

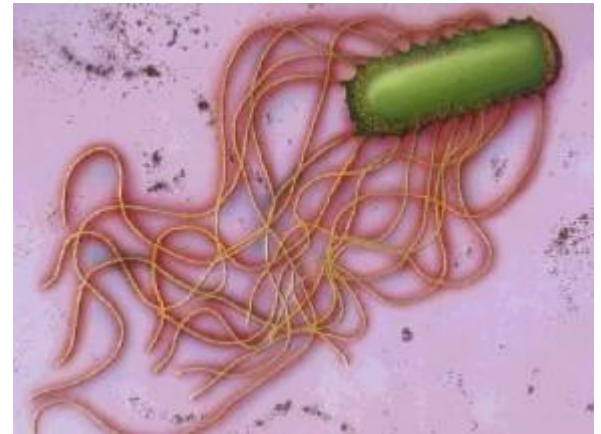
Salmonellosi

E. Marco

Marco Vetgrup; SLP

Salmonellosi

- Infezione causata da Salmonella
- Scoperto nel 1885 da uno scienziato nordamericano chiamato D.E. Salmon .
- Ben diffuso tra uomini e animali.



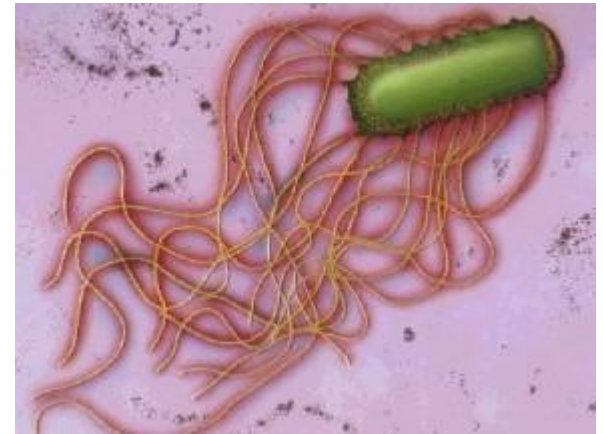
Salmonellosi

- Per molti anni è stata associata al CSF.
- Quando è stato identificato il CSF, tutti si sono dimenticati della salmonella.



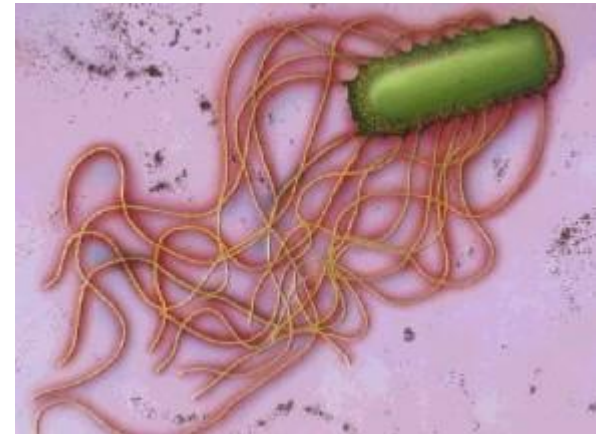
Salmonellosi

- La Salmonella è molto resistente:
 - si riproduce tra 7 e 45 ° C
- Sopravvive al congelamento o all'essiccazione.
- Può sopravvivere per settimane, mesi o persino anni nella carne.
- Sopravvive in molti organismi
- Le alte temperature (62°C) e un pH acido (<5) lo inattivano facilmente, così come molti disinfettanti.



Salmonellosi

- È una malattia suina
- È un problema di salute pubblica (tossinfezione alimentare)
 - Nell'UE vengono segnalati oltre 91.000 casi di salmonellosi ogni anno. L'EFSA (1) ha stimato che l'onere economico complessivo della salmonellosi umana potrebbe raggiungere i 3 miliardi di euro all'anno.
 - Il CDC stima che i batteri della Salmonella causano circa 1,35 milioni di infezioni, 26.500 ricoveri e 420 decessi negli Stati Uniti ogni anno. Il cibo è la fonte della maggior parte di queste malattie (2).
- È commercialmente importante

