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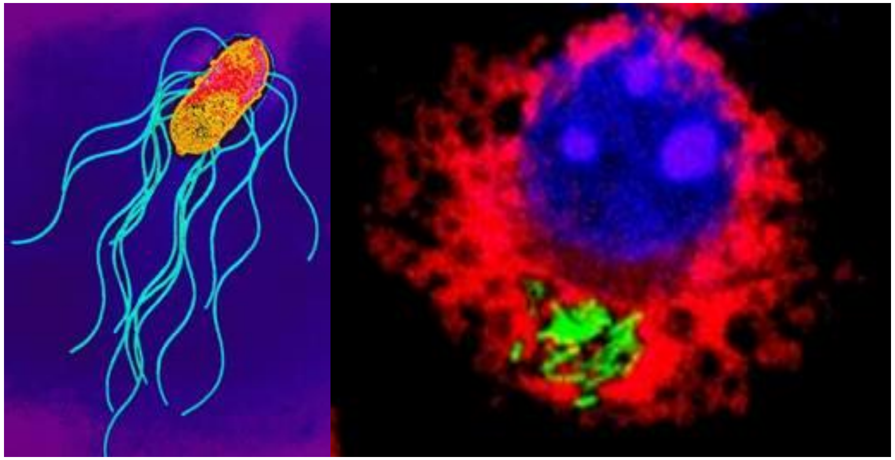
Salmonella – Taxonomie, Vorkommen, Serovare und Pathogenese



Workshop "Salmonellen – ein komplexes Thema für Human- und Veterinärmedizin"
Nationale Forschungsplattform für Zoonosen

Dr. Karsten Tedin
Institut für Mikrobiologie und Tierseuchen, Freie Universität Berlin

Introduction

- Salmonellose - Facts and Figures
- *Salmonella* - Taxonomy, Characteristics and Nomenclature
- Recent statistics human cases (RKI), monitoring studies, food screening (BfR)
- *Salmonella* pathogenesis and virulence Mechanisms
- Future concerns - Multi-Drug-Resistent (MDR) *Salmonella* serovars and changes in the pathogenesis of non-Typhoidal *Salmonella* infections

The Importance of *Salmonella enterica* Serovars in Infections and Disease

- According to the World Health Organization*, there are an estimated 20 million cases of Typhoid each year in the world (*S.* Typhi), resulting in approx. 600 000 deaths
- A further 1,3 billion (Mrd.) non-Typhoid human cases of Salmonellosis (*S.* Typhimurium, *S.* Enteriditis, *S.* Choleraesuis, *etc*.) are estimated to result in an additional 3 million deaths
- *Salmonella* infections account for ≥ 30% of all food-related deaths
- *Salmonella* infections can range from a self-limiting gastroenteritis to systemic infections
- Alone in the USA, nearly 7 billion (Mrd.) US-Dollar in economic losses yearly are attributed to contaminated food-related infections caused by *Salmonella* spp.
- Non-symptomatic infections and intermittent shedding in domestic animals provide a constant reservoir for infection and contamination of food

* Pang *et al*. (1995) Trends Microbiol. 3:253-255; Pang *et al*. (1998) Trends Microbiol. 6:131-133

Salmonella Taxonomy

- *Salmonella* spp. are members of the *Enterobacteriaceae*, which includes other Gram-negative bacterial genera such as *Enterobacter*, *Escherichia coli*, *Klebsiella*, *Shigella*, *Yersinia, etc*.
- These are all members of the "Proteobacteria", characterized by a high degree of metabolic diversity or "heterotrophy", *i.e.* aerobic and anaerobic respiration, fermentation, *etc*.
- High degree of metabolic flexibility
- Principal habitat of the *Salmonellae* is the intestinal tract of humans and animals (reservoir), but disseminate in the environment (water, soil, plants and food) through contamination with human or animal waste (faeces)

Salmonella Taxonomy

Salmonella spp. are identified through the presence of combinations of antigenic determinants, the Somatic, Surface and Flagellar antigens which are used to establish the serovar.

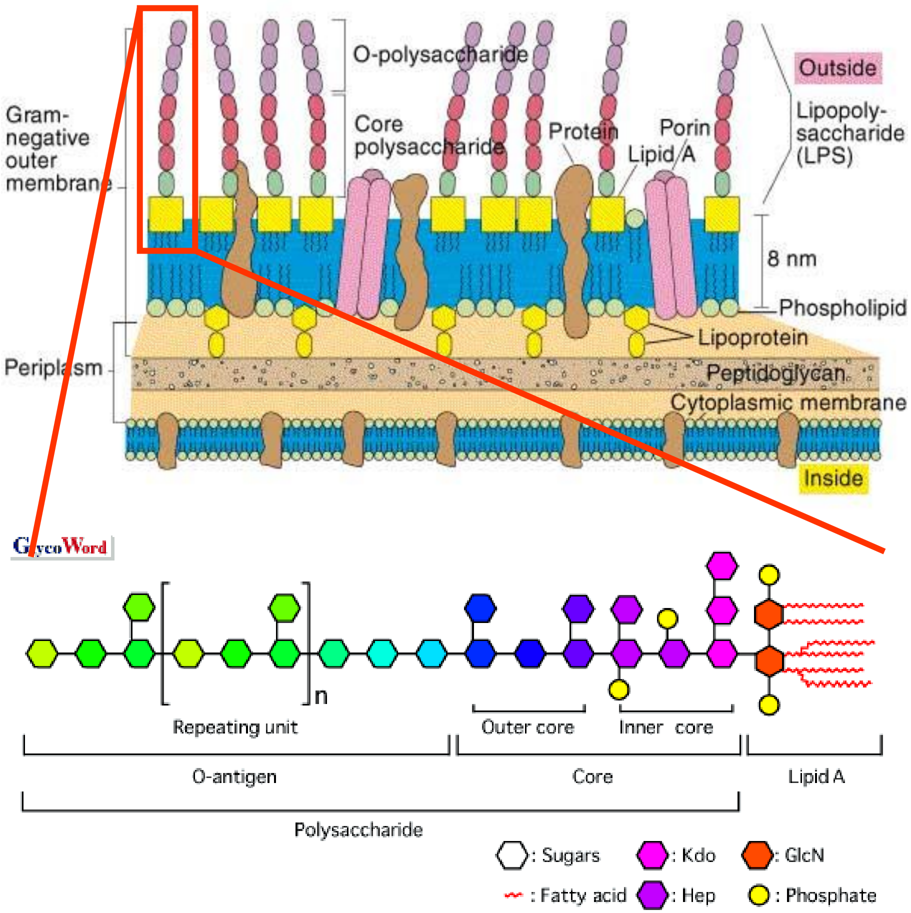
Currently, there are > 2500 serovars

Somatic (O) Cell Wall Antigens

- Heat stable, alcohol-resistant cell wall lipopolysaccharide (LPS) antigens
  ca. 70 different antisera used for serology
  the LPS component is the so-called "endotoxin"

Surface (Capsular) Vi Antigens

- Occurs in only a few *Salmonella* serovars (common in *E. coli* and *Klebsiella* = K antigen)
- May mask O antigens
- *Salmonella* Typhi, Paratyphi C and Dublin are the only serovars with the Vi antigen



Salmonella Taxonomy

Salmonella spp. are identified through the presence of combinations of antigenic determinants, the Somatic, Surface and Flagellar antigens which are used to establish the serovar.

