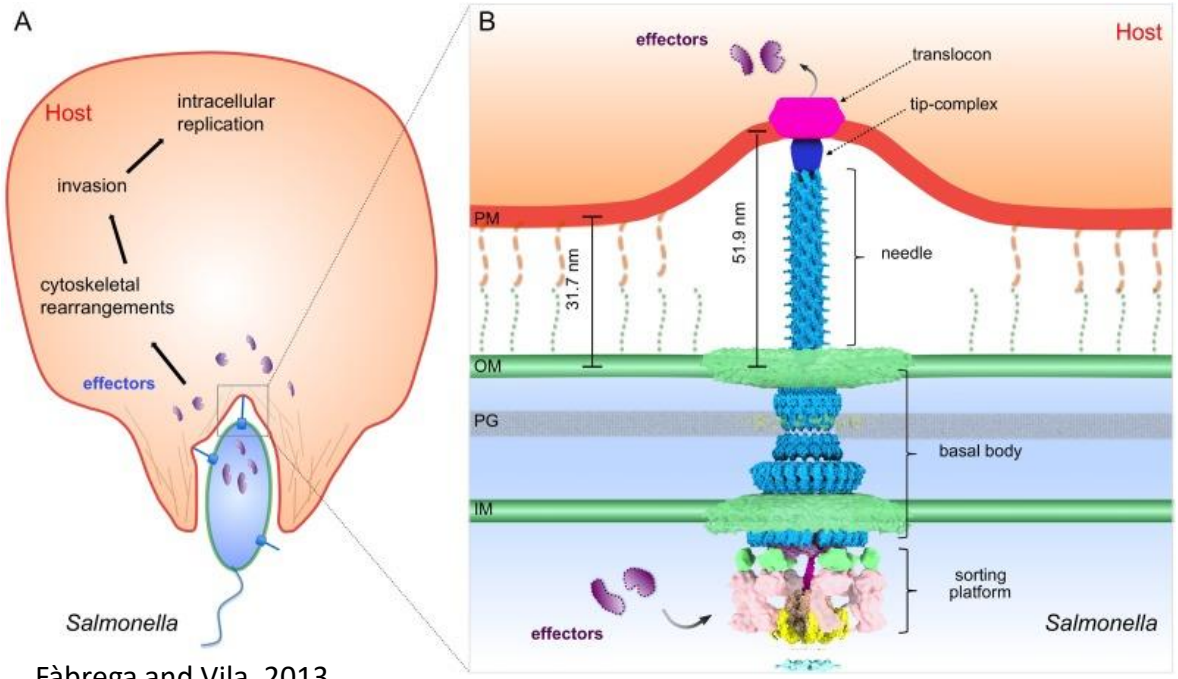


Cell invasion

Trigger mechanism → reorganisation of the actin cytoskeleton and ruffling of the membrane and bacterium enclosed within.