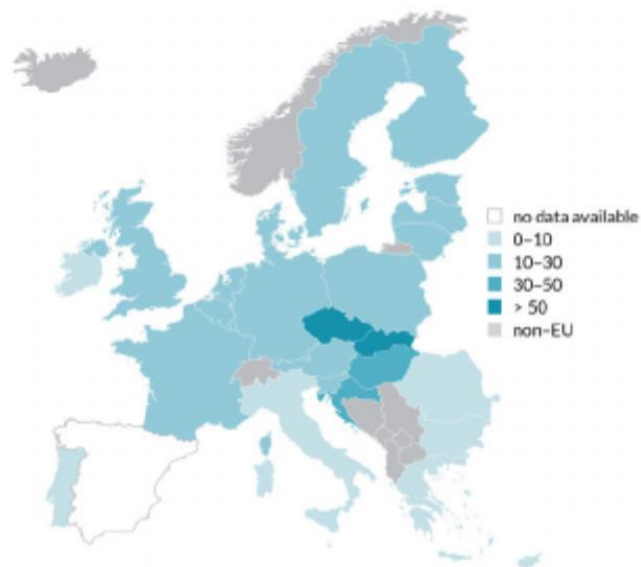


(1) <https://www.efsa.europa.eu/en/topics/topic/salmonella>

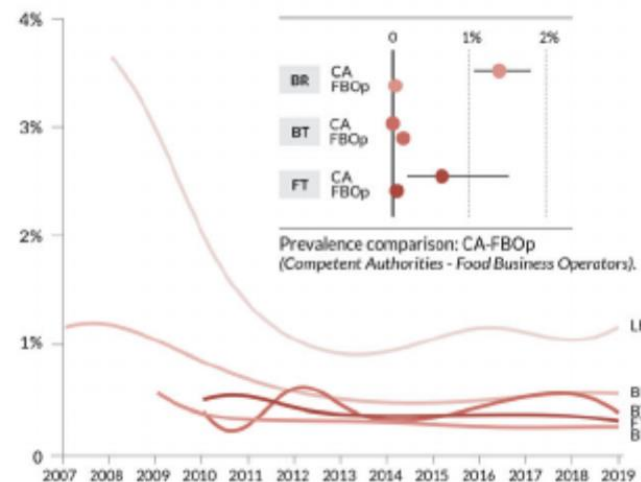
(2) <https://www.cdc.gov/salmonella/>

Salmonella in the EU, 2019

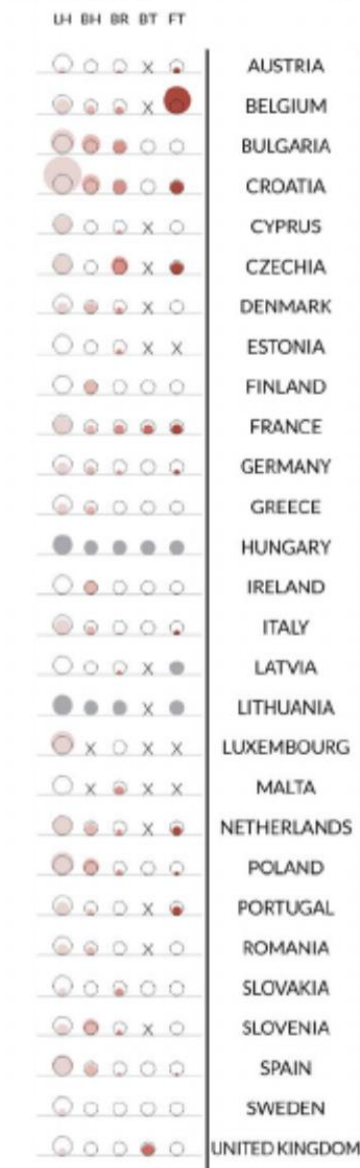
Human cases of Salmonellosis: notification rates per 100,000 population in the EU/EFTA.



Prevalence trend estimates of poultry flocks positive for target Salmonella serovars at the EU-level.



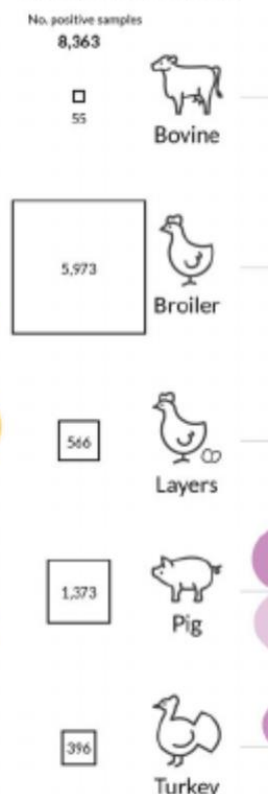
Prevalence of poultry flocks positive for target Salmonella serovars (NCP).



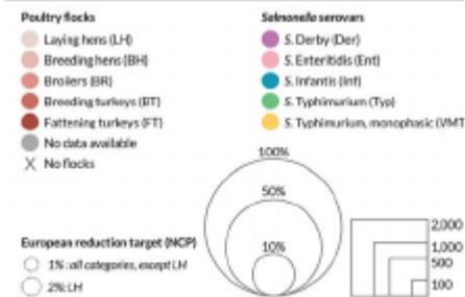
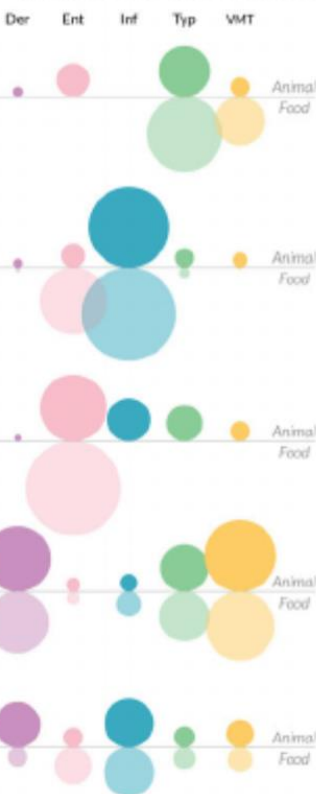
Geographical distribution (%) of the human EU top-5 Salmonella serovars isolates from food and animal matrices.



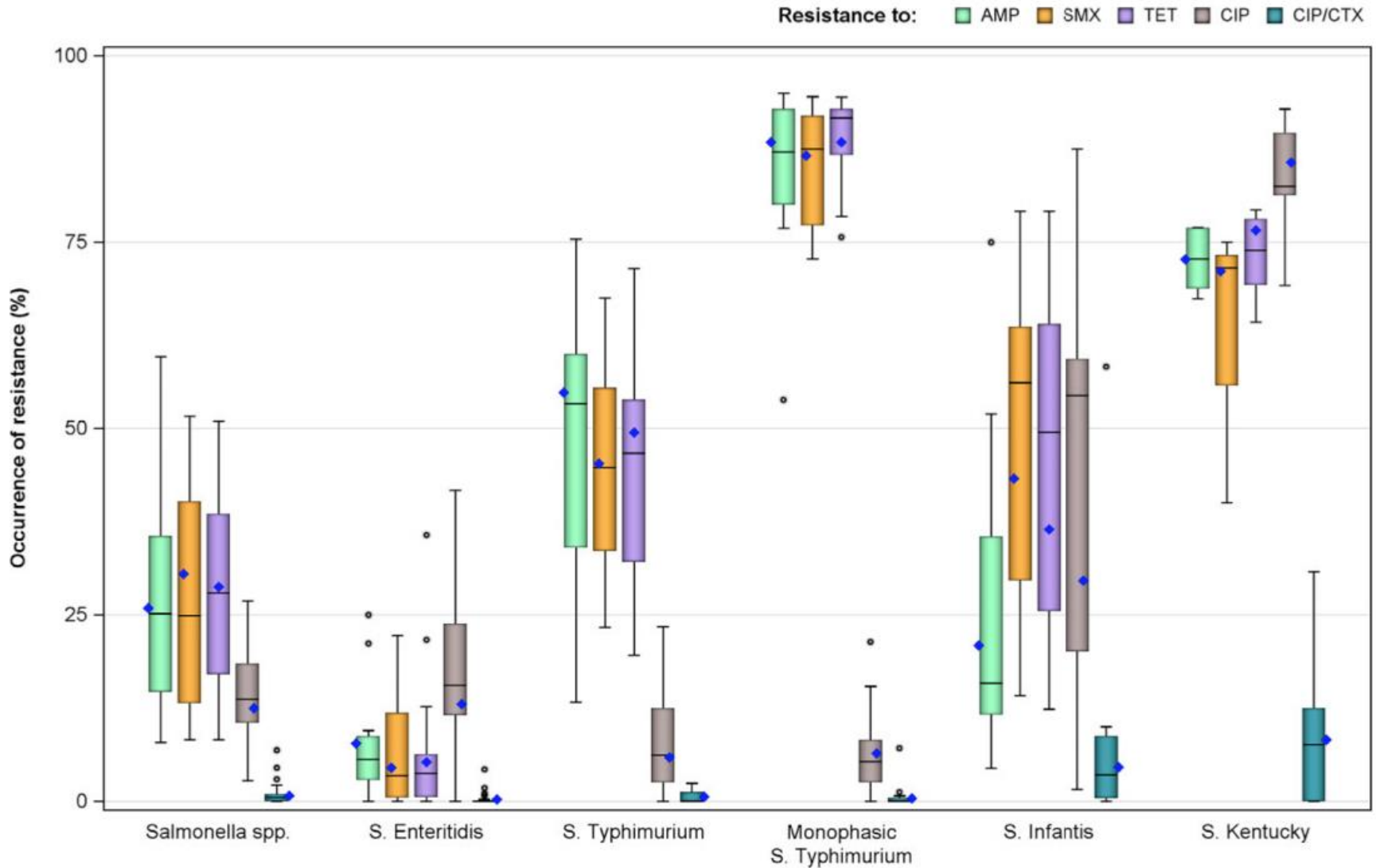
Distribution of the human EU top-5 Salmonella serovars among different sources.