Currently, there are > 2500 serovars

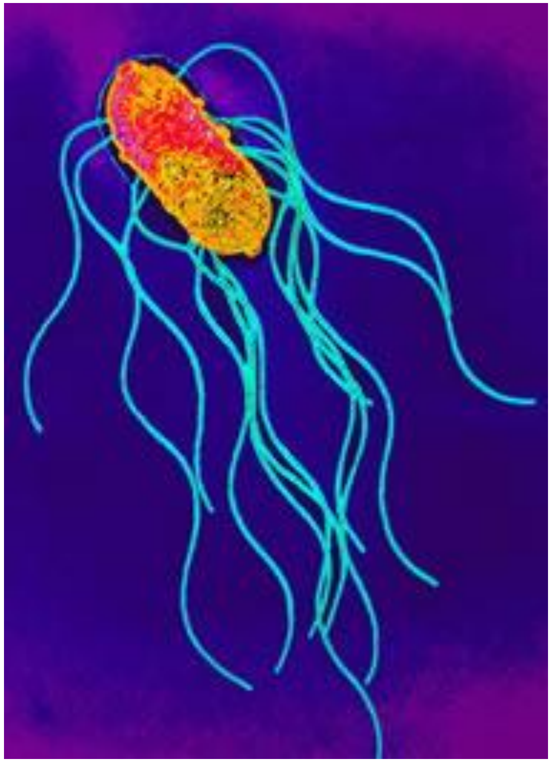
Flagellar (H) Antigens

- Some serovars have a constant (monophasic) H antigen (*S.* Enteriditis, *S.* Typhi), however most *Salmonella* serovars have two, different H antigens, 1 or 2 (diphasic)
- Serovars with one H antigen may acquire the other at a rate of 10^{-3} to 10^{-5} (phase variation)

- Example of a serological formula:

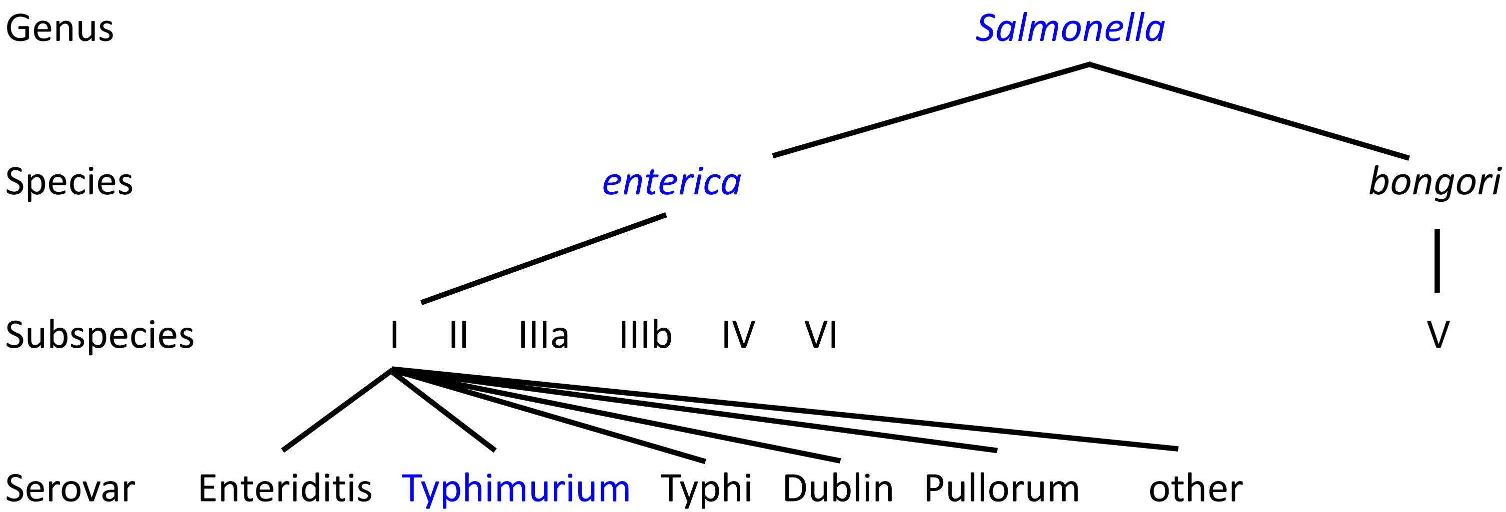
S. ser. Typhimurium – 1, 4, 5, 12 : i : 1, 2

O antigens detected: 1, 4, 5 and 12
H1 antigens detected – phase 1: i
H2 antigens detected – phase 2: 1 and 2



Salmonella Taxonomy

Due to the close genetic (DNA) relatedness of *Salmonella* strains, a new taxonomic system was proposed by Popoff and Le Minor in 1987 in which only two species are recognized:



The full taxonomic name for *Salmonella typhimurium* following this system is:

Salmonella enterica subspecies *enterica* serovar Typhimurium

Salmonella Taxonomy

Salmonella enterica Subspecies Group Designations:

Subspecies*	Group	Mal.	ONPG	Dulcit	Salicin	Galakt.	Gel.
S. enterica ssp. *enterica*	I	neg.	neg.	pos.	neg.	neg.	neg.
S. enterica ssp. *salamae*	II	pos.	neg.	pos.	neg.	pos.	pos.
S. enterica ssp. *arizonae*	IIIa	pos.	pos.	neg.	neg.	(±)	pos.
S. enterica ssp. *diarizonae*	IIIb	pos.	pos.	neg.	neg.	(±)	pos.
S. enterica ssp. *houtenae*	IV	neg.	neg.	neg.	pos.	pos.	pos.
S. enterica ssp. *indica*	VI	neg.	var.	var.	neg.	pos.	pos.
*S. bongori**	V	neg.	pos.	pos.	neg.	pos.	neg.

Abbreviations: (±), schwach; var., variabel; Mal., Malonat; ONPG, ; Galakt., Galakturonsäure; Gel., Gelatin.

* *S. bongori* eigene Spezies, zum Vergleich mit *S. enterica* ssp. aufgeführt.

Salmonella Taxonomy

Salmonella Subspecies Designations:

Group	Species/Subsp.	Comments*
I	*enterica*	99.5% of all *Salmonella* isolates in mammals/birds
II	*salamae*	
IIIa	*arizonae*	
IIIb	*diarizonae*	largely cold-blooded animal isolates
IV	*houtenae*	
V	*bongori*	*S. bongori* recognized as separate species
VI	*indica*	

* The pathogenesis and virulence of groups II – VI has not been well studied, and human infections are rare

Salmonella enterica subspecies *enterica* Serovars

Serovar		Host specificity / Disease
S. Typhi		Humans / Typhoid, Septicemia
S. Typhimurium		Broad Host Range
		Humans / Gastroenteritis, Diarrhea, Enteric Fever
		Mice / Typhoid, Septicemia
S. Abortusovis		Sheep / Goats Diarrhea, Septicemia
S. Arizonae	Reptiles	
S. Choleraesuis		Swine / Swine Paratyphoid, Septicemia
S. Dublin		Cattle / Diarrhea, Septicemia
S. Enteritidis		Broad host range / Gastroenteritis, Diarrhea
S. Gallinarum		Poultry / Fowl Typhoid, Diarrhea, Septicemia
S. Paratyphi A,B,C		Humans / Paratyphoid, Septicemia
S. Pullorum		Poultry / Pullorum Disease (young birds < 3 weeks old)
S. Typhisuis		Swine / Chronic Paratyphoid, Diarrhea

Salmonella Nachweis

(Anlehnung an DIN EN ISO 6579:2003; 6579-1:2014)

Lebensmittel / Futtermittel

■ Enrichment (Voranreicherung)	Buffered Peptone Water Nährbouillon	16 - 20 h, 37°C
■ Selective Enrichment (selektive Anreicherung)	Rappaport-Vassiliadis-Bouillon bzw. halbfesten RV Agars	0,1 ml + 10 ml "Rappaport" ≥24 h, 42°C (2. enrichment ≥24 h, 42°C)
■ Subculture (Subkultivierung)	Xylose-Lysin-Desoxycholat-Agar (XLD) Gassner Chromagar Nähragar	≥24 h, 37°C
■ Serology/Biochemistry/PCR (serologische u. biochemische Bestätigung)	meist Gruppen-spezifisch Antisera* Weiterleitung NRZ-*Salmonella* (RKI)	

*Serogroup	A:	Paratyphi A, u.a.
	B:	Typhimurium, Agona, Heidelberg, u.a
	C1:	Choleraesuis, Infantis, u.a.
	C2:	Newport, Hadar, Bovismorbificans, u.a.
	D:	Enteriditis, Dublin, Typhi
	E:	Anatum, u.a.
	G:	Poona, Havana, u.a.