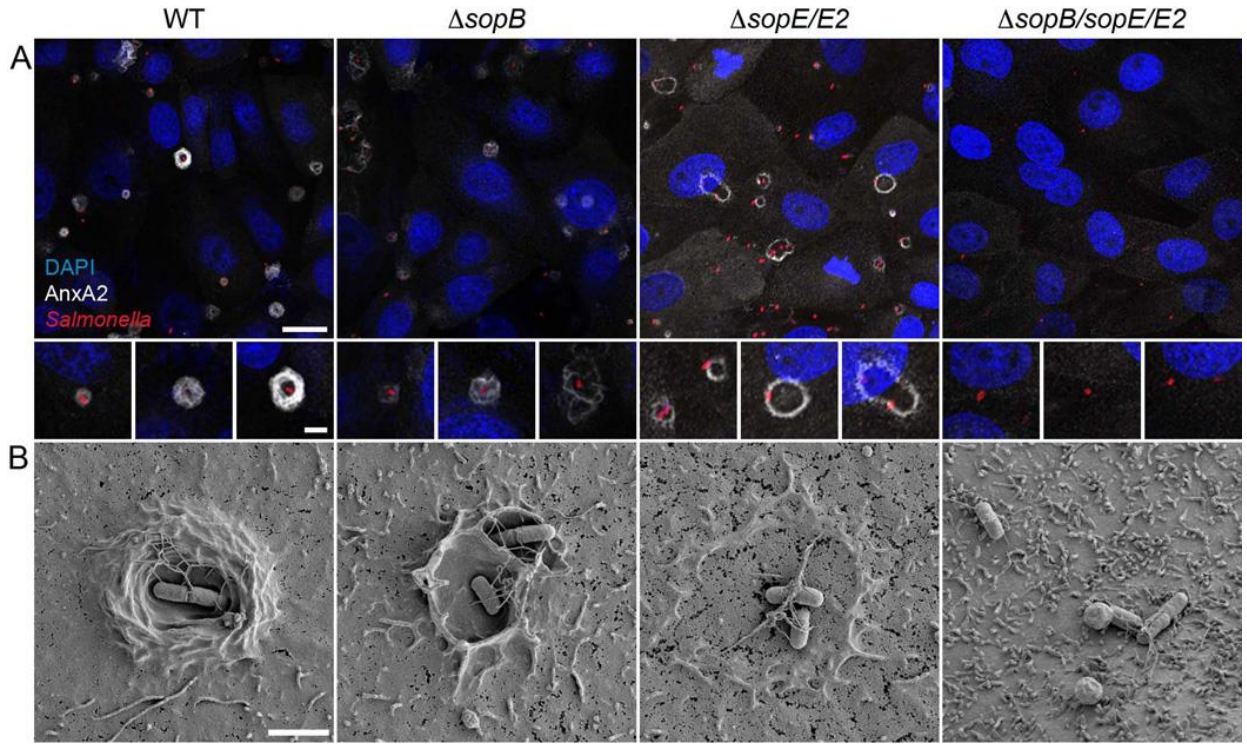
Davidson et al., 2023



Fàbrega and Vila, 2013

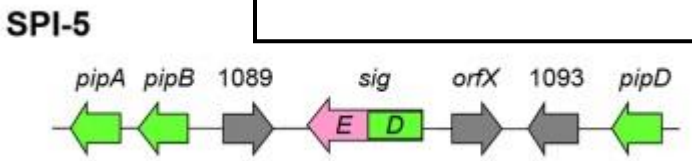
- **SipB** and **SipC** → responsible for inducing actin-cytoskeletal rearrangements and in T3SS-1 translocation of effector molecules
- **SipD**→ tip protein mediates the sensing phase
- **SipA** → translocon component promotes actin polymerisation
- **SipC** → recruits regulatory proteins to stabilise neosynthesised filaments, contributes to their localisation. Activates NF-kB and recruits neutrophiles.
- **SptP** → phosphotyrosine phosphatase; when injected into epithelial cells, alters the actin cytoskeleton
- **SopE1/SopE2** (SPI-5) → target Rho family GTPases (Rac-1 and Cdc42) that modulate the cytoskeleton (ramification) and, via NF-kB, induce pro-inflammatory cytokines (IL-8)
- **SopB** (SigD in ST) (SPI-5) → actin rearrangements

Cell invasion



Single mutants induce ripples with lower efficiency than WT (smaller and less distinct)

ΔsopB/sopE/E2 triple mutant does not create ripples (no invasion)



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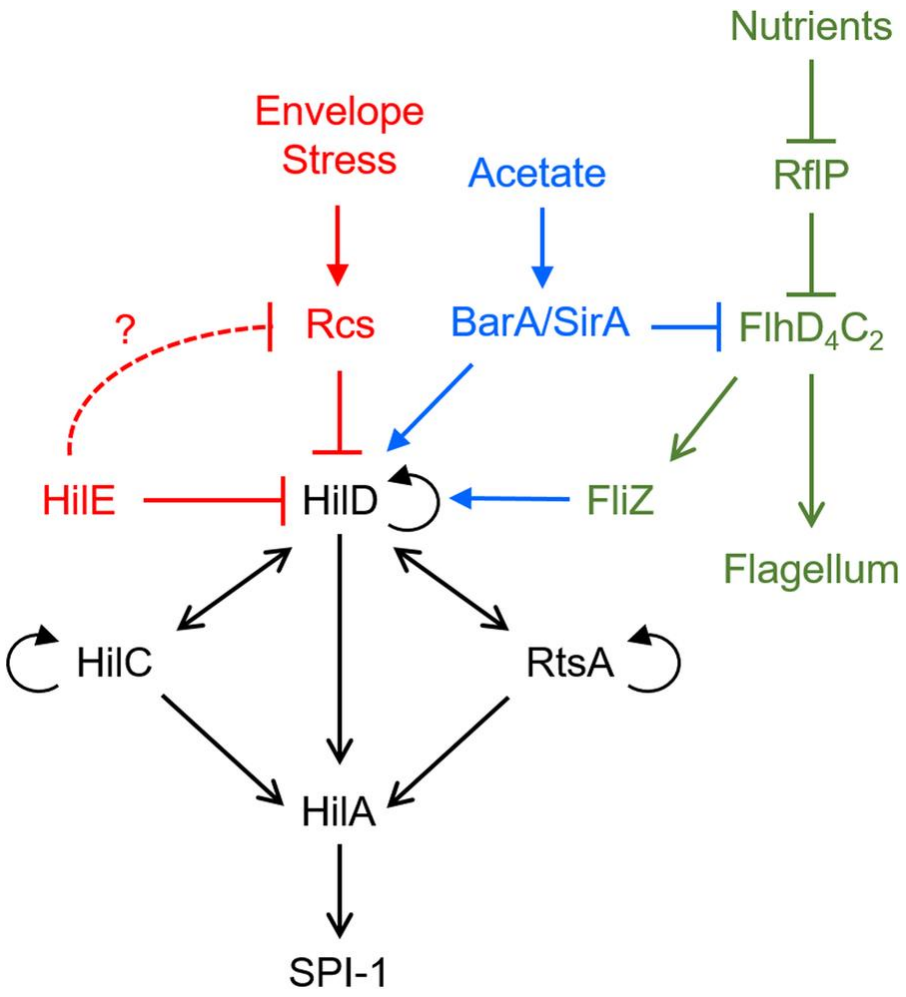
Regulation of SPI-1



Hamed et al., 2021

BarA/SirA TCS senses the intestinal lumen conditions: low oxygen, high osmolarity and mild alkaline pH