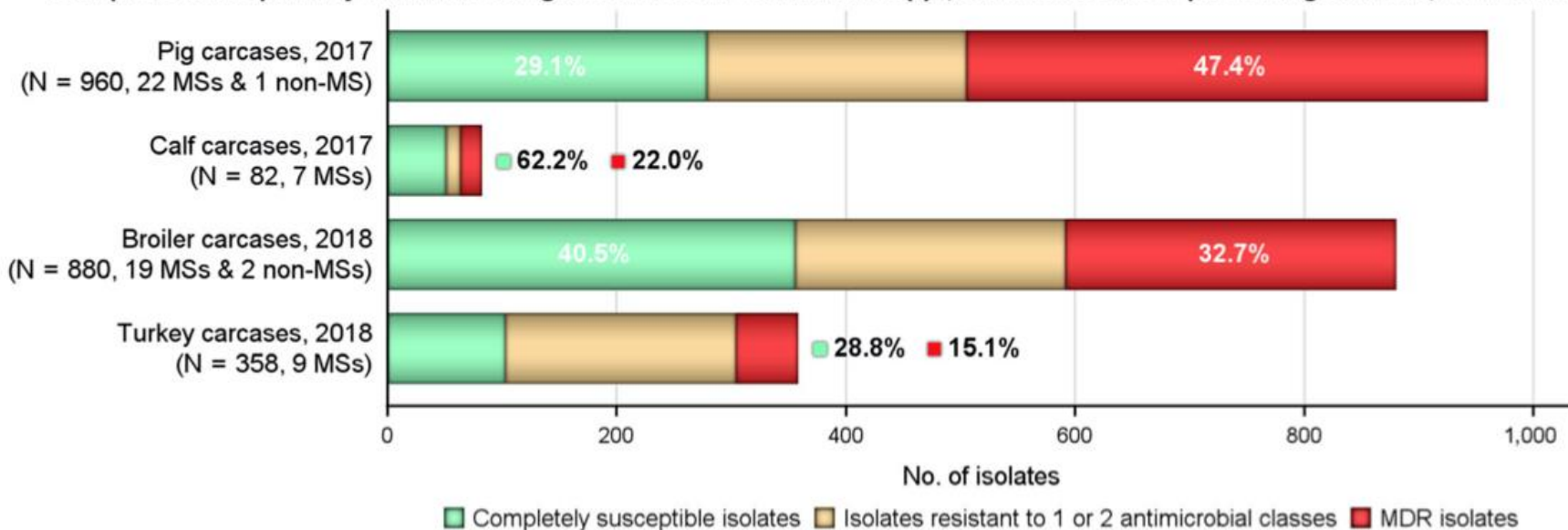
Distribution (%) of the human EU top-5 Salmonella serovars across different food and animal sources.



Occurrence of resistance to selected antimicrobials in *Salmonella* spp. and selected serovars isolated from humans, 2018



Complete susceptibility and multi-drug resistance in *Salmonella* spp., carcasses of food-producing animals, 2017/2018

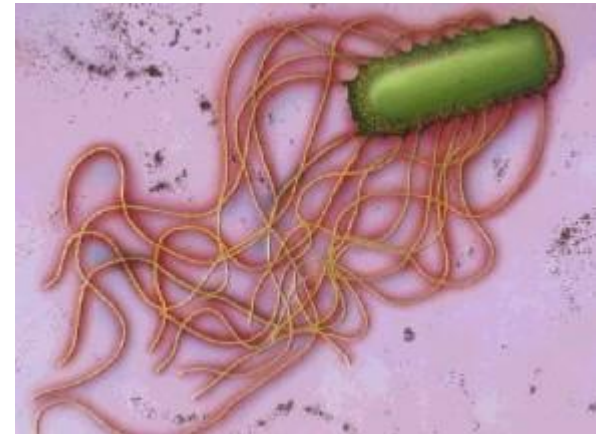


MDR and complete susceptibility levels are also expressed as a percentage; N: total number of *Salmonella* spp. reported by MSs and non-MSs.