Salmonella Serovars in Selected Animal Species/Food Products

	Salmonella-Nachweisrate (%)		
Tierart	2012	2013	Lebensmittel-2013
Hühner	2,6	2,1	4 - 12%
Rinder	2,8	3,0	0,09%
Schweine	13,5	17,4	2,7%
Schafherden	5,2	7,3	
Ziegenherden	1,3	1,3	
Pferde	3,3	1,1	
Hunden	2,3	2,4	
Katzen	1,2	1,7	

Bundesinstitut für Risikobewertung - Erreger von Zoonosen in Deutschland im Jahr 2013

Salmonella Serovars in Selected Animal Species

Salmonella-Nachweisrate in Tierkörpern (%)

Tierart	2009	2010	2011
Kälber	0/80 (0)	0/55 (0)	3/101 (2,9)
Kühe	1/36 (2,8)	2/96 (2,1)	0/26 (0)
Mastschweine	13/504 (2,6)	4/209 (1,9)	15/166 (9,0)
Schaf	1/52 (1,9)	0/43 (0)	0/38 (0)
Ziegen	0/21 (0)	0/12 (0)	0/9 (0)
Pferde	0/39 (0)	0/22 (0)	0/20 (0)
Hunden	0/149 (0)	0/66 (0)	6/58 (10,3)
Katzen	1/155 (0,6)	0/60 (0)	3/88 (3,4)
Reptilien	5/21 (23,8)	6/23 (26,1)	1/9 (11,1)
Tauben	10/48 (20,8)	6/53 (11,3)	12/56 (21,4)

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Salmonella Serovars in Selected Animal Species

Salmonella-Nachweisrate in Kotproben (%)

Tierart	2009	2010	2011
Kälber	9/267 (3,4)	10/283 (3,5)	19/302 (6,3)
Kühe	1/882 (0,1)	3/416 (0,7)	554/5266 (10,5)
Mastschweine	3/135 (2,2)	15/220 (6,8)	12/76 (18,8)
Schaf	0/18 (0)	0/5 (0)	0/4 (0)
Ziegen	0/17 (0)	0/4 (0)	0/6 (0)
Pferde	0/14 (0)	0/21 (0)	1/22 (4,5)
Hunden	4/187 (2,1)	3/188 (1,6)	13/113 (11,5)
Katzen	0/97 (0)	1/63 (1,6)	0/45 (0)
Reptilien	10/43 (23,3)	13/51 (25,5)	5/13 (38,5)
Tauben	17/267 (6,4)	10/229 (4,4)	5/330 (1,5)

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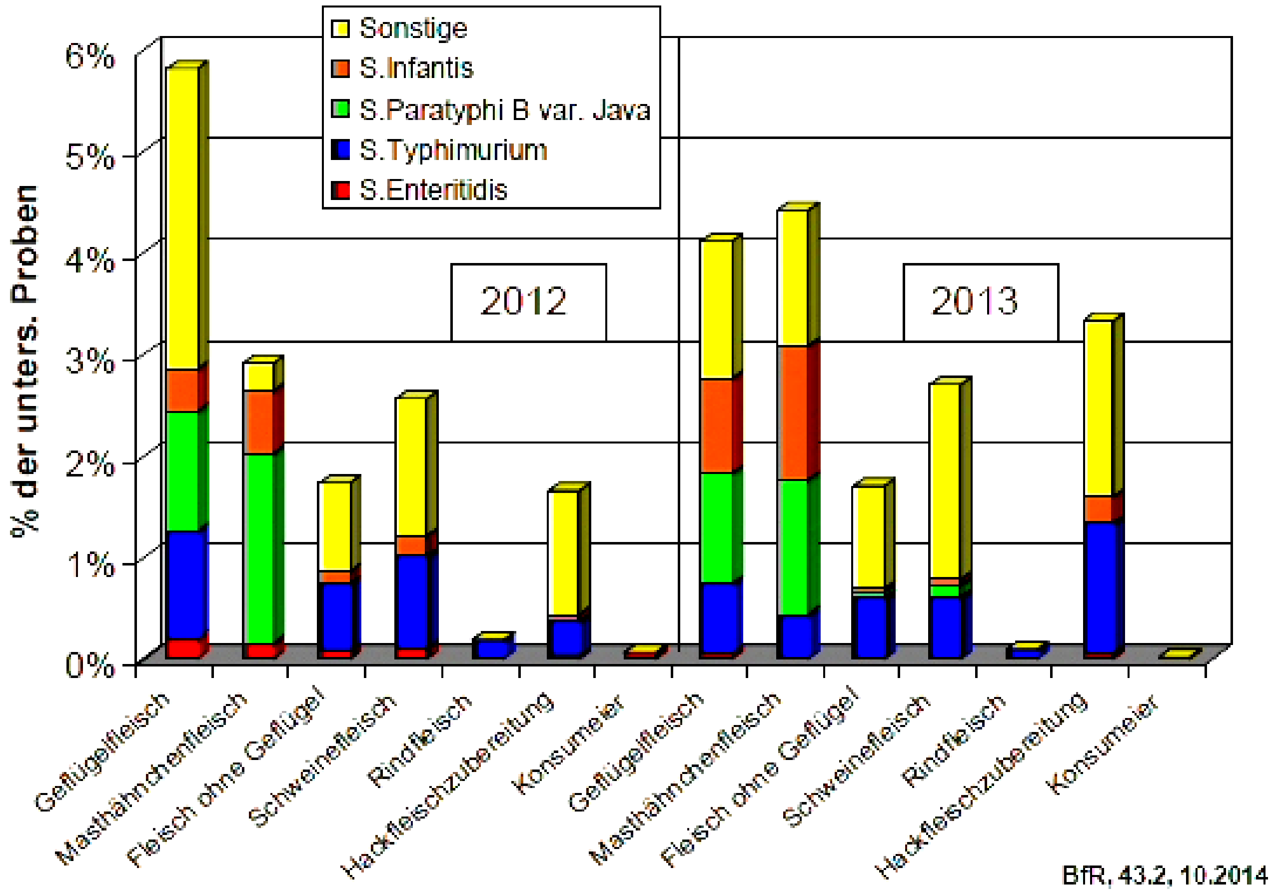
Salmonella Serovars in Selected Animal Species/Food Products



Salmonella Serovars in Food Samples of Animal and Plant Origins

Anzahl der zehn häufigsten Serovare aus Einsendungen von Lebensmitteln in den Jahren 2004 – 2008

(NRL BfR, 2010)

S. enterica Serovar	Anzahl der Isolate aus Lebensmitteln insgesamt		Anzahl der Isolate aus Lebensmitteln tierischer Herkunft		Aus pflanzlichen Lebensmitt.
Salmonella gesamt	6241	(100%)	5780		265
Typhimurium	1939	(31,1%)	1908	(33,0%)	10
Enteritidis	950	(15,2%)	805	(13,9%)	12
1,4,[5],12:I:-	373	(6,0%)	373	(6,5%)	0
Derby	323	(5,2%)	308	(5,3%)	12
Infantis	244	(3,9%)	239	(4,1%)	4
Paratyphi B (dT+)	229	(3,7%)	227	(3,9%)	1
S. Subspezies I, rau	177	(2,8%)	170	(2,9%)	3
Saintpaul	155	(2,5%)	150	(2,6%)	5
Bovismorbificans	135	(2,2%)	133	(2,3%)	2
Hadar	135	(2.2%)	133	(2,3%)	0