↓

HilA expression
member of the OmpR/ToxR transcriptional activators family

↓

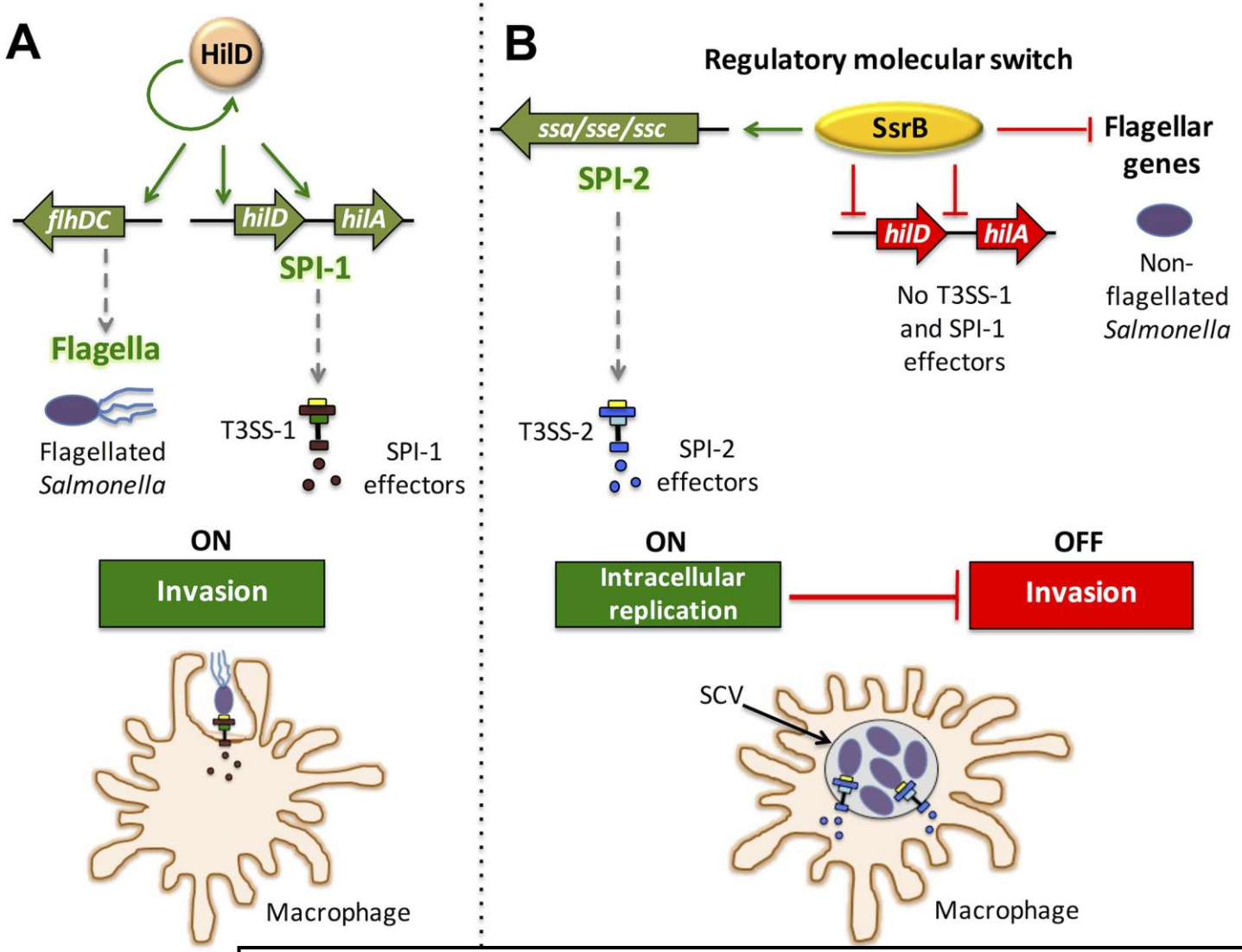
bind to prg, inv/spa and sip operon promoters

↓

activation of T3SS/SPI-1 and SPI-1 effectors

Transition to the intracellular lifestyle

PeÂrez-Morales et al., 2017



Invasion phase → **HilD** directly or indirectly activates the expression of

- SPI-1 genes
- many other genes located outside SPI-1 (T3SS)
- flhDC flagellar regulatory operon required for host cell invasion

Intracellular phase → After invasion, *Salmonella* in SCVs and here **SsrB** induces

expression of:

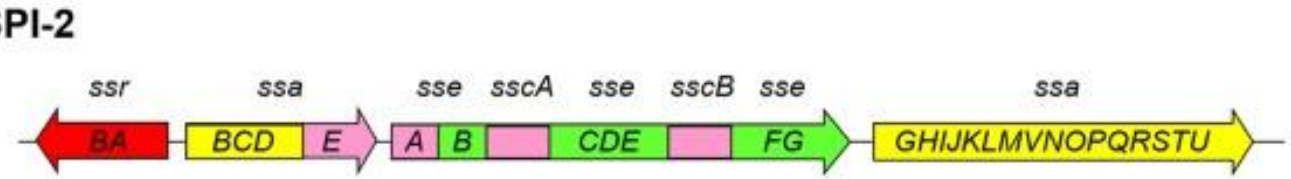
- SPI-2 genes
- other genes located outside SPI-2, which are necessary for survival and replication

