(1) Mucosal response to pathogenic enterobacteria. (2) Illustration of a peculiar host-Salmonella relationship: Intestinal carriage in birds.

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Mucosal response to pathogenic enterobacteria

Illustration of a peculiar host-*Salmonella* relationship: Intestinal carriage in birds.

1

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Mucosal response to pathogenic enterobacteria

- Microbial communities in the gut
- *Salmonella* as emblematic pathogenic enterobacteria
- First contact of *Salmonella* with intestinal epithelium
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3

Microbial communities in the gut

• **Commensal microorganisms** : non invasive normal residents.
4 major phyla: firmicutes (*Lactobacillus* spp., *Clostridiales)* spp.), *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*.

• **Pathobionts** : potentially harmfull when weakness of the barrier.
Enterococcus spp., *Clostridium difficile.*

• **Enteric pathogens** : 1.5 million deaths worldwide in 2012 (source WHO), by **diarrhoeal illnesses**.

Secretory diarrhoea: *Vibrio cholerae*, E.T.*Escherichia coli,* inhibit enterocytes Na+ transporter, increase Cl- secretion

Inflammatory diarrhoea: *Salmonella* spp*., Campylobacter* spp., neutrophilic infiltrate

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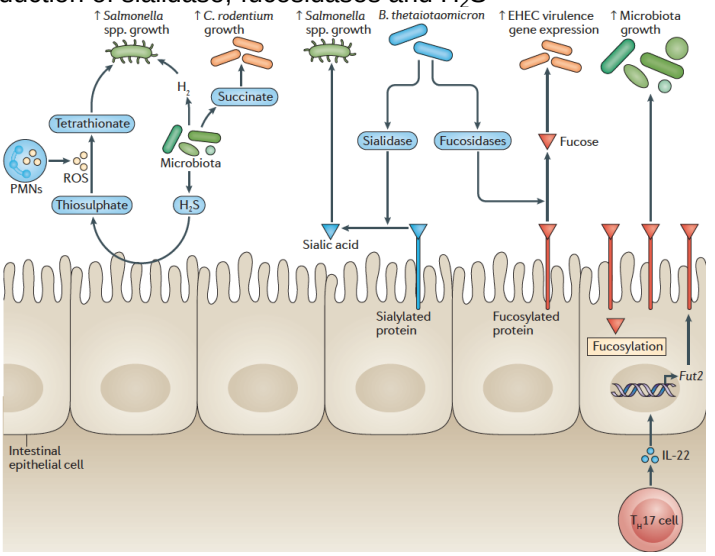
Microbial communities in the gut

Commensal microorganisms inadvertently feeds pathogens with sugars and other beneficial compounds :

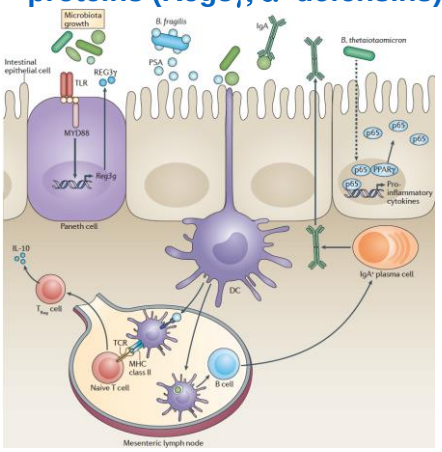
via production of sialidase, fucosidases and H_2S



From: Perez-Lopez et al., Nature Reviews Immunology, 2016, 16:135-148.

Microbial communities in the gut

Recognition of commensal motifs by host receptors down-regulates inflammation and induce production of antimicrobial proteins (Reg3γ, α–defensins) and IgA :



From: Perez-Lopez et al., Nature Reviews Immunology, 2016, 16:135-148.

- TLR2 of DCs recognizes polysaccharide A (PSA, capsular) of *Bacteroides f.* and induce regulatory T cells (Tregs, producing IL10).
- *Bacteroides t*. inhibits pro-inflammatory cytokines transcription by exporting NFκB (p65) via PPARγ from epithelial cell nucleus.
- Sampling of commensal bacteria by CX3CR1+ mucosal dendritic cells induce the production and secretion of sIgA (neutralizing bacteria and toxins in the lumen).

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Salmonella as emblematic pathogenic enterobacteria

• **Enteric pathogens** : Salmonellosis second most commonly reported zoonose, after campylobacteriosis, in humans (ECDC-EFSA source 2019: doi: 10.2903/j.efsa.2019.5926.

- Two major *Salmonella* serotypes (more than 2/3 recorded strains),
  S. Enteritidis
  S.Typhimurium
  Ubiquist: many host animal species (by contrast to host specific serotypes inducing systemic typhoid disease)
- 70% of foodborne outbreaks
- Stagnation of the sanitary situation since 5 years

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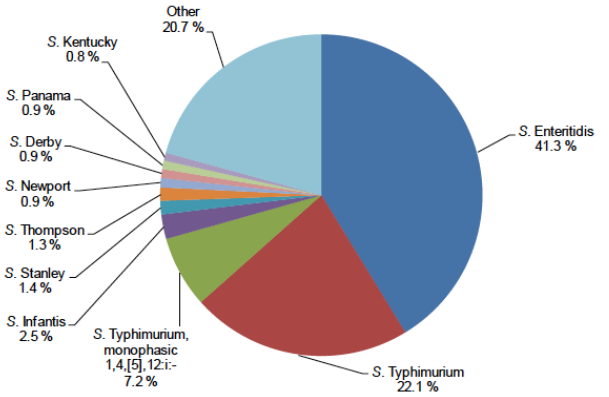
Salmonella as emblematic pathogenic enterobacteria

Salmonellosis, a major public health concern

Figure SA3. Distribution of the 10 most common Salmonella *serovars in humans in the EU, 2012 (N = 82,409)*



European Union Summary Report on Zoonoses, EFSA Journal 2014;12(2):3547

Salmonella as emblematic pathogenic enterobacteria

Salmonellosis, a major concern for veterinary and public health

- Associated to consumption of contaminated food products from animals
- Causative contamnated food products in Europe in 2012

Eggs and egg-derived products 22%	**Meet, ham, saussages… (poultry, pig, beef and others) 20,2%**	**Fish and derived products 9,2%**
Cheese 5,4%	**Fruits and vegetables 5,4%**	**Sea food 4,6%**