Figure 5: MDR and completely susceptible *Salmonella* spp. recovered from carcasses of pigs (fatteners), calves (under 1 year of age), broilers and fattening turkeys, for all reporting countries (including 1 non-MS in pig carcasses and 2 non-MSs in broiler carcasses) in 2017/2018

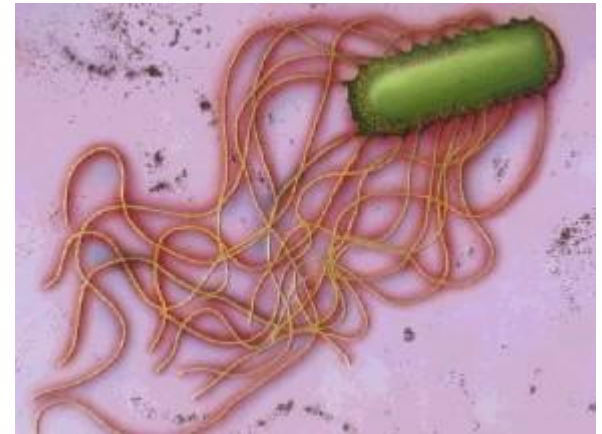
Salmonellosi

- La maggior parte sono sierotipi o sottospecie di *Salmonella enterica*, (più di 2400)
- i più importanti sono *typhimurium*, *enteritidis* e *typhi*: *Salmonella enterica* sierotipo *typhi* è la causa della febbre tifoide.
- *S. Typhimurium* e *S. Enteritidis* sono le cause dell'intossicazione alimentare.



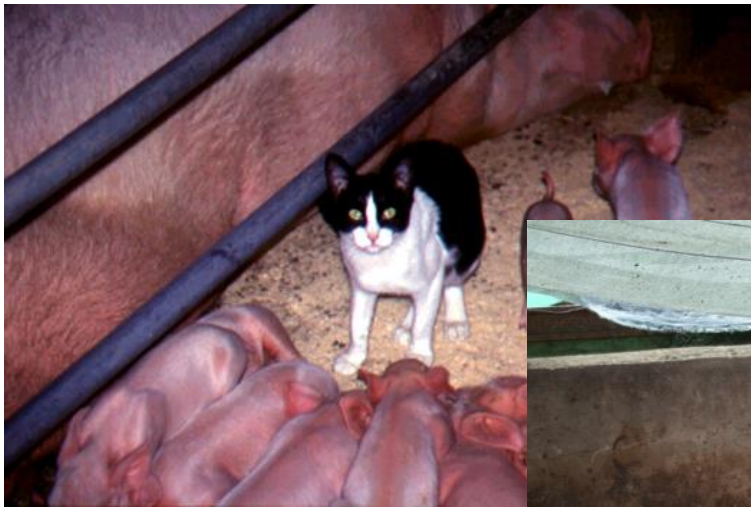
Salmonellosi

- Causa malattia clinica nei suini:
 - *Salmonella Choleraesuis*
 - *Salmonella Typhimurium*
 - *Salmonella Derby*.
- *S. Choleraesuis* è il sierotipo specifico adattato ai suini:
 - grave malattia generalizzata: febbre, depressione, setticemia, polmonite, meningite, artrite e diarrea
- *Salmonella Typhimurium* è il sierotipo più comune nei suini:
 - diarrea nei suini giovani e fonte comune di intossicazione alimentare nell'uomo.
- I suini sono portatori di *Salmonella* per lunghi periodi.



Fonti di contaminazione

- Mammiferi, uccelli e rettili sono suscettibili di essere infettati da Salmonella e, in molti casi, ne diventano portatori e quindi potenziali fonti di contaminazione.





Fonti di contaminazione

- Mammiferi, uccelli e rettili sono suscettibili di essere infettati da Salmonella e, in molti casi, ne diventano portatori e quindi potenziali fonti di contaminazione.
- Le salmonelle sono molto resistenti nell'ambiente, il che fa diffondere tali microrganismi facilmente quando sono trasportati da materiale contaminato.
 - L'acqua potrebbe essere un veicolo
- Il suino può essere infettato da un gran numero di sierotipi e rimanere come portatore, sebbene sia raro che sviluppi la malattia poiché pochi sierotipi sono patogeni per questo.

S. Choleraesuis

- Suini svezzati di età inferiore a 5 mesi
 - Ma occasionalmente visto
 - Suinetti lattanti
 - Stock riproduttivo adulto.
- Inappetenza
- Letargico
- Ipotermia (40,5-41,6°C)
- Dispnea
- Cianosi delle estremità e dell'addome
- La diarrea può essere vista dopo alcuni giorni (giallastro)