Reported Cases of *Salmonella* Infections of Humans 2001 - 2013

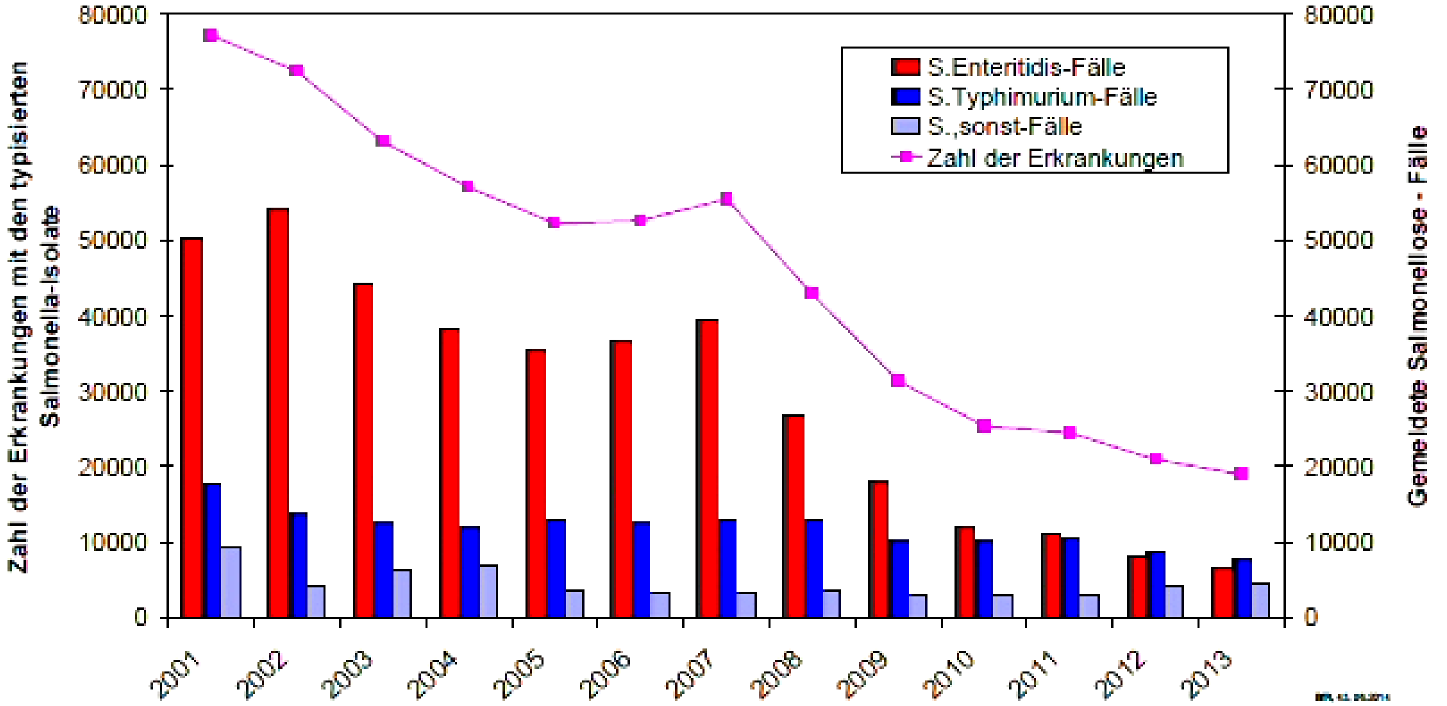


Abb. 4.2.1: Dem RKI gemeldete Fälle von Salmonellose beim Menschen 2001–2013 (n. RKI, 2013: nach IfSG, 2014)

Total Reported Cases of Human Infections 2014

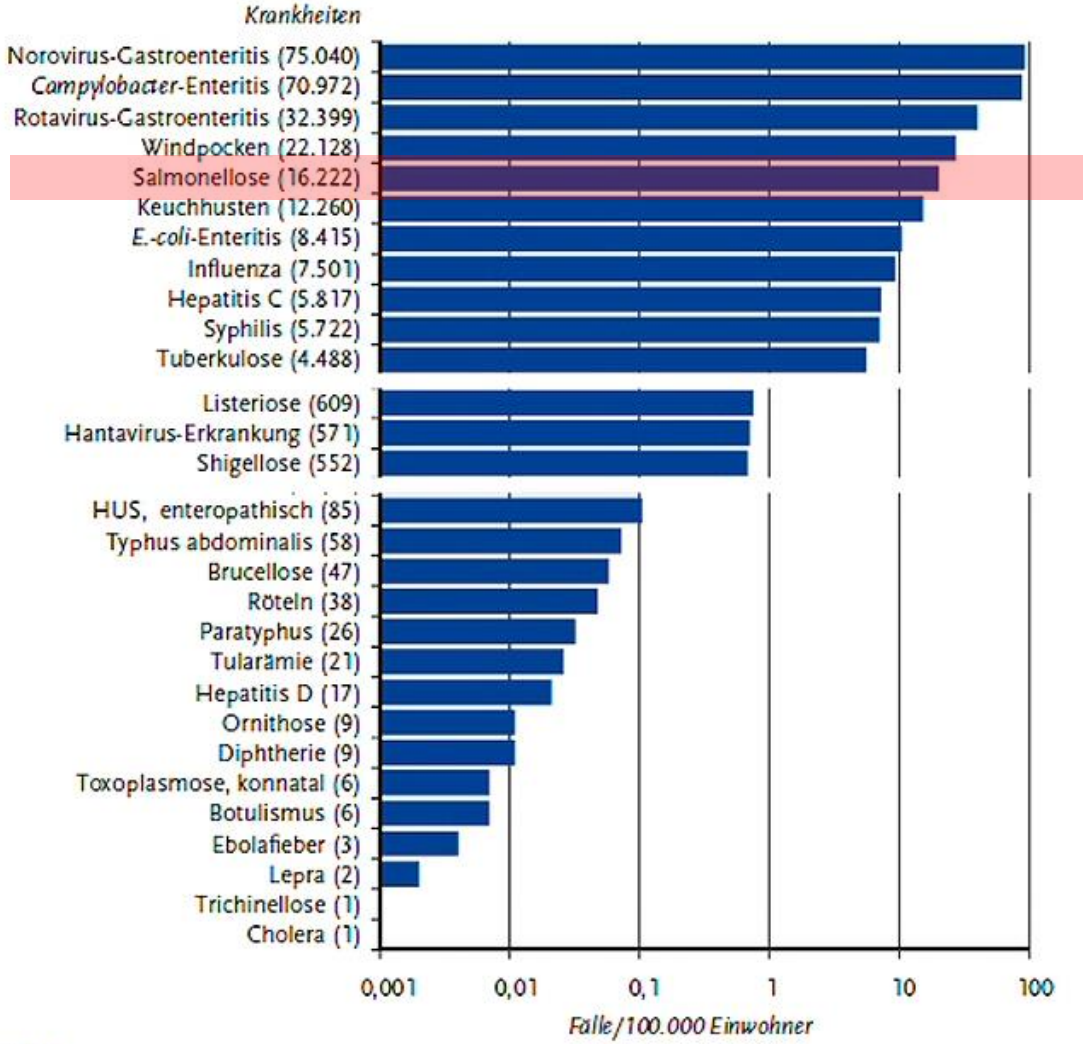
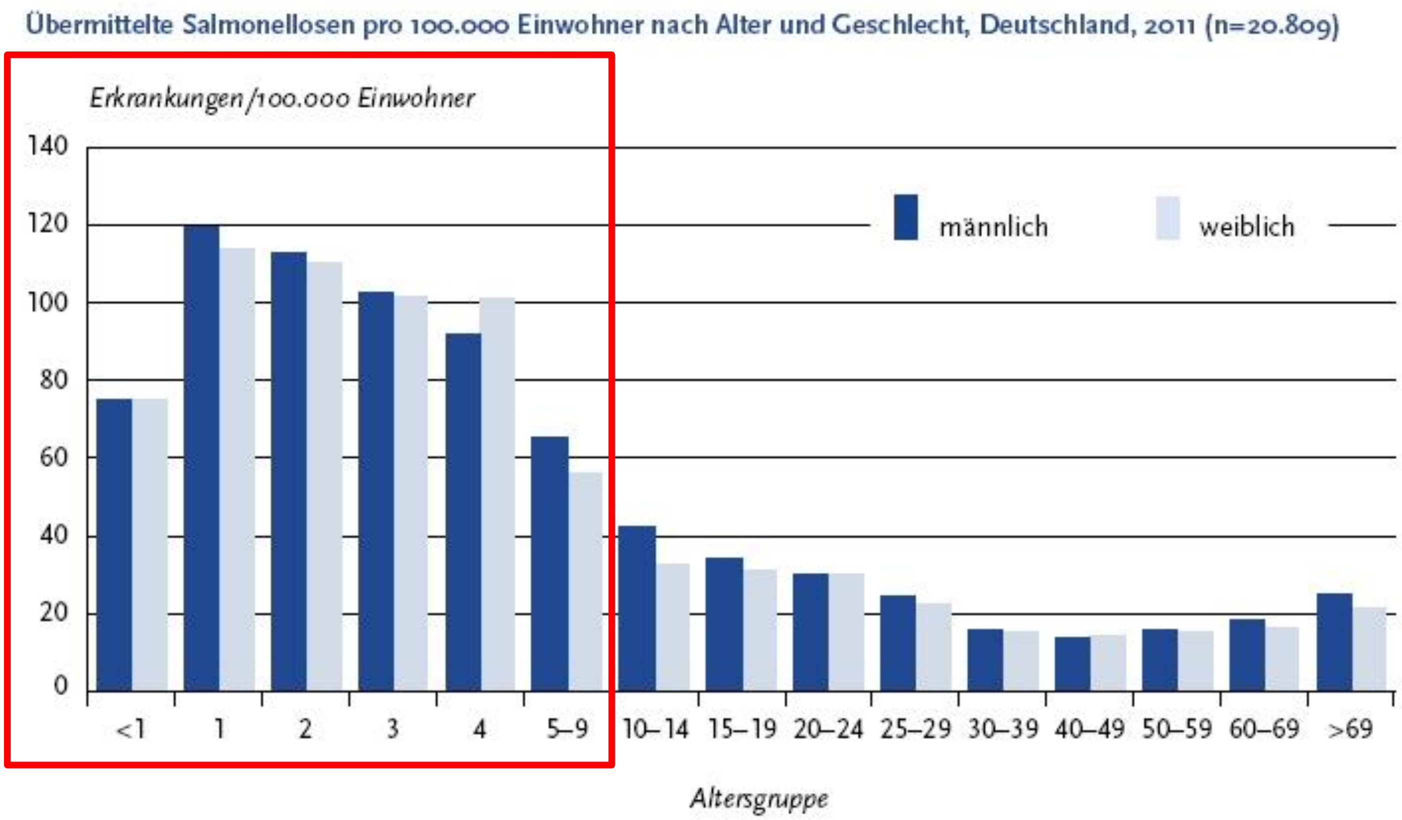