- In food, the highest levels of *Salmonella*-positive samples still occurred in poultry meat and other meat, intended to be cooked before consumption (ECDC-EFSA report, 2019)

Salmonellosis : a One Health problem

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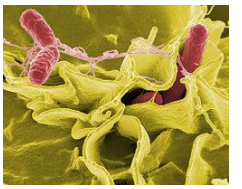
First contact of *Salmonella* with intestinal epithelium

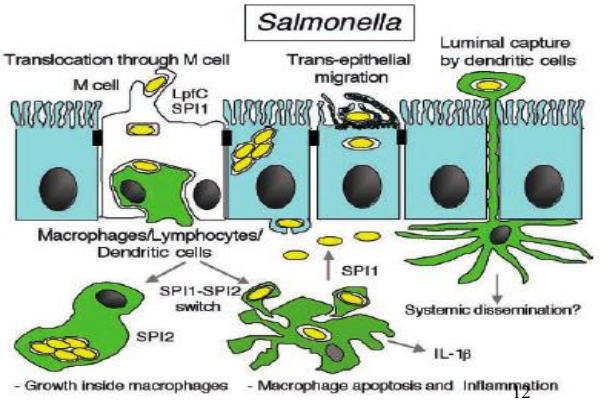
Initial steps



- Possible detection of bacterial products in the lumen: LPS by TLR4.
Bacterial amyloid curli fibrils and lipoproteins by TLR2.

- Luminal capture by dendritic cells (dendrites expressing tight junction components)[1].

- Active entry through M cells and epithelial cells: *Salmonella* TTSS



1 Rescigno M. et al., Nat. Immunol. 2001, 2: 361-367.

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First contact of *Salmonella* with intestinal epithelium

Initial steps

Epithelial cell surface remodelling by *Salmonella* : to favor internalization by triggering process

- Initiated by adhesion (fimbriae of *Salmonella*)
- Involvement of the Type Three Secretion System (T3SS) of *Salmonella*, encoded by SPI1:
  - •SipB + SipC form a « syringe » inside the host cell membrane.
  - •SopE injected activates GTPases .
  - •Actin polymerization stabilized par SipA , negative regulation by SptP.
- Induction of membrane remodelling, by modifications of cytoskeletton. Formation of pseudopodes and internalization

Remark: If *Salmonella* is devoid of TTSS, entry by « zippering process ».

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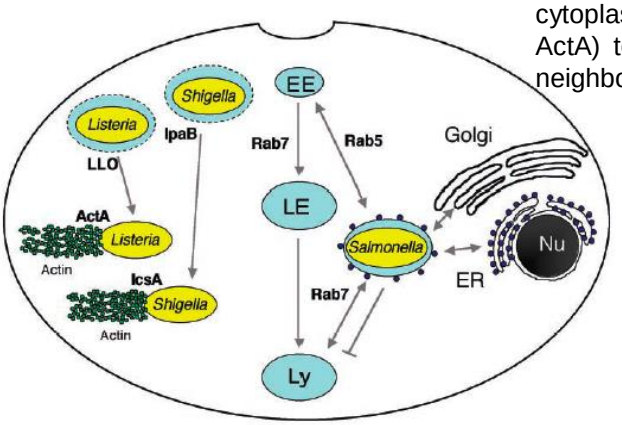
First contact of *Salmonella* with intestinal epithelium

Comparison between *Salmonella*, *Listeria* and *Shigella*

Shigella and *Listeria* engulf inside the cell, in a vacuole.

Once inside, *Listeria* destroys the vacuole (with LLO) to reach the cytoplasm, aggregates actin (with ActA) to move in the cell and to neighbor cells (like *Shigella*).



LLO: listeriolysin
EE: early endosome
LE: late endosome
Ly: lysosome

Salmonella in the vacuole, inhibits fusion with lysosome (via Rab5 et 7), survives and multiplies using genes encoded by SPI2.

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Mucosal response to pathogenic enterobacteria

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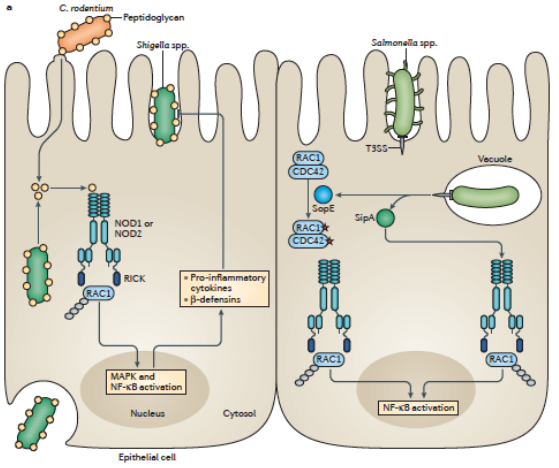
First contact of *Salmonella* with intestinal epithelium

Epithelium sensing of *Salmonella*

At the surface: LPS sensing by TLR4 -> signal transduced via MyD88 and TRIF. Intracellularly: Muramyl dipeptide (MDP) sensing by NLR NOD2; T3SS molecules sensing by NLR NOD1 and NOD2 -> signal transduced via CARD Kinase (RICK)



Results: Activation of NF-κB pathway and transcription of NF-κB-controlled inflammatory response genes.