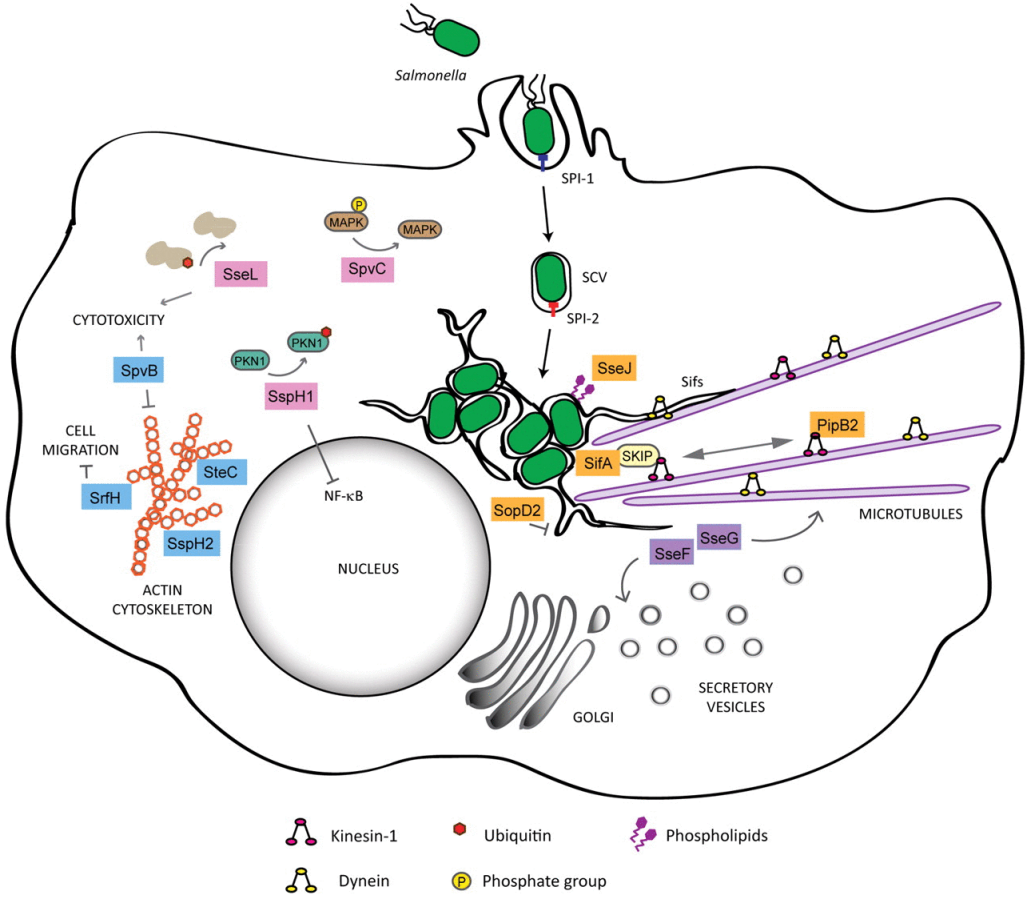
repression of

- *hilD* and *hilA* regulatory genes
- SPI-1 genes
- flagellum-based motility genes

SsrB molecular regulatory switch that helps *Salmonella* transition to an intracellular lifestyle

SPI-2





Figueira and Holden, 2012

Includes more than 40 genes organised in 4 operons:

- *ssa* → type III secretion apparatus (T3SS-2)
- *ssr* → secretion system regulators (SsrAB TCS)
- *ssc* → molecular chaperones
- *sse* → effector proteins

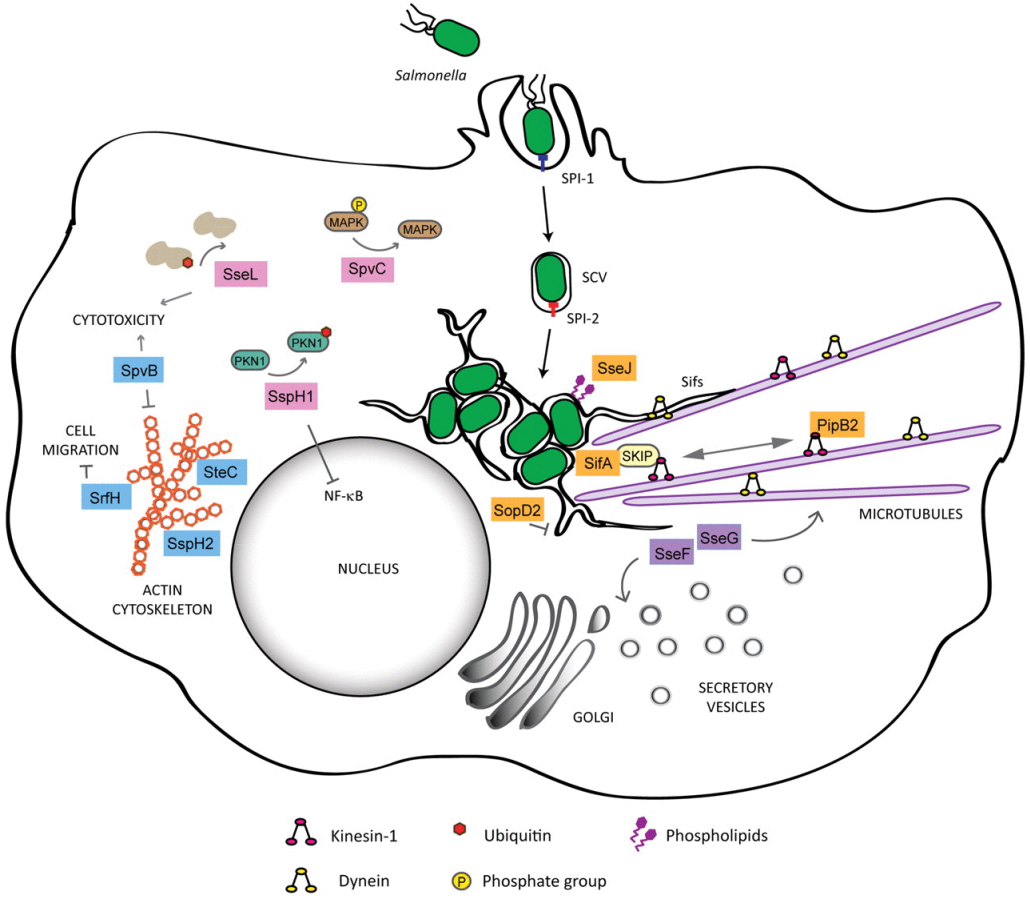
Main functions:

1. Survive and replicate within phagocytes and cells (4 hours after invasion)

2. Evade host phagosome oxidation mechanisms

3. Persist in target organs such as spleen and liver (systemic virulence in typhoid fever)

SPI-2

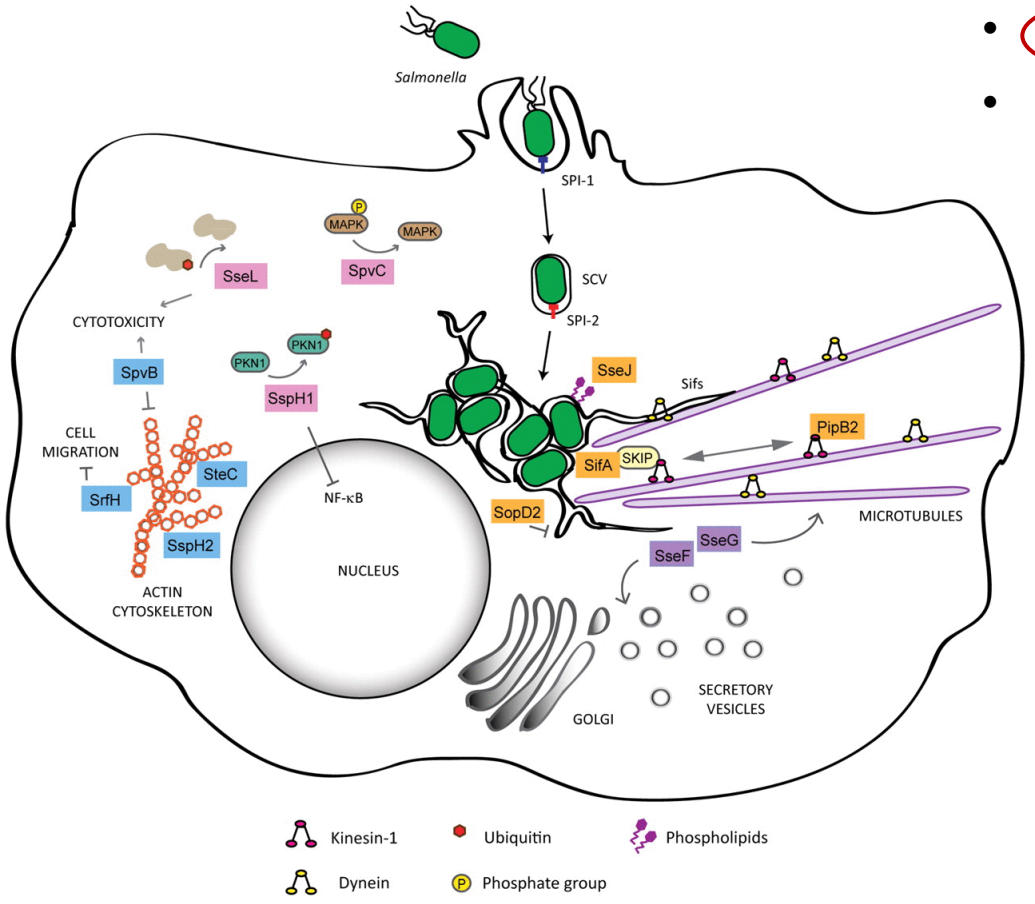


Figueira and Holden, 2012

T3SS-2 (Type III Secretion System) → translocation of effector proteins (around 30) into the host cytoplasm leading to a series of bacterial adaptations

- Vacuolar remodelling
- Intracellular survival (maintenance of SCV integrity)
- Intracellular replication
- Interference with immune signalling
- Localisation in the peri-Golgi region
- prevents trafficking from the phagocyte NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase) towards the SCV → prevents a phagocytic burst

T3SS-2 effectors



Figueira and Holden, 2012

Salmonella adapts to the intracellular environment and SPI-2 genes are differentially expressed.

Expression regulated by two-component systems:

- OmpR-EnvZ → regulates the expression of SsrA
- SsrA-SsrB → encoded by SPI-2
- PhoP-PhoQ → activated by the low pH intraphagosomal environment (PhoP regulates > 20 genes including SsrB)

regulates

- **SifA** → main effector, *Salmonella*-induced filament formation (SIF). Together with **PipB2** interacts with SKIP protein (binds kinesin-1) for anterograde transport on microtubules (SCV localisation)
- **SpiC** → prevents fusion with phagolysosome
- **SseF** → SCV localisation, microtubule clustering and SIF Formation
- **SpvC** → anti-inflammatory effect (MAPK)