Abb. 4.1.1:
Inzidenz (logarithmisch) und Anzahl der Fälle aller meldepflichtigen Krankheiten mit mindestens einem Fall, Deutschland, 2014

- RKI Infektionsepidemiologisches Jahrbuch 2014

Total Reported Cases of *Salmonella* Infections 2011 by Age of Patients



- Majority of reported cases among the young

Four Main Forms of Salmonellose

Salmonella infections can have a number of differing consequences, often serovar-dependent:

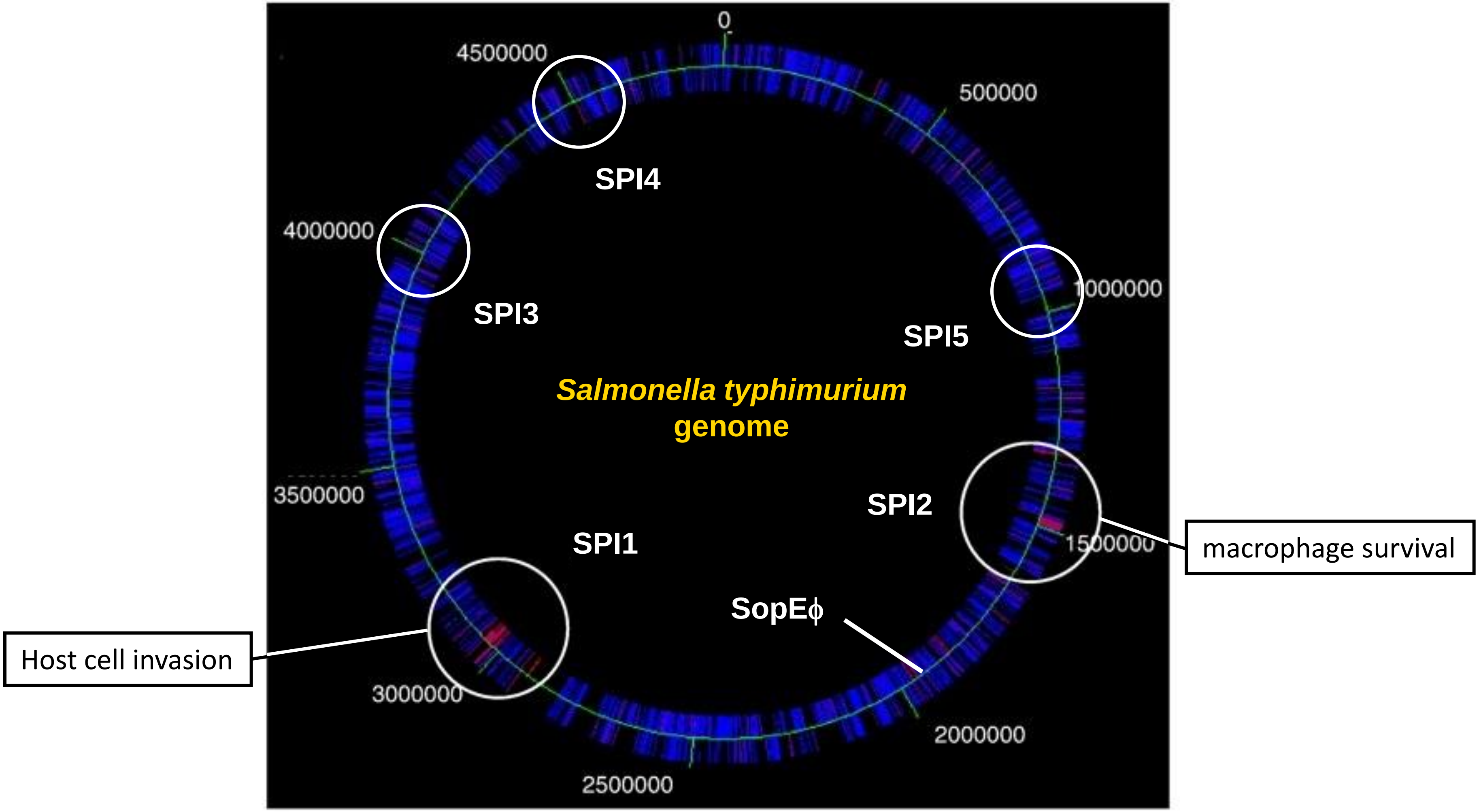
- Acute Enterocolitis: short-term, intestinal inflammation, diarrhea
- Chronic Enterocolitis: long-term intestinal inflammation, often recurring diarrhea
- Acute Septicemia: involvement of the liver, spreading to the circulatory (blood) system
  may involve abortion in animals, including recurring abortion
- Clinically Asymptomatic Shedding:

  Active carriage - Constant or intermittent shedding of *Salmonella* in the faeces
  Latent carriage - Persistent infection of the Lymph nodes or Tonsils without shedding
  (stress can result in clinical symptoms and "active" infection)
  Passive carriage - Continuous uptake and shedding of *Salmonella* from/into the environment
  without invasion/infection of the host. Removal of contaminated materials
  results in reduction or loss of further infections

Salmonella is a pathogen of the intestinal tract, but gains access to

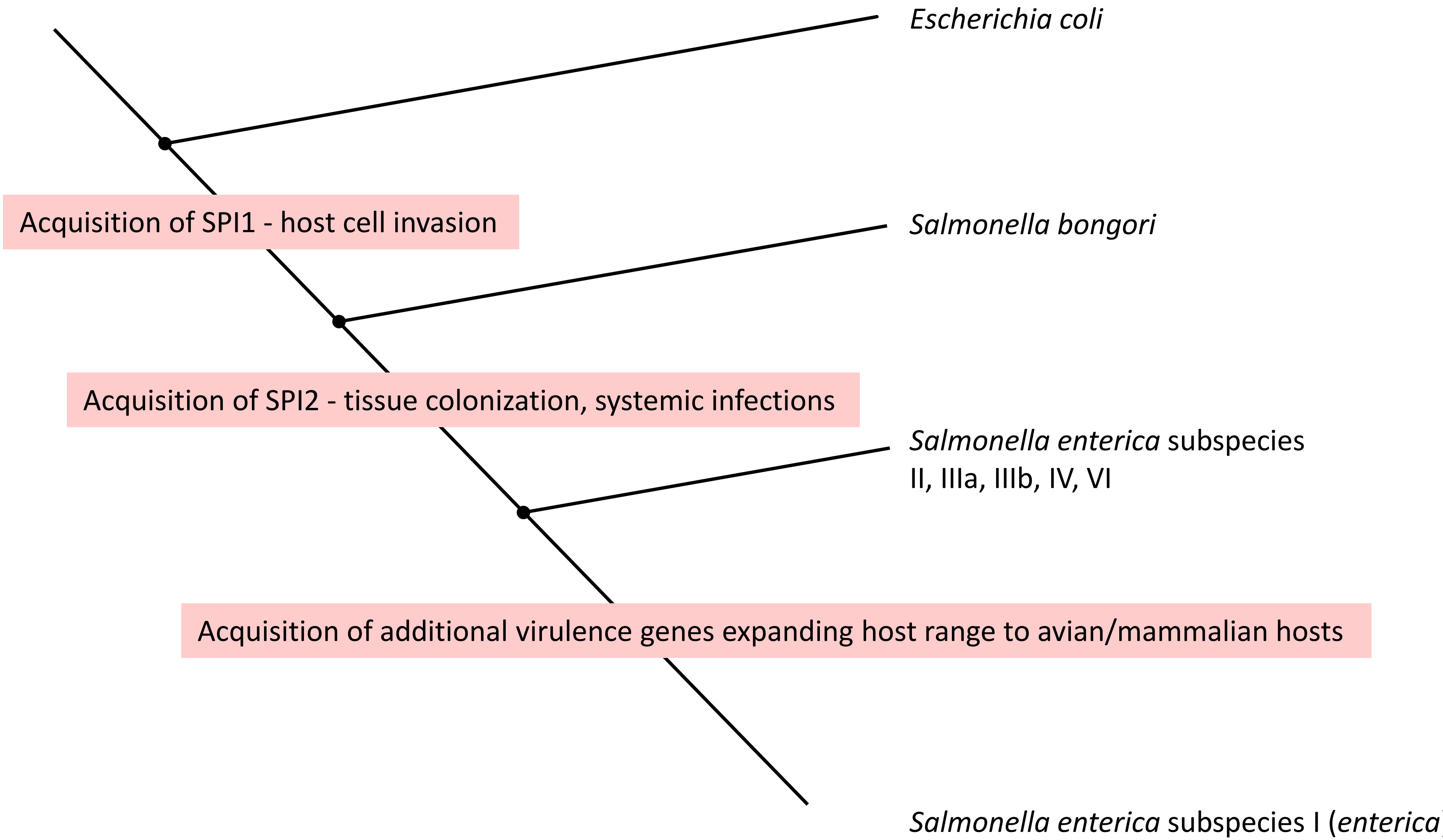
lymphatic / immune tissues for proliferation and persistence within the host…