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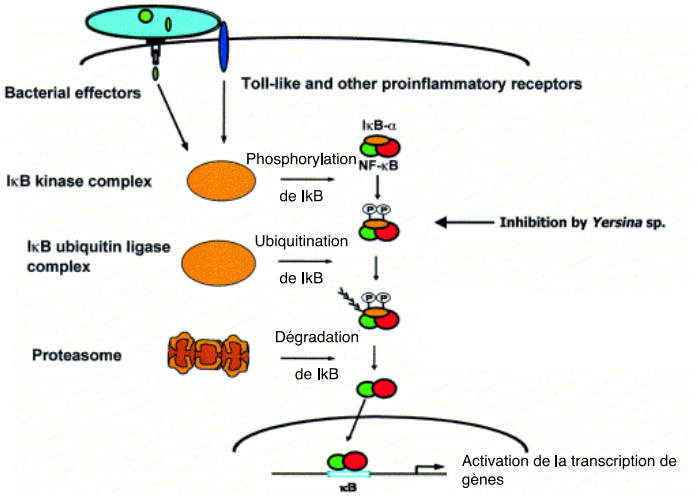
First contact of *Salmonella* with intestinal epithelium

NF-κB pathway

From TLRs and NLRs → **Production of cytokines, defensins and chemokines attracting immune cells**



First contact of *Salmonella* with intestinal epithelium

Epithelium sensing of *Salmonella* flagellin

When *Salmonella* reaches the sub-epithelial area:

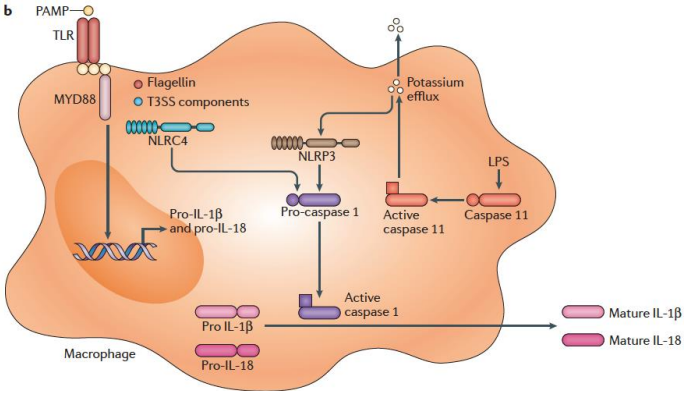
→ Flagellin sensing by TLR5 expressed

- at the basolateral surface of epithelial cells => CCL20 prod [1]
- at the surface of dendritic ¢ (CD11c+) of the lamina propria => IL23 prod

Activation of NF-κB pathway

→ Flagellin sensing inside resident macrophages by NLRC4 [2, 3]

Activation of Caspase 1 (IL1 and IL18 secretion)



1 Sierro et al, 2001, PNAS, 98 (24):13722-27.

2 Mlao EA *et al*, 2006, Nat Immunol, 7:569-75.

3 Franchi L. *et al.* 2006. Nat. Immunol, 7:576-82.

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First contact of *Salmonella* with intestinal epithelium

Epithelium response to *Salmonella*

Production of inflammatory cytokines:

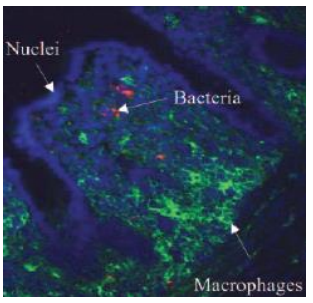
- IL-1 and IL-18 (via caspase 1), IL-23
- IL-6
- TNF-α

Production of chemokines attracting immune cells:

- IL-8 (CXCL8) for neutrophils
- MCP-1 (CCL2) for monocytes
- MIP-1α (CCL3) for neutrophils and macrophages
- CCL20 (MIP3α) for immature dendritic cells

→ **Correlates with histological observation of phagocytes infiltrate in infected intestinal lamina propria.**

→ **Phagocytes called to fight.**



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First contact of *Salmonella* with intestinal epithelium

Epithelium response to *Salmonella*

The IL-23 – T_H17 axis:

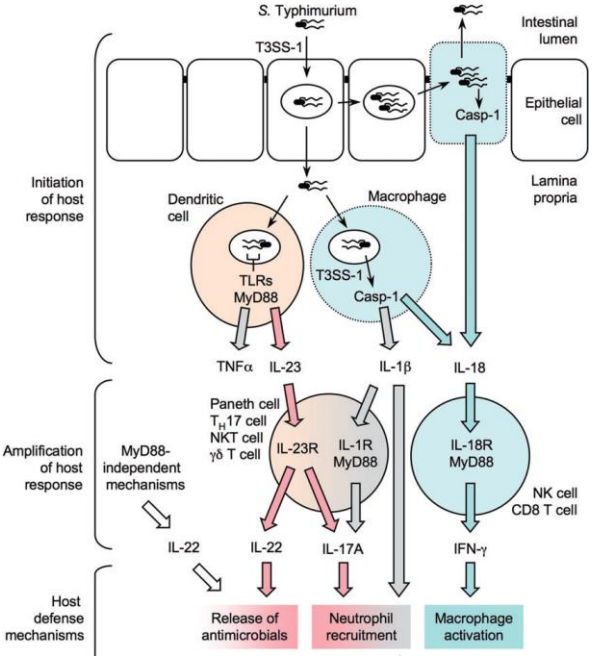
- **IL-23**: production by TLR and NLR stimulated mucosal DCs
- IL-23: stimulates T_H17, NKT, γδT and ILC to produce **IL-17** and **IL-22**
- IL17 and IL22: stimulate epithelial cells to proliferate (tissue repair), to produce lipocalin2 and calprotectin [1,2], Fe3+ and Zn2+ chelators, and to produce antimicrobial defensins
- IL-17 induces CXC chemokines-dependent recruitment of neutrophils

1 Behnsen J *et al*, 2014, Immunity, 40 (2): 262-73.
2 Dixon BR *et al*, 2016, PLOS One, 11(2):e0148514. doi: 10.1371

First contact of *Salmonella* with intestinal epithelium

Epithelium response to *Salmonella*

The IL-23 – T_H17 axis: **amplification of host defense mechanisms**



Godinez I *et al*, 2011, Cellular Microbiology, 13(11): 1639–47.

First contact of *Salmonella* with intestinal epithelium

Defensin response: important antibacterial weapon of epithelium

- FliC (flagellar protein) of *Salmonella* Enteritidis induce HBD2 expression in Caco-2 epithelial cells
- Matrilysin MMP7 (maturation of α-defensins) KO mice

  → Susceptibility to lethal infections by *Salmonella* Typhimurium

  → Low efficiency in the clearance of entero-pathogenic bacteria

- Transgenic mice for the gene of human α-defensin HD5

  → Resistant to pathogenisc effects of *Salmonella* Typhimurium [1]

1 Salzman *et al,* 2003, Nature, 422:522

First contact of *Salmonella* with intestinal epithelium

Salmonella strategies of escape from defensins

- *Salmonella* Typhimurium: acylation of lipid A, modification with aminoarabinose.
- *Salmonella* Typhimurium: negative regulation of α-defensins and lysozyme expression (dependent on PhoP virulence regulator).