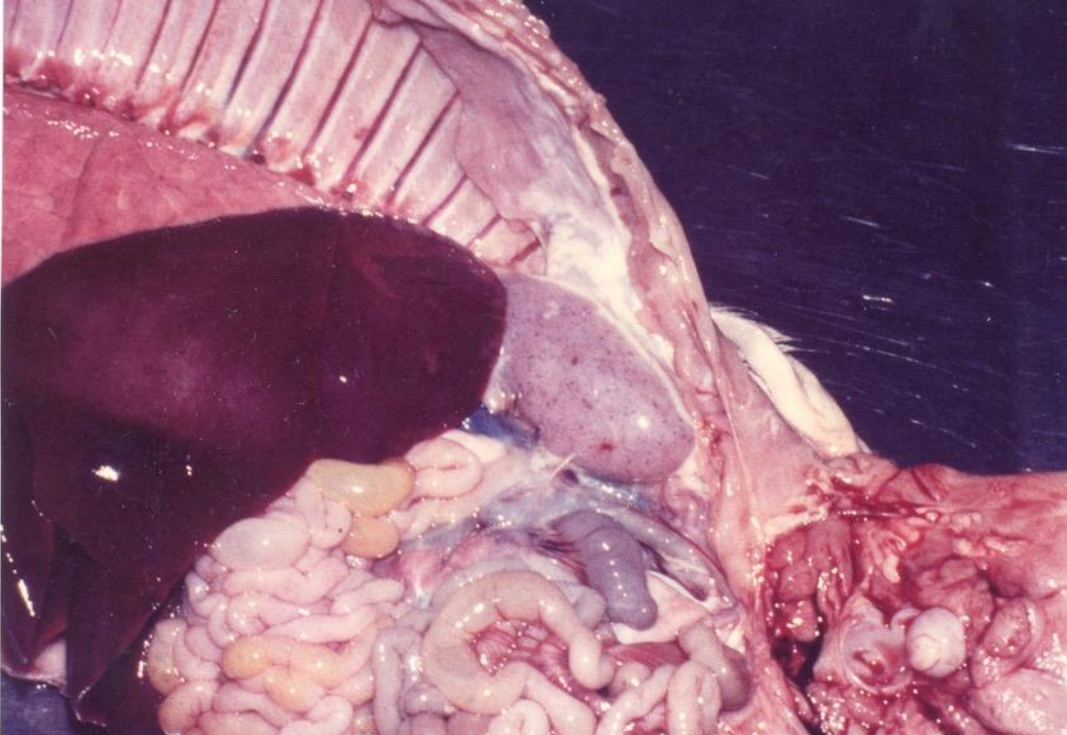
S. Choleraesuis











S. Typhimurium

- Suini svezzati di età inferiore a 5 mesi
 - Ma occasionalmente visto
 - Suinetti lattanti
 - Stock riproduttivo adulto.
- Diarrea giallastra acquosa (inizialmente senza sangue o muco)
- Il sangue appare dopo alcuni giorni (ma non in grande quantità)
- Ipotermia (40,5-41,6°C)
- Diminuzione dell'assunzione di mangime
- Disidratazione
- Occasionalmente si può sviluppare stenosi rettale

S. Typhimurium

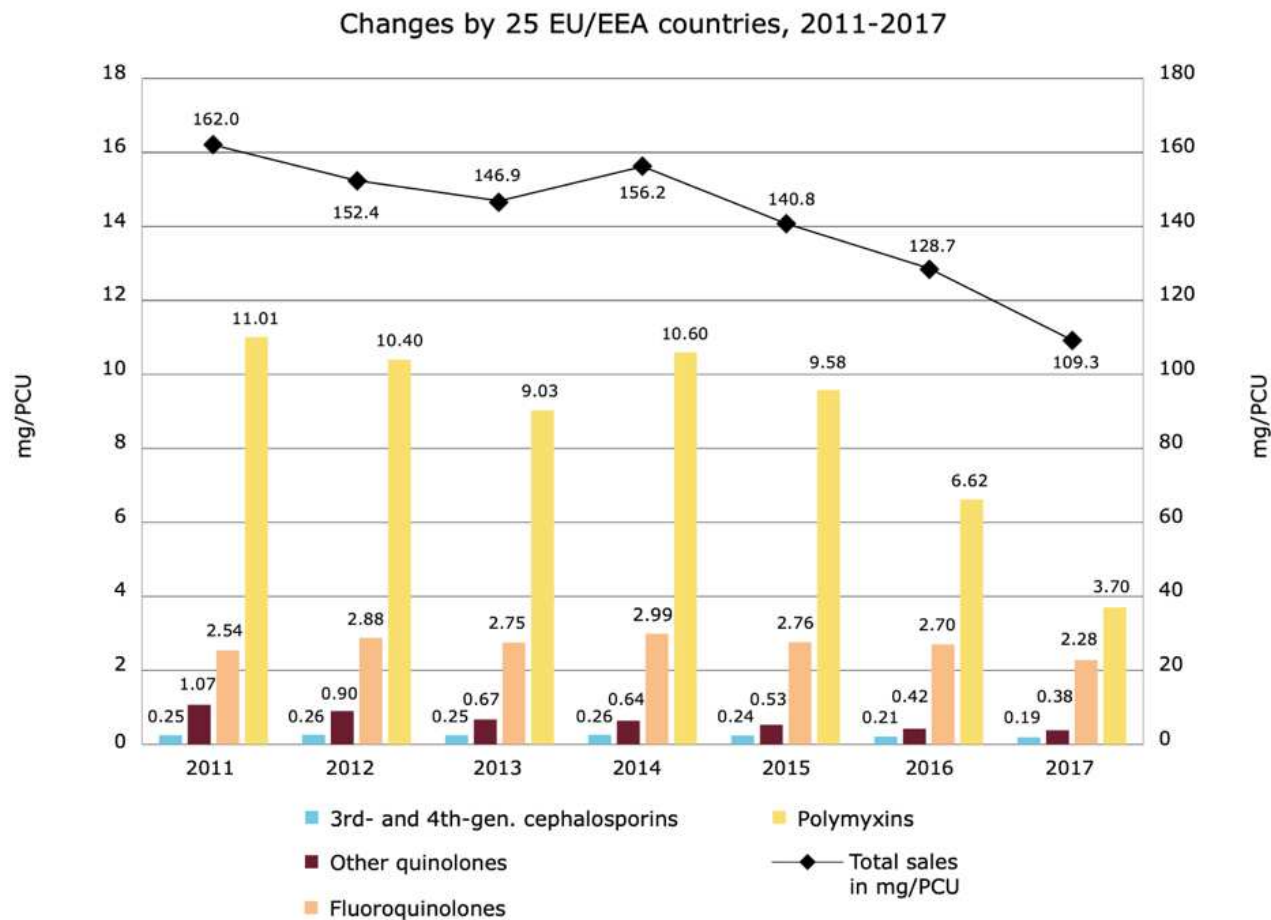








Figure 24. Changes in aggregated overall sales, as well as sales of fluoroquinolones, other quinolones, 3rd- and 4th-generation cephalosporins and polymyxins, for 25 EU/EEA countries¹, from 2011 to 2017 (note the differences in the scales of the Y axes)



¹ Austria, Belgium, Bulgaria, Cyprus, Czechia, Denmark, Estonia, Finland, France, Germany, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden and United Kingdom.

Biosicurezza

- Esterna
- Interna
 - Tutto pieno- Tutto vuoto
 - Integrità delle bande
 - Pulizia e disinfezione
 - Conduzione di acqua
 - Salmonella può rimanere al biofilm
 - Controllo di topi e ratti
 - Altri animali



Pulizia e disinfezione

Pen Floors		Enterobacteriaceae ^a					Salmonella ^b			
Category	Farm	Samples Tested (n)	Before washing		After washing		Before washing	After Washing		
			Range	Median	Range	Median	Positive samples (n)	Range	Positive samples (n)	Range
3	A	64	1.7-6.6	4.5	0-5.8	0.8	- ^c	-	- ^c	-
High 2	B	64	3.5-6.1	4.6	0-1.6	0	- ^c	-	- ^c	-
High 2	C	72	0-5.1	1.2	0-1.6	0	1	1.1	- ^c	-
1	D	84	2.6-6.1	4.6	0-3.6	0.8	26	36->106	1	7.2
1	E	84	0-3.6	1.6	0-3.2	0	- ^c	-	- ^c	-
1	F	60	1.2-5.1	3.3	0.7-4.2	2.9	- ^c	-	- ^c	-
1	G	72	0-6.0	2.0	0-3.6	0	- ^c	-	1	0.36
1	H	48	0.8-4.2	3.7	0.7-4.1	2	- ^c	-	- ^c	-

Table 1. Effect of cleaning procedure on levels of *Salmonella* and *Enterobacteriaceae* on the pen floors. ^aLog₁₀ cfu/cm². ^bMPN/cm²; detection limit, 0.36 MPN/cm². ^cNegative for *Salmonella* (detection limit, <0.36 MPN/cm²).