Salmonella Pathogenicity Islands (SPI)*



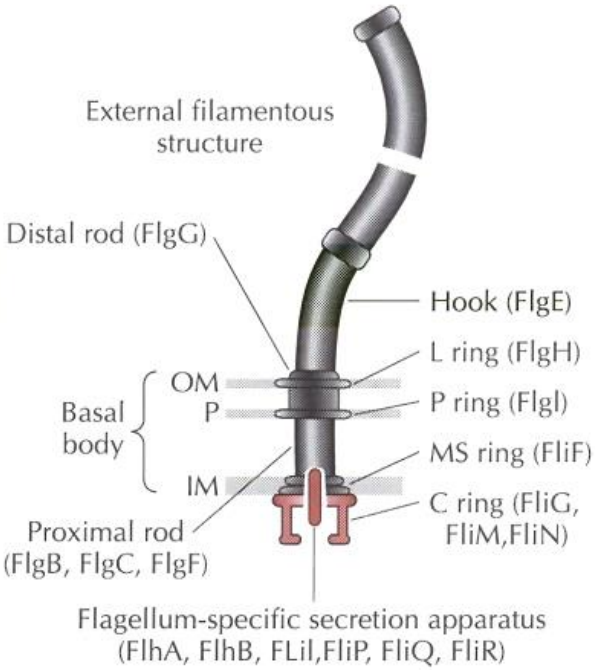
*SPI1 present in all serovars, SPI2 not in *S. bongori*. Other SPI serovar/isolate-dependent

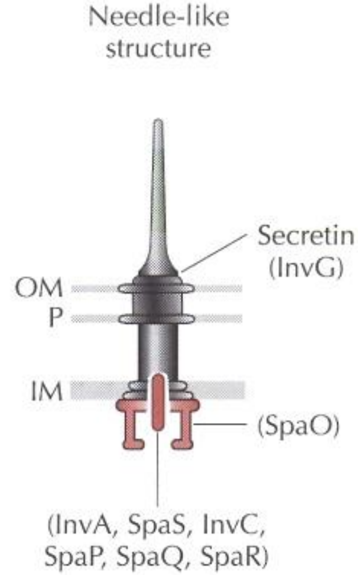
Model for the Evolution of Virulence in the Genus *Salmonella*

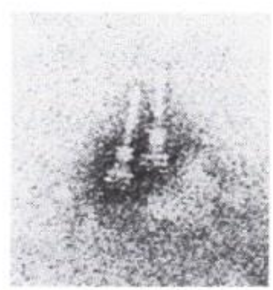


Nach Bäumler (1997) *Trends Microbiol*. 5:318; Bäumler *et al.* (1998) *Infect. Immun*. 66:4579

Salmonella Secreted VIrulence/Effector Proteins





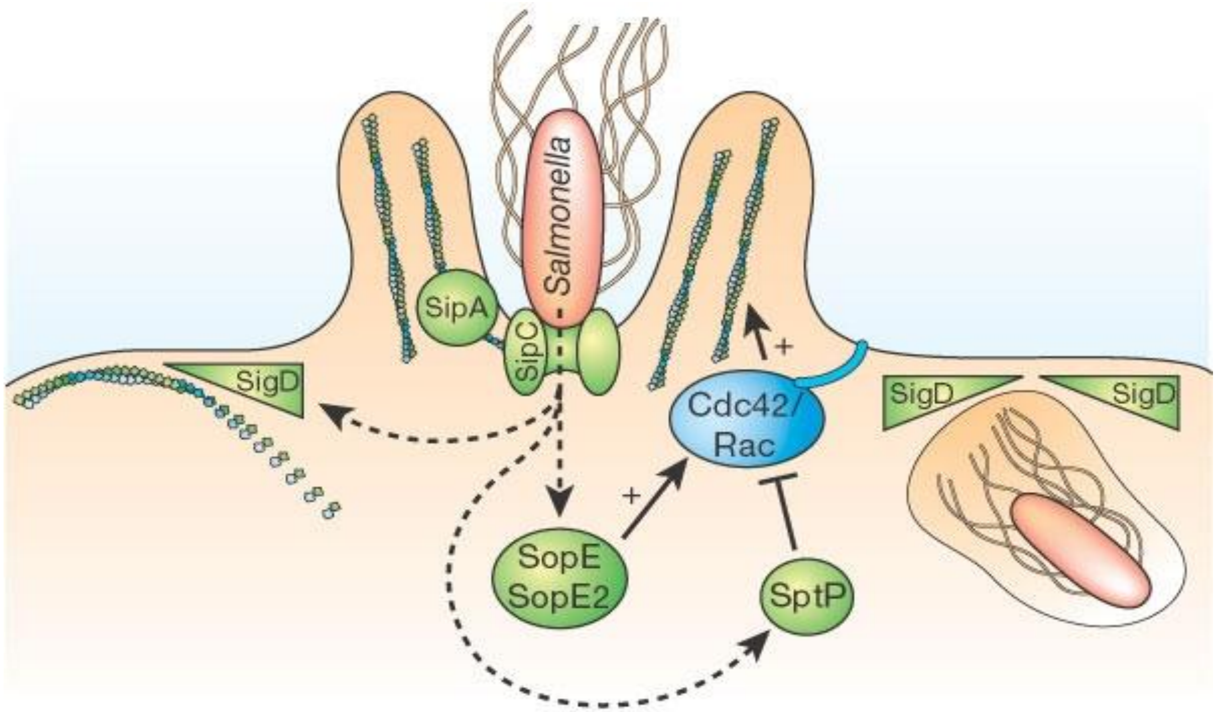


The SPI1- and SPI2-encoded Type III Secretion Apparatus show homology with the bacterial flagellar apparatus

Like the flagellar apparatus, the type III secretion apparatus is essentially a transport system for proteins

The *Salmonella* type III secretion system is designed for the delivery (secretion) of virulence proteins into eukaryotic host cells

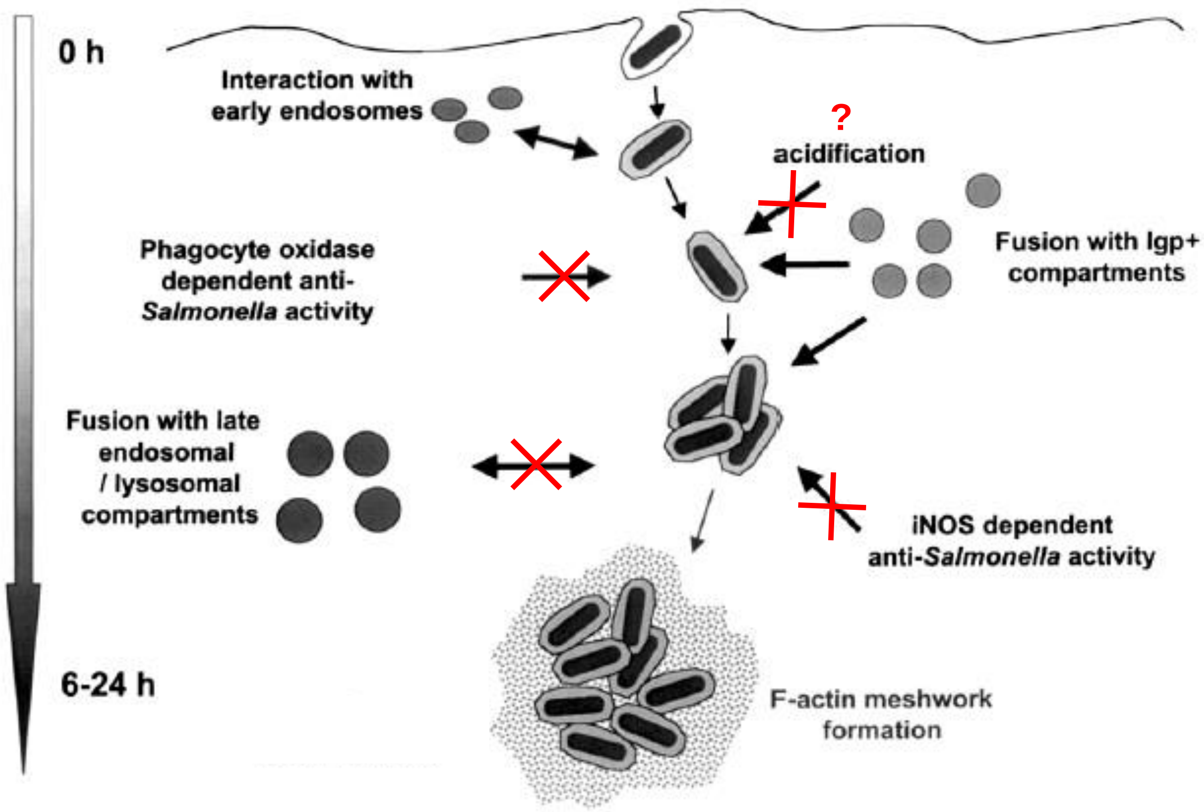
Salmonella Invasion of Host (Intestinal Epithelial) Cells