Salmonella strategies of survival and dissemination

Live happy, live hiding : stay invisible from anti-bacterial factors

- Persistance inside macrophages within phagosomes: inhibition of the fusion with lysosome.
- Probability of dissemination via circulating macrophages and dendritic cells.

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The fate of *Salmonella* after epithelial crossing

Dependent on the activation state of phagocytes: neutrophils, macrophages and dendritic cells

- Important bacterial burden reduced by neutrophils: by production of ROS, nitric oxide, antimicrobial peptides,
- *Salmonella* is taken up by macrophages and/or dendritic cells.
- TLR4 is critical for early cytokine production and killing of bacteria by macrophages. TLR4 and TLR2 are involved in the control of bacterial burden and the host survival [1, 2]
- Bacterial survival in macrophages if non activated. *Salmonella* blocks oxidative burst (inhibition of NADPH oxidase) by SPI2.
- Induction of apoptosis/pyroptosis in macrophages (via SipB on caspase1) and/or inflammatory response (IL-1).

1 Weiss DS *et al*, 2004, J Immunol, 172:4463.
2 Sivick KE *et al*, 2014, Cell host Microbes, 15:203.

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The fate of *Salmonella* after epithelial crossing

Salmonella in circulating cells

- Apoptotic bodies generated after macrophage infection and induced cell death: taken up by bystander dendritic cells [1].

- Extra-intestinal dissemination of *Salmonella* within phagocytes/DC CD18+ [2] according to the serotype.

- *Salmonella* does not multiply within DC but may stay and multiply within inactivated macrophages.

→ **Phagocytes may serve as vehicle**

1 Yrlid and Wick, 2000, J. Exp. Med. 191:613-624.
2 Vazquez-Torres A. et al., Nature 1999, 401: 804-808.

The fate of *Salmonella* after epithelial crossing

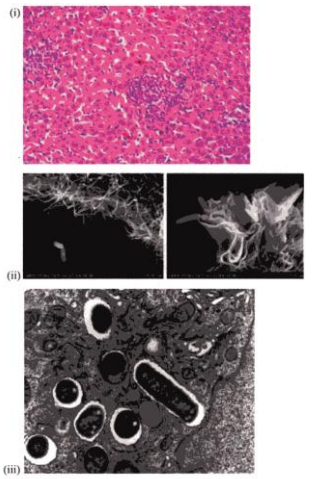

Fig. 2. Histochemical and electron microscopic images of Salmonella associated with the host. (i) A H&E stain showing clear evidence for a *Salmonella* associated granuloma in the liver of an infected mouse. (ii) Scanning electron micrograph of salmonella attaching to a macrophage. (iii) Transmission electron micrograph of *Salmonella* residing within an adapted vacuole within a murine cell.

Infected liver: Hematoxylin/Eosine staining. Granuloma aspect (neutrophils), full of bacteria.

Infected macrophage : Scanning electron microscopy. *Salmonella* attached.

Murine infected cell: Transmission electron microscopy. Intra-vacuolar *Salmonella*.

→ **Phagocytes are in the heart of the fight**

The fate of *Salmonella* after epithelial crossing

Interaction host - *Salmonella*: phagocytes in the heart of the fight

Dendritic cells DC:	An alternative entry way/ a mean to stimulate adaptative immunity
Macrophages:	A niche for systemic infection and persistance / a way to eliminate bacteria if activated.
Neutrophils:	A good weapon against extracellular bacteria, production de defensins…

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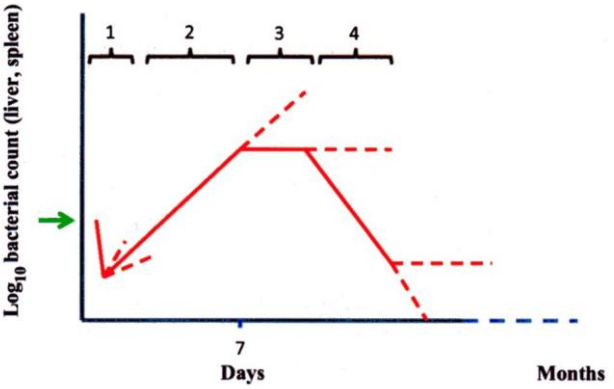
The fate of *Salmonella* after epithelial crossing

Systemic phase of infection after inoculation by oral route in mice



1: Reduction of l'inoculum
2: Bacterial multiplication
3: Plateau phase
4: Bacterial clearance

- *Salmonella* found in Peyer's patches, 6h post infection per os.
- *Salmonella* found in mesenteric lymph nodes (24-48h p.i.) then in spleen and liver.

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Systemic response to infection, adaptive immunity

Lessons learned from murine models of *Salmonella* infection

Bacterial multiplication which does not stay unseen,

cells of the secondary lymphoid organs wake-up:

- Decrease of the NK population (NK1.1+, TCR αβ-)
- Decrease of the NKT population (NK1.1+, TCR αβ+)

In the spleen, 2 days after oral infection (in mice)

- Decrease of T-cell populations (TCRαβ+, NK1.1-).

In the spleen, 5 days after oral infection (in mice)

➡ Activation, probable emigration towards mucosal effector sites.

Kirby et al. J. Immunol. 2002, 169:4450-4459.

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Systemic response to infection, adaptive immunity

Lessons learned from murine models of *Salmonella* infection

Epithelial receptors towards immune cells:

- ICAM1: constitutive and up-regulated by *Salmonella* ⟷ LFA1 of leucocytes
- LFA3: constitutive and up-regulated at the epithelial level ⟷ CD2 of T lymphocytes (costimulation of IEL)
- gp180 ⟷ CD8
- CD1, CD1d: Presentation of bacterial lipid antigens (mycobacteria)

→ **Contribute to interaction and activation of coming lymphocytes**

Systemic response to infection, adaptive immunity

Lessons learned from murine models of *Salmonella* infection

- Primed T cells detected 6-12h in Peyer's patches and 9-12h in mesenteric lymph nodes of mice after oral infection [1].
- Priming initiated by CD11c+ CCR6+ dendritic cells attracted to the infected epithelium by CCL20.
- Mice lacking T-bet+ Th1 cells are unable to resolve the infection [2].
- Th1 cells are major source of IFN-γ.
- Neutrophils have been recently pointed out as a source of IFN-γ [3].
- Human genetic disorders linked to fatal salmonellosis : invalidated IFN-γ and IFN-γR genes [4].