Pulizia e disinfezione

Feeder/Drinker Units			Enterobacteriaceae ^a				Salmonella ^b			
			Before washing		After washing		Before washing		After Washing	
Category	Farm	Samples Tested (n)	Range	Median	Range	Median	Positive samples (n)	Range	Positive samples (n)	Range
3	A	16	3.0-5.6	4.4	2.4-6.8	5.2	— ^c	—	1	4600
High 2	B	16	3.7-5.7	5	0.7-6.8	5.6	— ^c	—	3	11-240
High 2	C	24	0-6	2	2.0-6.0	5	— ^c	—	— ^c	—
1	D	27	3.7-6.1	5.5	0-6.1	4.9	6	0.92-105	2	0.6-7.2
1	E	36	1.5-4.9	3.4	0-5.5	3	— ^c	—	— ^c	—
1	F	24	0.5-4.9	3.5	3.2-5	4.4	— ^c	—	— ^c	—
1	G	24	0-5..5	2.8	0-4.1	2.8	— ^c	—	— ^c	—
1	H	20	3.4-4.9	3.9	3.3-6	4	— ^c	—	— ^c	—

Table 2. Effect of cleaning procedure on levels of *Salmonella* and *Enterobacteriaceae* in feeder/drinker units
^aLog₁₀ cfu/cm². ^bMPN/cm²; detection limit, 0.36 MPN/cm². ^cNegative for *Salmonella* (detection limit, <0.36 MPN/cm²).



Acqua

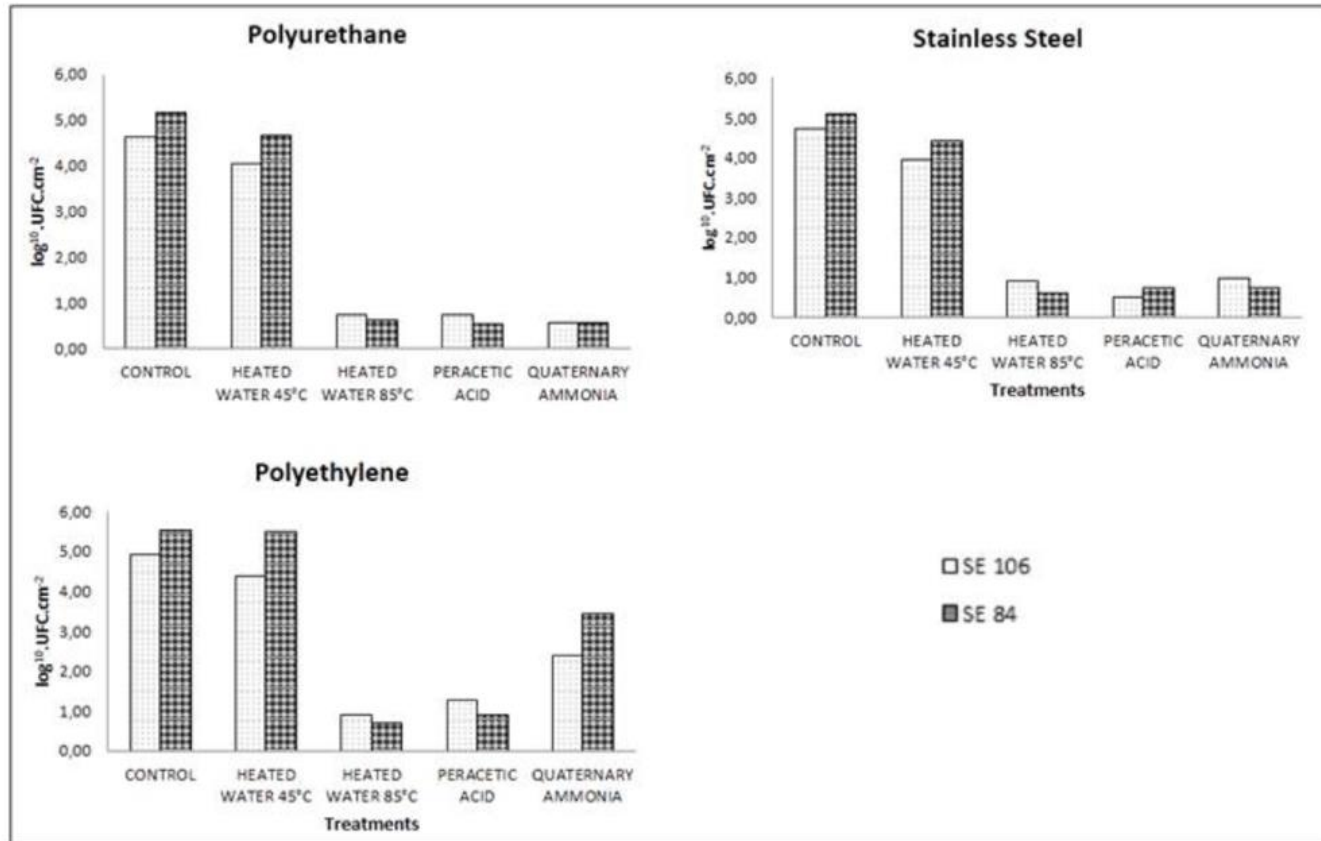


Figure 4 Removal of biofilm formed by SE of poultry origin on stainless steel, polyethylene and polyurethane surfaces by different hygiene procedures. Abbreviations: Heat water 45 °C: sterile water heated at 45 °C; Heat water 85 °C: sterile water heated at 85 °C; Peracetic acid (0.5%); Quaternary ammonia (1%).