- SigD weakens the actin cytoskeleton attachment to the membrane. SipA and SipC act as actin nucleating proteins - the stage is set for re-structuring the actin cytoskeleton

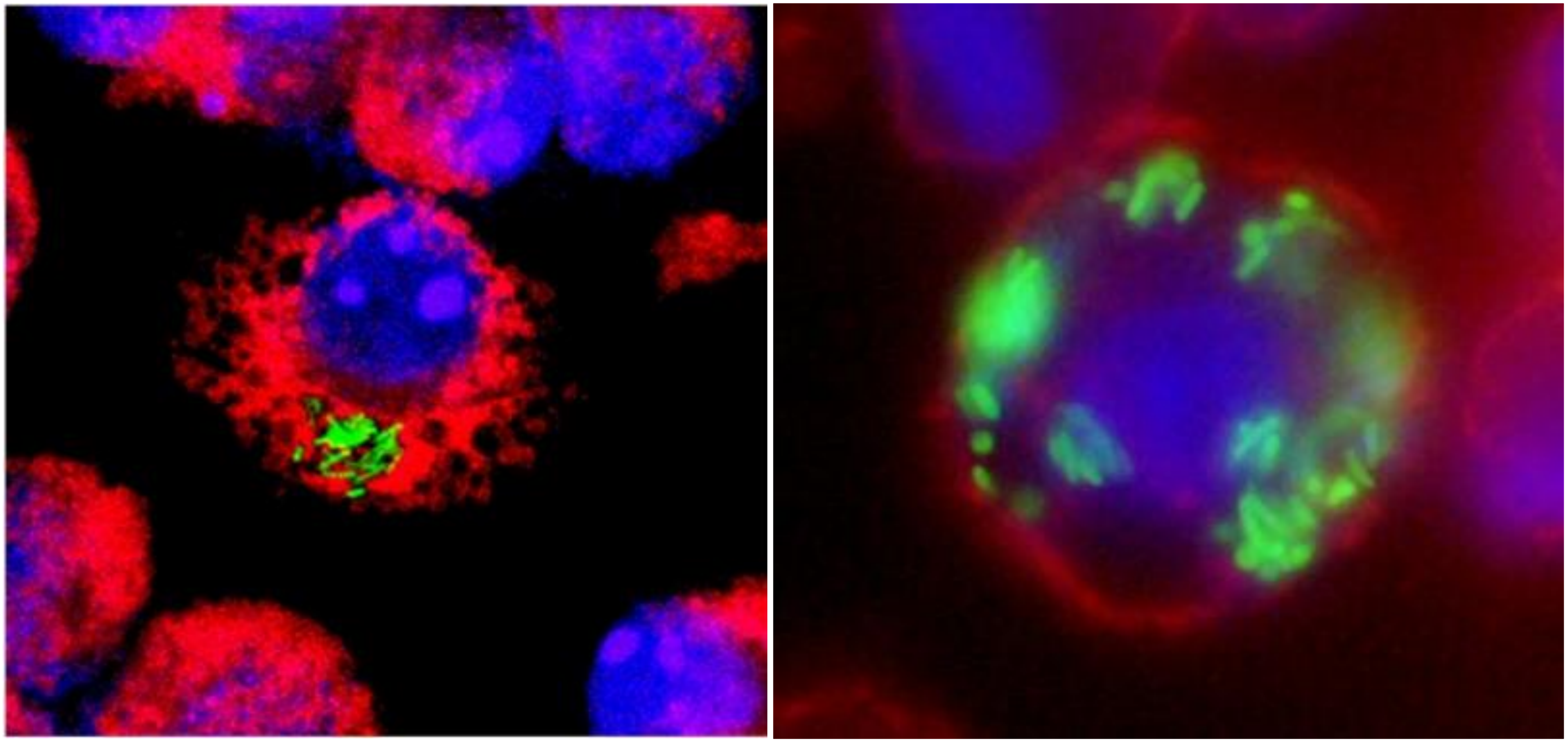
- SopE and SopE2 activate the host Rho-family GTPases CDC42 and Rac1 leading to activation of Arp2/3 Complex and actin polymerization - membrane movement - uptake

Salmonella Infection of Host Cells and Establishment of the Intracellular Vacuole



✕ Processes inhibited or reduced by *Salmonella*

Salmonella Survives and Proliferates within Macrophage



Murine (left) and human (right) macrophage harboring GFP-expressing *S.* Typhimurium (**green**) and counter-stained with Lysotracker (**red**) and nuclear stain DAPI (**blue**)

Photos: Dr. M. Nordhoff, Institut für Mikrobiologie und Tierseuchen, Freie Universität Berlin

How *Salmonella* Copes With Macrophage / Phagosome Defenses

- Acquisition of the vATPase by the *Salmonella*-containing phagsome results in a reduction of the vacuolar/phagosomal pH to approx. pH 5 - 6

- *Salmonella* has a number of genes involved in an Acid Tolerance Response, and is capable of surviving acidic conditions for long periods, particularly if allowed to acclimate

- The host NADPH Oxidase produces reactive oxygen species (superoxide), which is antibacterial due to strong oxidizing effects

- However, *Salmonella* encodes at least 4 Superoxide Dismutases, two cytoplasmic (SodA/MnSod and SodB/FeSod) and two periplasmic (SodCI and SodCII), which detoxify superoxide

- In addition, *Salmonella* also encodes a number of Catalases, which can detoxify hydrogen peroxide, the intermediate reaction species from superoxide detoxification

Pathogenesis of Different *Salmonella* Serovars in Different Species

- Broad Host-Range Serovars - *S.* Typhimurium, *S.* Enteritidis
- Host-Restricted Serovars - *S.* Typhi, *S.* Gallinarum, *etc*.
- Host-Adapted Serovars - *S.* Dublin, *S.* Choleraesuis, *etc*.

Host \ Serovar	*S.* Typhimurium / *S.* Enteritidis	*S.* Typhi	*S.* Dublin	*S.* Choleraesuis	*S.* Gallinarum
Humans	Enterocolitis/ Diarrhea	Typhus/ Septicemia	Septicemia	Septicemia	-
Poultry	Diarrhea/ Septicemia*	-	-	-	Fowl Typhoid/ Septicemia
Cattle	Diarrhea/ Septicemia*	-	Diarrhea/ Septicemia	-	-
Swine	Diarrhea/ Asymptomatic*	-	-	Septicemia	-
Mice	Septicemia	-	Septicemia	Septicemia	-

* age-dependent pathogenesis; - non-pathogenic. Here, "septicemia" includes bacteremia and/or sepsis

Uneven Distribution of Systemic Forms of Salmonellosis in Humans

Serovar	Total Isolates	Blood Samples	Ratio (%)
Enteriditis	563.221	6.775	1.2
Typhimurium	183.542	2.692	1.5
Typhi	4.636	2.825	60.9
Paratyphi A	3.125	1.727	55.3
Paratyphi B*	2.463	308	12.5
Paratyphi C	37	20	54.1
Choleraesuis	147	48	32.7
Dublin	1.283	530	41.3

* *S.* Paratyphi A and *S.* Paratyphi C are host-restricted (humans), *S.* Paratyphi B isolates are also infectious for animals

Certain serovars show a much higher rate of systemic infections in humans, including host-adapted serovars such as *S.* Choleraesuis (swine) and *S.* Dublin (cattle)

Questions:

- What bacterial or host factor(s) are responsible for these differences?
- If bacterial, can the traits be acquired by the widespread, broad host-range *Salmonella* serovars?