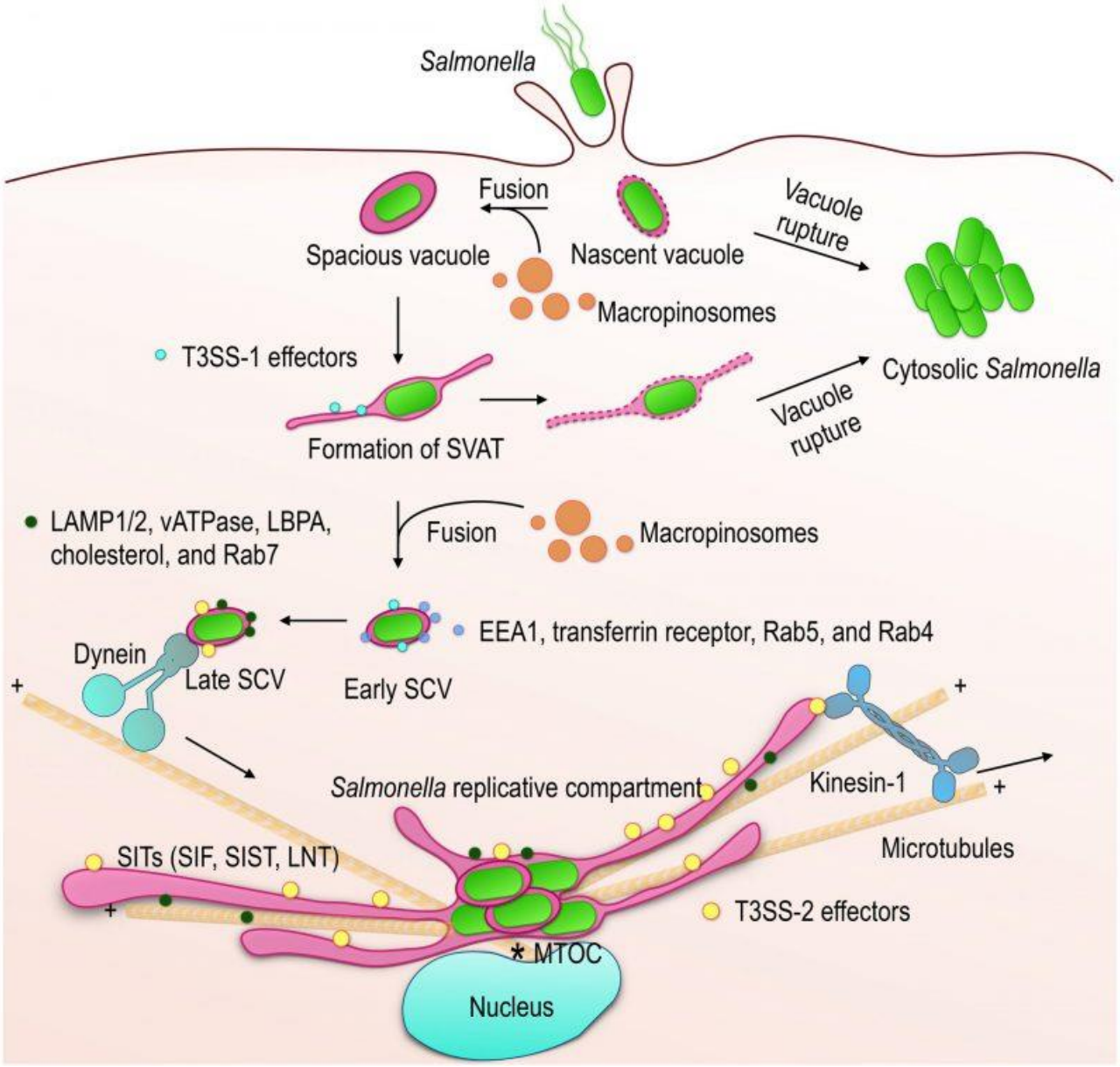
SCV maturation

Evading antimicrobial activities arsenal by staying within the SCV

SCV undergo a maturation process (latency 2-3 hours) before cell replication takes place:

- volume growth by fusion with endocytic vesicles
- membrane remodelling → lysosomal membrane glycoproteins (LAMP1 and Rab7)
- lumen acidification (vacuolar-type V-ATPases)
- no fusion with lysosome (no acid hydrolases, no mannose 6- P receptors) → remains in late endosome state

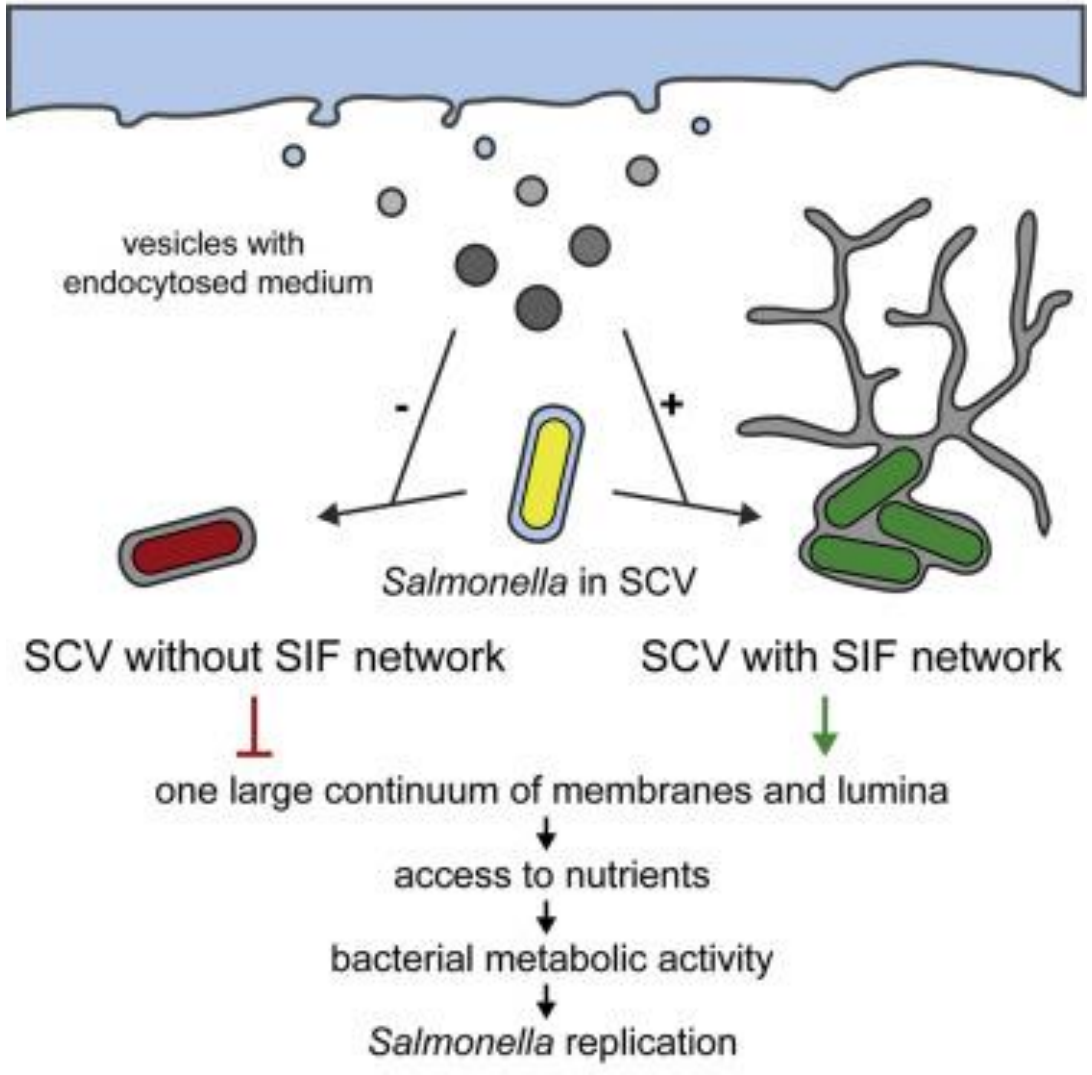
Movement from the cell periphery to the perinuclear region → pH decrease→ inducing expression of T3SS-2 and its effectors