Igiene quotidiana

Pediluvio in nuova soluzione per 2 minuti

Table 3: Post-treatment summary statistics for bacterial counts per 75-mm² area of boot sole cultured in Experiment One

Treatment (n=5)	Mean bacterial count / 75-mm ² area	Standard deviation	95% confidence interval	
			Lower bound	Upper bound
No boot bath	1.36 x 10 ⁸ a	4.8 x 10 ⁷	1.1 x 10 ⁸	1.6 x 10 ⁸
Cidex Formula 7*	6.79 x 10 ⁶ b	3.6 x 10 ⁶	-2.2 x 10 ⁷	3.6 x 10 ⁷
1Stroke Environ®	7.96 x 10 ⁷ a	3.5 x 10 ⁷	5.1 x 10 ⁷	1.1 x 10 ⁸
Chlorox®	5.30 x 10 ⁷ a	2.9 x 10 ⁷	2.4 x 10 ⁷	8.2 x 10 ⁷
Betadine solution	3.98 x 10 ⁷ a	1.2 x 10 ⁷	1.1 x 10 ⁷	6.9 x 10 ⁷
Roccal™-D Plus	5.54 x 10 ⁷ a	2.4 x 10 ⁷	2.6 x 10 ⁷	8.4 x 10 ⁷
Nolvasan® solution	9.32 x 10 ⁷ a	3.9 x 10 ⁷	6.4 x 10 ⁷	1.2 x 10 ⁸

a,b Different superscripts indicate statistical differences ($P < .0001$).

Igiene quotidiana

Table 4: Cumulative percentage of weaned pigs¹ from which *Escherichia coli* strain M1823B (challenge strain) was isolated during sample collection periods on Day 0 (prior to exposure), and Days 2, 4, 7, and 11 after initial exposure by inoculation (Inoculated Pigs) or direct (Pen Sentinels) or indirect exposure to the Inoculated Pigs.²

Treatment Group	n	No. of pigs (%) from which <i>E coli</i> strain M1823B was isolated ³				
		Day 0 (prior to exposure)	Day 2	Day 4	Day 7	Day 11
Inoculated Pigs	20	0 (0)	18 (90)	19 (95)	20 (100)	20 (100)
Pen Sentinels	5	0 (0)	2 (40)	5 (100)	5 (100)	5 (100)
Direct Sentinels	25	0 (0)	0 (0)	12 (48)	17 (68)	20 (80)
Hand-wash Sentinels	25	0 (0)	0 (0)	0 (0)	13 (52)	23 (92)
Shower Sentinels	25	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Non-exposed Pigs (negative controls)	25	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

¹ Inoculated Pigs were orally inoculated Days 0 and 2, and Pen Sentinels were housed with them except during inoculation procedures. On Days 1 through 10, Direct, Hand-wash, and Shower Sentinel groups (Table 1) were contacted according to the schedule in Table 2. When diarrhea was observed (except in Inoculated Pigs), affected pigs were immediately euthanized for cultural and histological examination. Pigs determined to be positive on a designated sample collection day or on the day of euthanasia were counted as positive for all subsequent sample collection periods.

² Inoculated Pigs were individually offered 1.36 to 8.92×10^{10} colony forming units of *E coli* M1823B in liquid strawberry gelatin.

³ Strain M1823B was identified on the basis of antimicrobial sensitivity.

Amass SF, Halbur PG, Byrne BA, et al. Mechanical transmission of enterotoxigenic *Escherichia coli* to weaned pigs by people, and biosecurity procedures that prevented such transmission. *J Swine Health Prod.* 2003;11(2):61-68.

Prevenzione

- I suini portatori o l'ambiente contaminato sono le più comuni fonti di infezione.
- Ridurre al minimo l'esposizione a fattori stressanti
 - Ridurre al minimo i rimescolamenti
 - Densità corretta
 - Temperatura e ventilazione confortevoli
- Controllare altri processi infettivi
- Alimentazione
 - Farina grossolana
 - Additivi
- Vaccinazione
- Il trattamento preventivo funziona ma sempre più difficile

Ridurre il
mix delle
provenienze

Raul Mainar 2010.
Samonellosis en
Explotaciones Porcinas.
Jornadas Técnicas AVPA,
Zaragoza 25 Marzo 2010

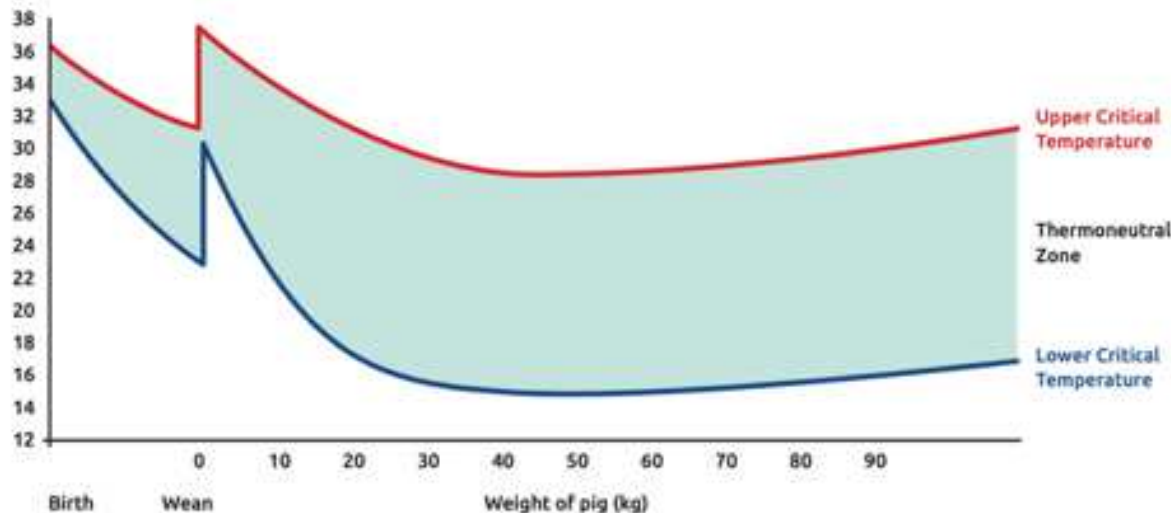
Prevalenza di Salmonella Spp. (53 allevamenti ingrasso)

Numero allevamenti di provenienza	Prevalenza salmonella spp. (%)
1	29,43
2	22,67
>2	43,50