1 Mc scorley SJ *et al*, 2002, Immunity, 16(3):665-77.
2 Ravindran R *et al*, 2005, J Immunol, 175(7): 4603-10.
3 Spees AM *et al*, 2014, Infect. Immun. 82: 1692–97.
4 Ottenhoff TH *et al*, 2003, Adv Exp Med Biol. 531:279-94.

Systemic response to infection, adaptive immunity

Lessons learned from murine models of *Salmonella* infection

In gamma-interferon gene knock out mice (IFN-$\gamma^{-/-}$), oral inoculation with an attenuated strain of *Salmonella* * :

- No resolution of the infection but septicemia in 2 weeks
- No increase in expression of MHCII and VCAM1
- No increase in CD4+ and CD8+ T cell populations

➡ Deficiency of intestinal and systemic immunity without IFN-γ

* Bao S. et al., Immunology 2000, 99: 464-472.

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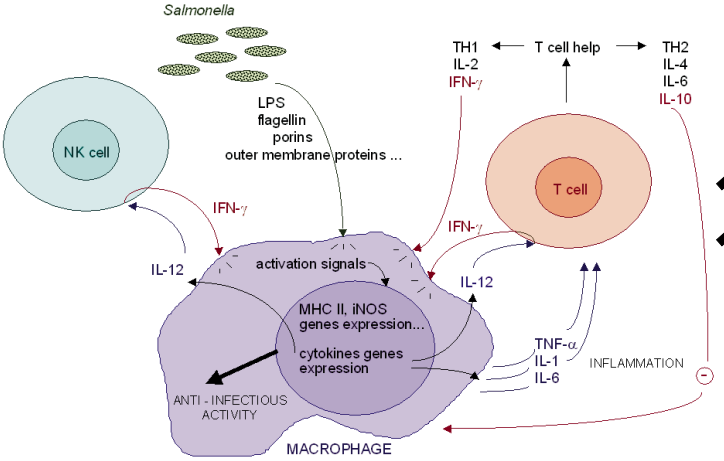
Bacterial clearance under the control of host IFN-γ

Macrophages as major host target cells of *Salmonella*: the success of the host fight depends on their activation by IFN-γ.



From Lalmanach AC and Lantier F, Microbes and Infection, 1999.

Microbicidal activity of macrophages is induced by IFN-γ:

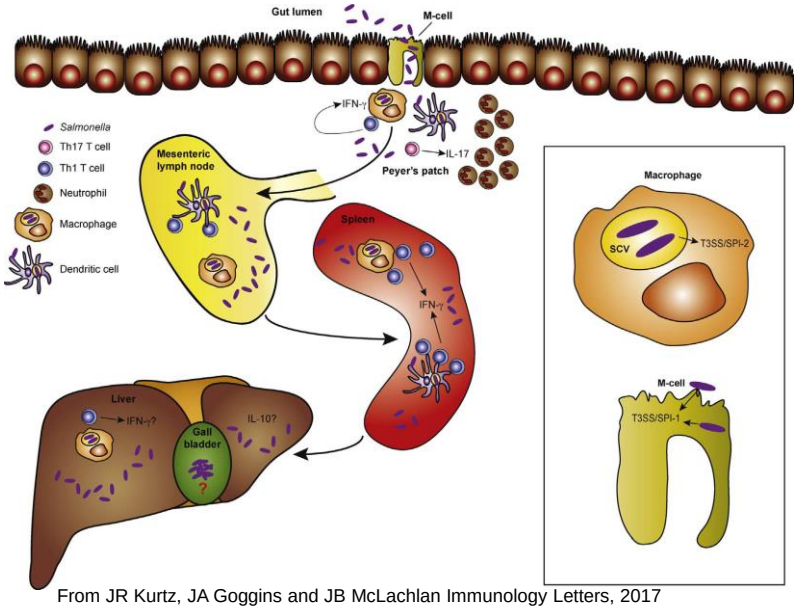
- ⬈ Activity NRAMP1[1]
- ⬈ Production of NO

The quantity of bacteria in tissues is proportional to the number of infected phagocytes.

1 Jabado N. et al., J. Exp. Med. 2000, 192: 1237-1248.

Bacterial clearance under the control of host IFN-γ

General overview of what happens in infected sites



From JR Kurtz, JA Goggins and JB McLachlan Immunology Letters, 2017

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Illustration of a peculiar host-*Salmonella* relationship: Intestinal carriage in birds.

- **Intestinal carriage of *Salmonella* in birds**
- Resistance of birds to *Salmonella* intestinal carriage
- Role of defensins in the protection of birds
- Insights into protective role of microbiota

Intestinal carriage of *Salmonella* in birds

Poultry is a reservoir for *Salmonella*:

- Colonisation of the digestive tract of birds by *S.* Enteritidis, contamination poultry flocks.
- Chicks are susceptible to the infection : more young the animals are, more high is the level of mortality.
- Chickens and adults : intestinal persistence in the caecum, asymptomatic with fecal shedding.
- Horizontal transmission (between congeneers).
- Vertical transmission (hen to eggs).
- Contamination of the food chain (eggs, meat).

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Intestinal carriage of *Salmonella* in birds

Means of fight:

- Genetic selection of resistant animals
- Vaccination, improvement of immune protection
- Alternative treatments to conventional antibiotics
- Microbiota driven preventive measures…

Common necessity: a better knowledge of immune factors of resistance in poultry

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Resistance of birds to *Salmonella* intestinal carriage

Identification of host factors involved in the resistance: genes differentially expressed between resistant (R) and susceptible (S) birds

Approach:

Comparison of chicken inbred lines divergently selected according to the level of *Salmonella* colonization in the caecum.