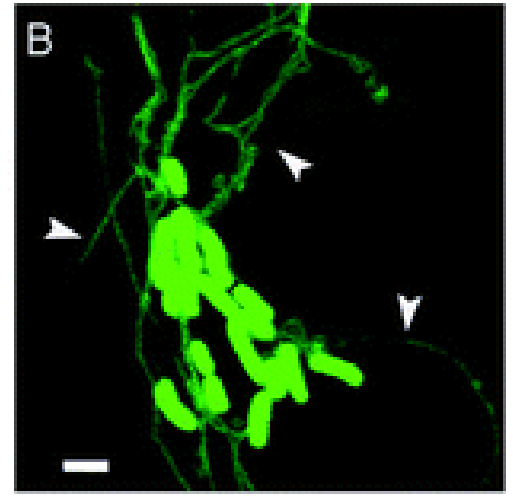
Fang and Meresse, 2021

Salmonella-induced filaments (SIFs)

Crucial for intracellular proliferation and survival



Liss, 2017



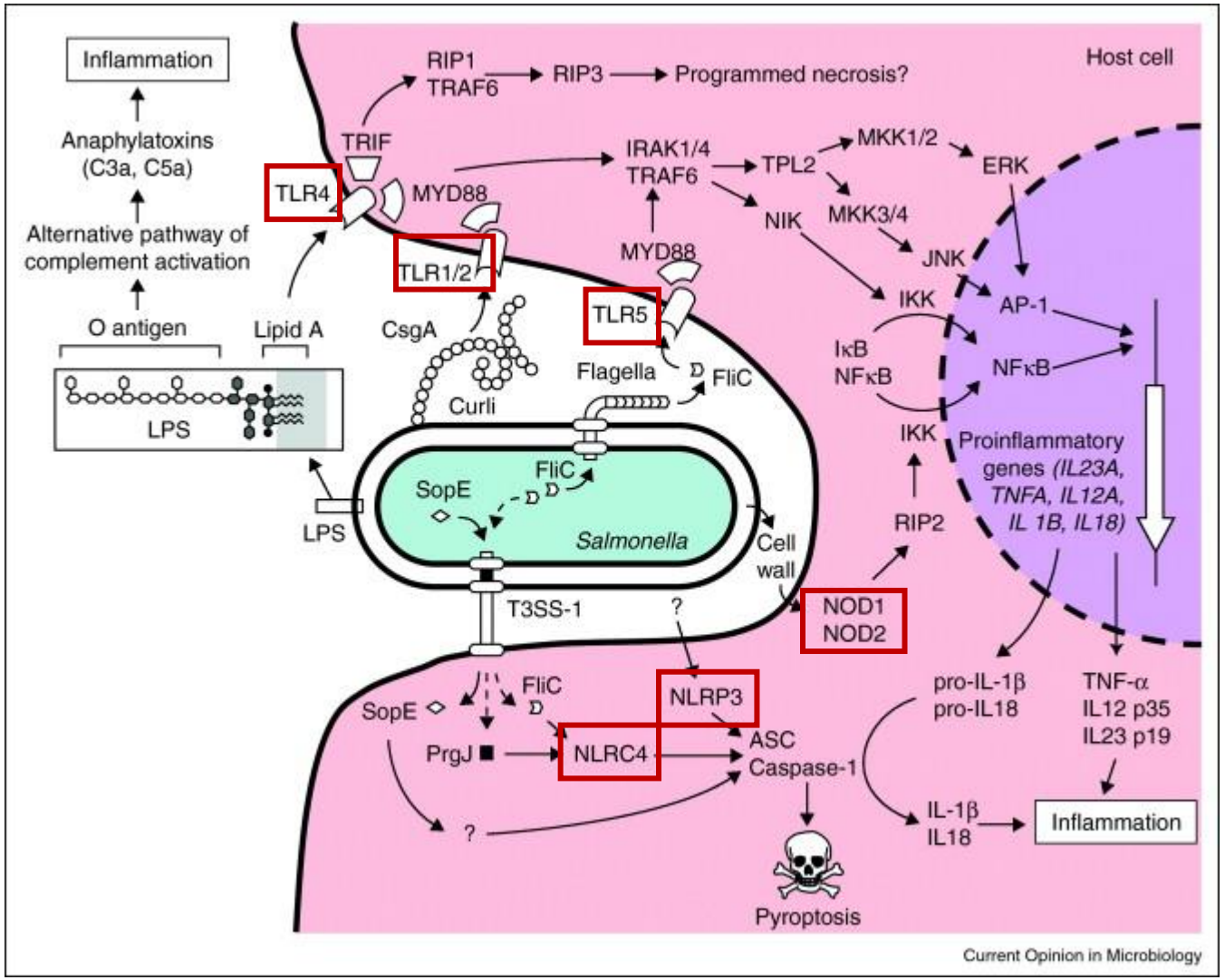
- *Salmonella* converts host cell endosomes into interconnected tubular vesicles called SIFs
- SIFs contain endocytosed medium compounds available to *Salmonella*
- Intracellular nutrition of *Salmonella* depends on access to endocytosed nutrients
- Connection to SIFs promotes intracellular replication of *Salmonella*

SIF formation coincides with initiation of *Salmonella* cell replication

Immune response

Host-*Salmonella* complex dialogue culminating in induction of host immune response

- Lipopolysaccharide (**lipid A**) → TLR4
- **FliC** of the flagellum → TLR5
- Cell wall (**PG**) → NOD1/NOD2
- **Curls** (biofilm fimbriae) → TLR1/2
- T3SS-1-dependent cytosolic process causes inflammasome activation (NLRC4 and NLRP3)
- Enterocyte-bacterium contact, flagellin release (FliC) → inflammatory response with NF-kB activation → proinflammatory cytokines for neutrophil and macrophage recall



Thiennimitr et al., 2012

Experimental models developed to study *Salmonella* infections

TABLE 1 | Salmonella infection models.