Data from 1994 – 2004. Modified from Wollin, R. (2007) *Euro Surveill*. 12:pii 3275; http://www.eurosurveillance.org/

Infection and Persistence of *Salmonella* in Host Cells

Microbial Pathogenesis 2000; **29**: 121–126
doi:10.1006/mpat.2000.0367

Experimental *Salmonella typhi* infection in the domestic pig, *Sus scrofa domestica*

Eleanor S. Metcalf[a], Glen W. Almond[b], Patricia A. Routh[b], John R. Horton[c], Richard C. Dillman[c] & Paul E. Orndorff[c]*

- Studies with the host-restricted serovar *S*. Typhi showed carriage in porcine tonsils for up to 3 weeks with no clinical signs of infection.

- Other studies show that transport stress results in shedding of *S*. Typhimurium by market weight pigs despite no signs of infection nor faecal shedding prior to transport (*e.g*. Isaacson *et al*., 1999. *Am. J. Vet. Res*. 60:1155-1158).

- Finishing pigs can show high levels of shedding of *S*. Typhimurium without developing signs of infection. *Salmonella* persist in the mesenteric lymph nodes. (Rostagno *et al*., 2011. *Foodborne Pathog. Disease* 8:623).

Increase in Multi-Drug Resistent *Salmonella* Serovars

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2002, p. 2813–2822 Vol. 40, No. 8
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.8.2813–2822.2002

Molecular Characterization of Multidrug-Resistant *Salmonella enterica* subsp. *enterica* Serovar Typhimurium Isolates from Swine

Wondwossen Abebe Gebreyes[1]* and Craig Altier[2]

Chiu *et al. BMC Microbiology* 2010, **10**:86
http://www.biomedcentral.com/1471-2180/10/86

BMC Microbiology

RESEARCH ARTICLE Open Access

Characterization of 13 multi-drug resistant *Salmonella* serovars from different broiler chickens associated with those of human isolates

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Animal Health Advisory

Multi-drug Resistant *Salmonella* Dublin in Cattle

Systemic Forms of non-Typhoidal Salmonellosis in Humans

Intracontinental spread of human invasive *Salmonella* Typhimurium pathovariants in sub-Saharan Africa

Chinyere K Okoro[1,20], Robert A Kingsley[1,20], Thomas R Connor[1], Simon R Harris[1], Christopher M Parry[2,3], Manar N Al-Mashhadani[3], Samuel Kariuki[4], Chisomo L Msefula[5,6], Melita A Gordon[7], Elizabeth de Pinna[8], John Wain[8,9], Robert S Heyderman[5,10], Stephen Obaro[11,12], Pedro L Alonso[13,14], Inacio Mandomando[14,15], Calman A MacLennan[16,17], Milagritos D Tapia[18], Myron M Levine[18,19], Sharon M Tennant[19], Julian Parkhill[1] & Gordon Dougan[1]

VOLUME 44 | NUMBER 11 | NOVEMBER 2012 **NATURE GENETICS**

Recall the question posed earlier regarding serovar-specific tendencies for systemic infections:

Certain serovars show a much higher rate of systemic infections in humans, including host-adapted serovars such as *S*. Choleraesuis (swine) and *S.* Dublin (cattle)

Questions:

- What bacterial or host factor(s) are responsible for these differences?
- If bacterial, can the traits be acquired by more widespread, broad host-range *Salmonella* serovars?

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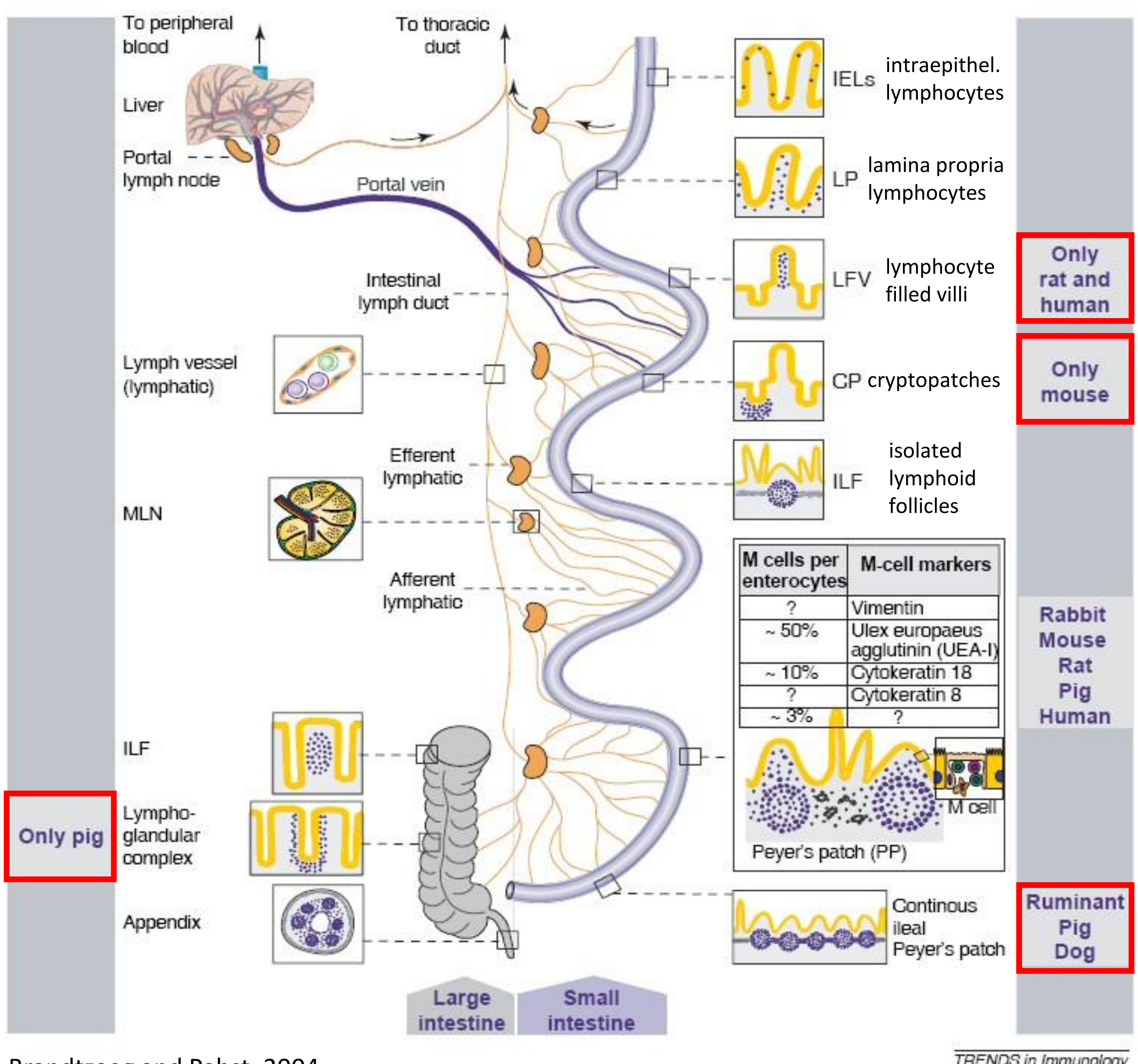
Bundesministerium für Bildung und Forschung (BMBF)

- FBI-Zoo

The Gastrointestinal Associated Lymphoid Tissue (GALT)



M cells per enterocytes	M-cell markers
?	Vimentin
~ 50%	Ulex europaeus agglutinin (UEA-I)
~ 10%	Cytokeratin 18
?	Cytokeratin 8
~ 3%	?

Brandtzaeg and Pabst, 2004

The GALT includes many other, additional regions of accumulated immune cells

Many of these regions are species-specific, *e.g*. the cryptopatches in mice or the lympho-glandular complex in swine

Other regions, such as the extended Continuous Peyer´s Patch, is found only in certain animal species

In addition, the M Cells show specific differences in surface and structural aspects in different animal species and humans

Suggest differences in antigen presentation or immune responses