- Oral inoculation of *S*. Enteritidis.
- Analysis of bacterial burden: bacterial counts in the caecum.
- Analysis of expression of immune response target genes (in the caecal lymphoid area) by real time qRT-PCR

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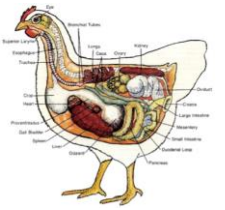
Resistance of birds to *Salmonella* intestinal carriage

Comparative analysis of the caecal bacterial load between divergently selected inbred lines

- **Methods** : Oral inoculation of *S*. Enteritidis (10^8 CFU/hen); bacterial counts in the caecum, real-time qRT-PCR for target genes (avian defensins)



- **Results** : Bacterial load in the caecum

Weeks pi	Line 6 Log CFU/g	Line 6 Positive animals	Line 15 Log CFU/g	Line 15 Positive animals
1	4.37 ± 0.53	7/7	5.03 ± 0.50	7/7
2	**0.96 ± 0.14**	6/6	**2.25 ± 0.44**	8/8
4	ND	**0/8**	ND	**4/6**

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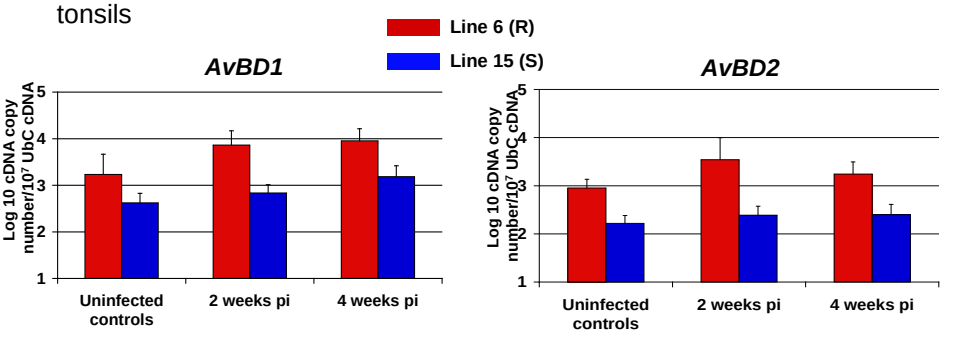
Resistance of birds to *Salmonella* intestinal carriage

Comparative analysis of defensin genes expression between S and R birds

- **Results** : Defensin genes (AvBD1 and AvBD2) expresssion in caecal tonsils



Expression of AvBD1 and of AvBD2 correlated with the phenotype of resistance to *Salmonella* carriage.

Sadeyen J.R. et al., 2006, Microbes &Infection, 8, 1308-14.

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Master 2 I²VB: Infectiologie Immunité Vaccinologie Biomédicaments
Master 2 IDOH: Infectious Diseases and One Health

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Illustration of a peculiar host-*Salmonella* relationship: Intestinal carriage in birds.

- Intestinal carriage of *Salmonella* in birds
- Resistance of birds to *Salmonella* intestinal carriage
- **Role of defensins in the protection of birds**
- Insights into protective role of microbiota

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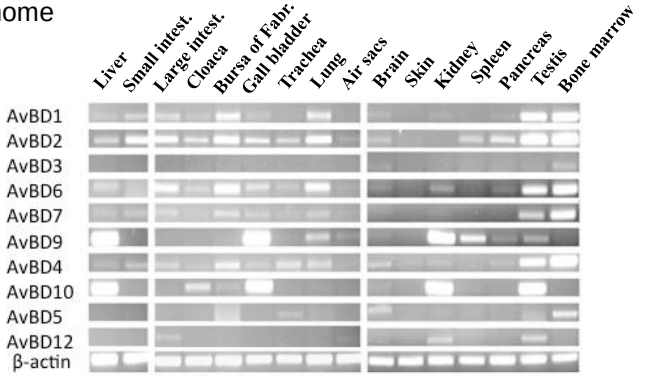
Role of defensins in the intestinal protection of birds

History of AvBDs

❖ **1994:** Gallinacin1 and 2, first avian beta-defensins (AvBDs) discovered in the granules of heterophils (chicken neutrophils) (Evans et al, J. Leukoc. Biol., 1994; Harwig et al, FEBS Letters, 1994)

❖ **2004:** First chicken genome sequence: cluster of 13 genes for ß-defensins (on *Gallus gallus* chromosome 3)

❖ **2004:** First transcriptomic analysis on the entire repertoire (Lynn et al, Immunogenetics, 2004, 56: 170; Xiao et al, BMC Genomics, 2004, 5: 56)



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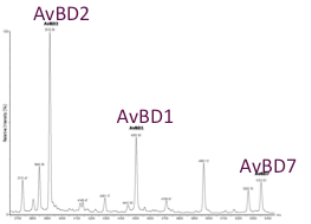
Role of defensins in the intestinal protection of birds

Avian defensins producing cells:

❖ **2009 Heterophils peptide map**

Heterophils purified (chicken peritoneal wash)

Wright staining



Mass spectrometry profiling from intact cells

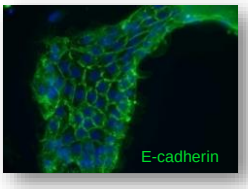
Derache C. et al. 2009. Antimicrob Agents Chemother. 53: 4647-55.

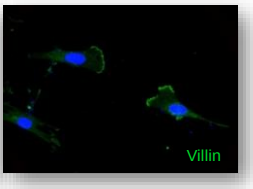
Role of defensins in the intestinal protection of birds

Avian defensins producing cells

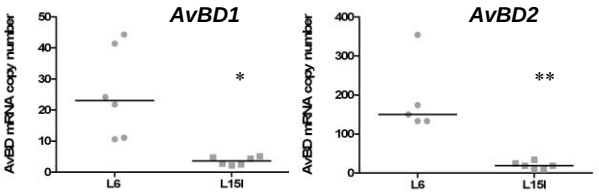
❖ **Epithelial cells of the caecum**

- Embryonic caecal epithelium explants: cell monolayer with epithelial markers





- Expression of AvBD1 and AvBD2



Derache C. et al. 2009. Dev Comp Immunol. 33: 959-66.