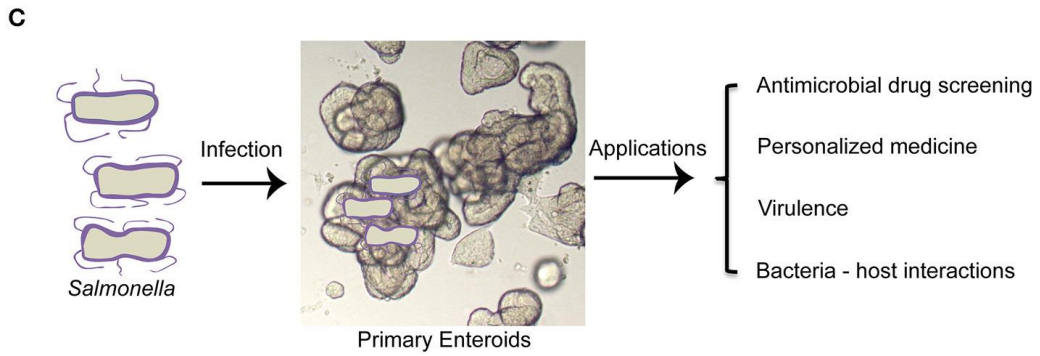
	Year	Author	*Salmonella* type	Model
In vitro	2001	Nickerson et al.	*Salmonella* Typhimurium	3D organotypic model based on the human embryonic intestinal epithelial cells (Int-407) (Barrila et al., 2010)
	2006	Zu Bentrup et al.	*Salmonella* Typhimurium	3D organotypic model based on the human colon adenocarcinoma cell line (HT-29 cell line) (Höner Zu Bentrup et al., 2006)
	2008	Isabel Martinez-Argudo and Mark A. Jepson	*Salmonella enterica*	M cell model (Martinez-Argudo and Jepson, 2008)
	2009	Le Blay et al.	*Salmonella* Typhimurium	Colonic fermentation model (Le Blay et al., 2009)
	2012	Tang et al.	Clinical non-typhoid *Salmonella* (NTS) isolates	RAW 264.7 murine macrophage cell line (Tang et al., 2012)
	2014	Dostal et al.	*Salmonella* Typhimurium	Gut fermentation-cell model (Dostal et al., 2014)
	2014	Zhang et al.	*Salmonella* Typhimurium	Crypt-derived mouse intestinal organoids (Zhang K. et al., 2014)
	2015	Forbester et al.	*Salmonella* Typhimurium	Intestinal organoids derived from human induced pluripotent stem cells (hIPSCs) (Forbester et al., 2015)
	2016	Newburg et al.	*Salmonella* Typhimurium	Immature human normal fetal intestinal epithelial cell (H4), mature human metastatic colonic epithelial cell (T84) and human normal colon mucosal epithelial cell (NCM-460) (Newburg et al., 2016)
	2017	Fang et al.	*Salmonella* Typhimurium	HeLa cells, Caco-2 cells, THP-1 cells and LS174T cells (Fang et al., 2017)
ex vivo	1997	Frost et al.	*Salmonella* Typhimurium	Calf ileal epithelium (Frost et al., 1997)
	2004	Haque et al.	*Salmonella* Typhimurium TML	Human intestinal *in vitro* organ culture (IVOC) (Haque et al., 2004)
	2012	Tsilingiri et al.	*Salmonella* Typhimurium	Organ culture model (intestinal mucosa) (Tsilingiri et al., 2012)
	2015	Boyle et al.	*Salmonella* Typhimurium	Perfusion of the isolated rat small intestine (Boyle et al., 2015)
	2016	Newburg et al.	*Salmonella* Typhimurium	Immature human intestinal tissue (Newburg et al., 2016)
In vivo	1973	Giannella et al.	*Salmonela* Typhimurium	The ligated rabbit ileal loop model (Giannella et al., 1973)
	2003	Barthel et al.	*Salmonella* Typhimurium	C57BL/6 mice (Barthel et al., 2003)
	2007	Woo et al.	*Salmonela* Typhimurium	SLC11A1 wild type mice (Woo and Berk, 2007)
	2009	Ren et al.	*Salmonella* Typhimurium	C57BL/6 mice (Ren et al., 2009)
	2011	Mian et al.	*Salmonella* Typhi	Humanized mice (alymphoid RAG-2-/-γc-/- mice engrafted with human leukocytes) (Firoz Mian et al., 2011)
	2012	Özkaya et al.	*Salmonella* Typhimurium	BALB/c mice (Özkaya et al., 2012)
	2012	Mathur et al.	*Salmonella* Typhi	A mouse model (tlr11-/+ mice) (Mathur et al., 2012)
	2014	Zhang et al.	*Salmonella* Typhimurium	Neonate mice (Zhang Y. G. et al., 2014)

Yin and Zhou *et al.*, 2018

In vitro cell culture lines are relatively easy to maintain and provide a more consistent environmental niche for evaluating bacterial survival and replication than most animal hosts. Genetic manipulations in these cell lines greatly aided the investigation of how *Salmonella* interact with host epithelial and macrophage cells

Animals possess the complex cell types, architectural organizations, and specialized organ structures. More importantly, the intact immune systems of the animals have obvious advantages over all other models and therefore are considered the closest to clinical settings over in vitro cell or ex vivo organ and tissue models.

Organoid and Enteroid Modeling of *Salmonella* Infection



Yin and Zhou *et al.*, 2018

It has been shown that *Salmonella* quickly attaches and invades the enteroids causing the typical morphologic changes of the host cells during *Salmonella* invasion as well as the disruption of epithelial tight junctions.

https://figshare.scilifelab.se/articles/media/Time-lapse_Movies_for_Geiser_et_al_2021_mBio_Salmonella_enterica_Serovar_Typhimurium_Exploits_Cycling_through_Epithelial_Cells_to_Colonize_Human_and_Murine_Enteroids_/12998570/1?file=25766672