➡ **Intestinal epithelial cells express AvBD1 et AvBD2 according to the R/S phenotype of chicken line they originate from.**

Role of defensins in the intestinal protection of birds

Detection of AvBDs in intestinal segments

❖ **Bottom-up M/S analysis from jejunum (J), ileum (I), caecum (C) and tonsils (To)**

	Number of Unique Peptides				Protein Identification Probability				Exponentially Modified Protein Abundance Index			
Identified proteins	**To**	**C**	**I**	**J**	**To**	**C**	**I**	**J**	**To**	**C**	**I**	**J**
AvBD2	4	3	5	4	100	100	100	100	197.4	259.4	30.8	67.4
AvBD7	3	3	2	2	100	100	100	100	4.5	2.6	2.8	1.6
Cat D precursor, [*Gallus gallus*]	3	3	3	0	100	100	100	42	0.6	0.1	0.5	0
Cat B, [*Homo sapiens*]	1	0	1	0	98	99	100	99	0.1	0	0.1	0
Cat B precursor, [*Gallus gallus*]	0	0	1	1	0	98	100	98	0	0	0.1	0.2
AvBD1 precursor	1	1	0	1	86	84	99	99	0.8	0.3	0	0.6
Cat H, [*Gallus gallus*]	1	0	0	0	99	32	38	33	0.2	0	0	0
Cat L , [*Homo sapiens*]	1	0	0	0	100	27	0	0	0.1	0	0	0

➡ **Co-detection of defensins and proteases (cathepsins, Cat) in caecal tonsils => Test AvBD7 resistance to proteolysis**

Bailleul G et al, PLOS One, 2016

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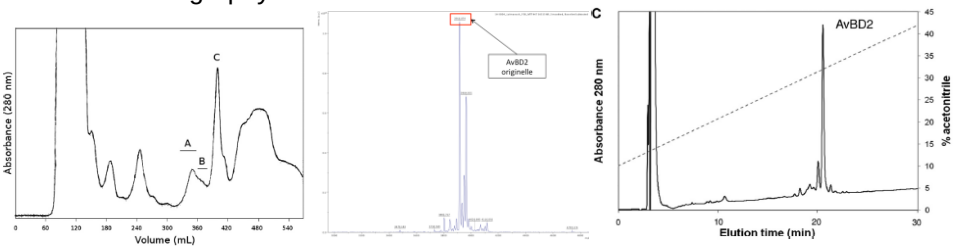
Role of defensins in the intestinal protection of birds

Preparation of AvBDs for structural and functional analysis

❖ **Extraction and purification from chicken bone marrow, rich in AvBDs**

Separation and analysis procedures:

Size Exclusion Chromatography → M/S MALDI-TOF → RP-HPLC



➡ **Three major defensins of the bone marrow : AvBD1, AvBD2 and AvBD7 (in agreement with data from heterophils ICM/MS)**

Derache C. et al. 2009. Antimicrob Agents Chemother. 53: 4647-55.

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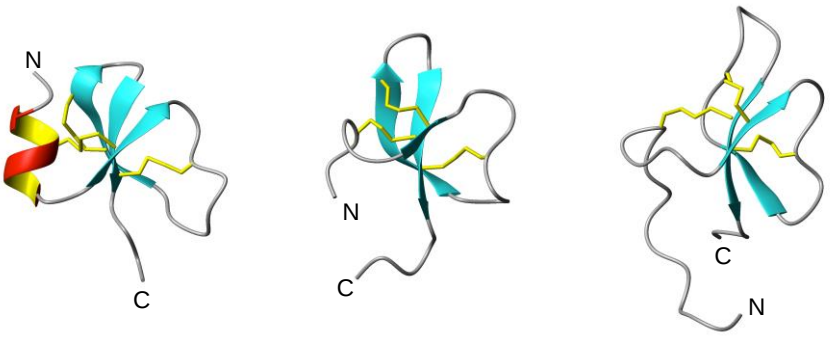
Role of defensins in the intestinal protection of birds

Structural insights on AvBD2 and AvBD7

❖ **3D-structure of AvBD2 and of AvBD7 in solution, solved by NMR**



AvBD103b **AvBD2** **AvBD7**

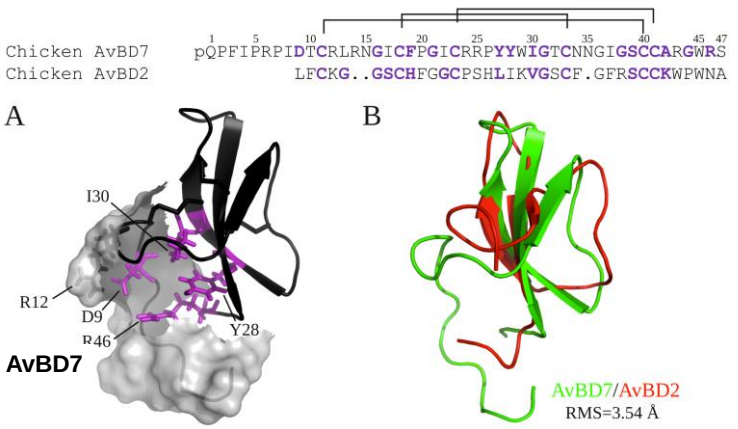
Landon C. et al. 2004. J Biol Chem. 279(29):30433-9.
Dearche C. et al. 2012. J Biol Chem. 287(10):7746-55.
Bailleul G. et al. 2016. PLOS One. 11(8): e0161573. doi:10.1371/journal.pone.0161573.

➡ **Highly compact structure of AvBDs, similarly to hBDs.**

Role of defensins in the intestinal protection of birds

Structural insights on AvBD2 and AvBD7

❖ **3D-structure of AvBD2 and of AvBD7 in solution, solved by NMR**



Bailleul G. et al. 2016. PLOS One. 11(8): e0161573. doi:10.1371/journal.pone.0161573.

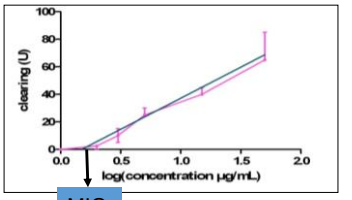
➡ **AvBD7 N-term extremity is stabilized by a salt bridge (D9-R12).**
AvBD7 N-term extremity covers the C-term extremity.

Role of defensins in the intestinal protection of birds

Antimicrobial activity of AvBD7

❖ **Capacity of AvBD7 to resist Cat K cleavage**

Bacterial strains	AvBD7, MIC (µM)	Ile4-AvBD7 (post-catK incubation) , MIC (µM)
Streptococcus salivarius	**0.69** (0,35–1,02)	**0,98** (0,27–1,69)
Listeria monocytogenes	**1,55** (0.07–3,03)	**0,16** (0.07–0,25)
Staphylococcus aureus	**0,45** (0.03–0,87)	**>9,19**
Escherichia coli	**0,99** (0.93–1.05)	**0,54** (0.40–0.68)
Salmonella Typhimurium LT2	**2,59** (1,73–3,45)	**1,4** (0,06–2.74)
Pseudomonas aeruginosa	**0,7** (0.26–1.14)	**0,24** (0.20–0.28)



Bailleul G. et al. 2016. PLOS One. 11(8): e0161573. doi:10.1371/journal.pone.0161573.

Cat K is the only cathepsin that cleaves AvBD7 without impairing antimicrobial activity.

➡ **AvBD7 good therapeutic candidate**

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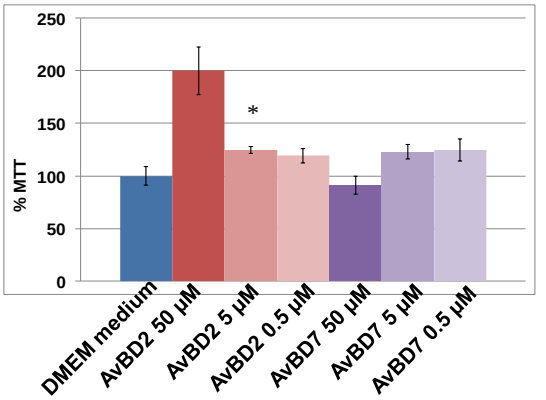
Role of defensins in the intestinal protection of birds

Effect of AvBD7 on chicken epithelial cells (viability measured by metabolism assay)



* P<0.05; One-way ANOVA/Bonferroni

➡ **AvBD2 or AvBD7 were not cytotoxic for epithelial cells.**
AvBD2, at 50 µM, was able to stimulate epithelial cell proliferation.

➡ **AvBD7 good candidate as an alternative to antibiotics.**

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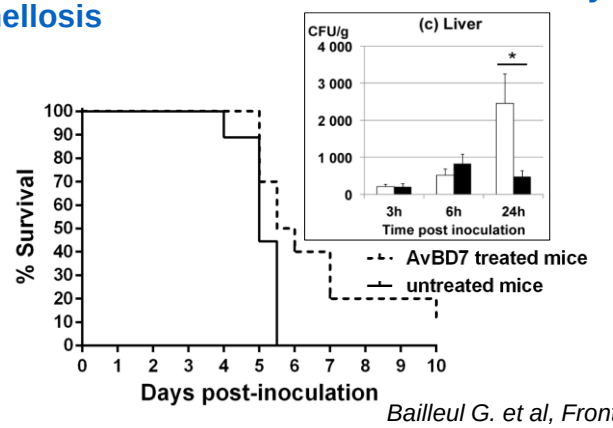
Role of defensins in the intestinal protection of birds

First proof of concept in a small animal model
Effect of AvBD7 *in vivo* in a murine model of systemic salmonellosis



Bailleul G. et al, Front Microbiol, 2019

Administration i.p. of AvBD7 improves survival of mice and reduces significantly the systemic bacteria load.

Bottleneck: Large scale production of AvBD7 remains unsuccessful for application in poultry!

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Illustration of a peculiar host-*Salmonella* relationship: Intestinal carriage in birds.

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- **Insights into protective role of microbiota**

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Insights into protective role of microbiota

First elements for consideration

- **Salmonella *Enteritidis* is able to persist in the caecum of individual isolated chickens** [1] :
  - In absence of former systemic and of collateral infection**.**
- **Heterogeneous levels of intestinal colonisation (Low Shedders/ Super Shedders)** [1] :
  - When transmission is reduced between congeners.
- **Low shedding phenotype is intrinsic : linked to the chick**
  - By blockage of the colonisation.

Menanteau P et al., Environmental Microbiology, 2018.

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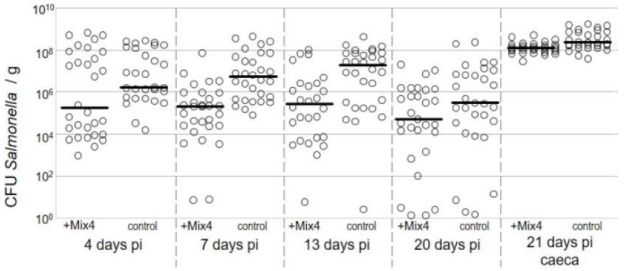
Insights into protective role of microbiota

First elements for consideration

- **Low shedding / Super shedding phenotype linked to the microbiota composition**
  - Transferable trait, through transfer of commensal consortia:



A mix of four commensal bacteria (*Escherichia coli*, *Enterococcus faecium*, *Lactobacillus rhamnosus* and *Clostridium butyricum*) administered orally at day 1 of age reduce by 10 to 100 fold *Salmonella* excretion in the feces (following inoculation at day 7 of age).

Kempf F et al., Environmental Microbiology, 2020.

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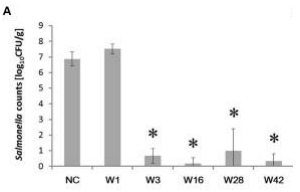
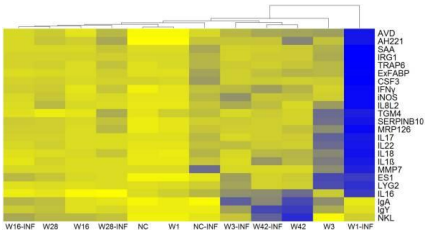
Insights into protective role of microbiota

First elements for consideration

- **Phenotype of resistance to caecal infection : linked to the microbiota composition**
  - Transferable trait, through transfer of adult microbiota:





An adult flora (from at least 3 weeks old chickens) given to newly hatched chicks protects against subsequent infection and does not induce inflammation

Varmuzova K et al., Frontiers in Microbiology, 2016.

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Insights into protective role of microbiota

Future prospects

- **Standardize and preserve an efficient commensal consortium against *Salmonella* infection**
- **Deciphering mechanisms of protection of the host**
  - **Bacterial metabolites involved,**
  - **Immune response factors involved,**
  - **Crosstalk between them…**

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