Trattamento e controllo

Esigenze di temperatura:

Ideale 3°C su LCT (temperatura critica inferiore) e sempre inferiore a ECT (temperatura critica di evaporazione)

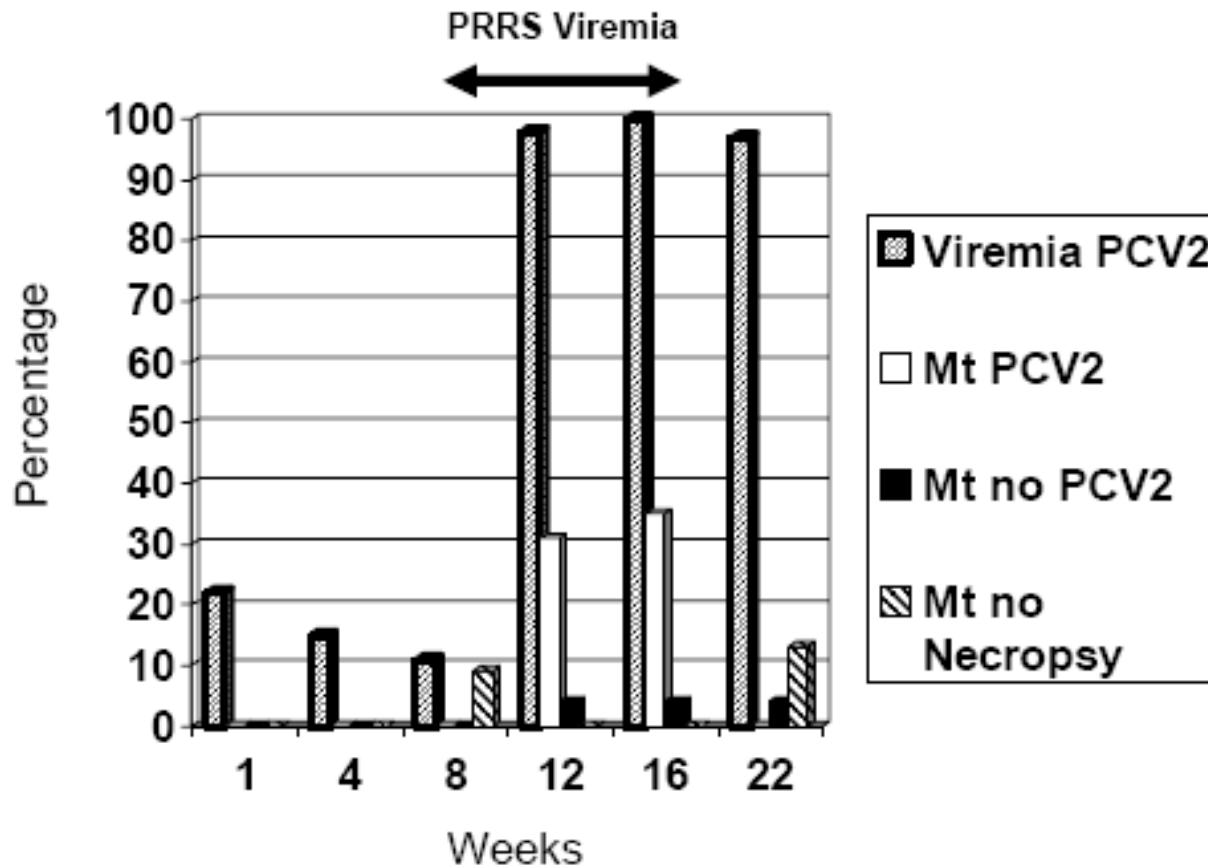




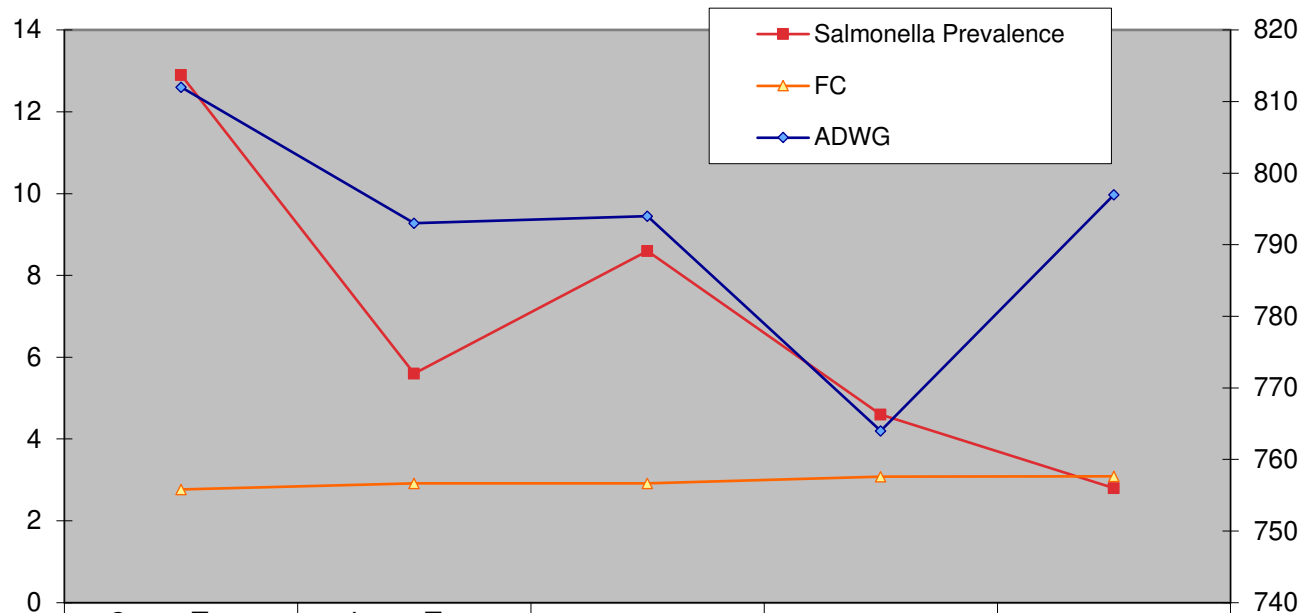




Alta mortalità >30% (n=2)



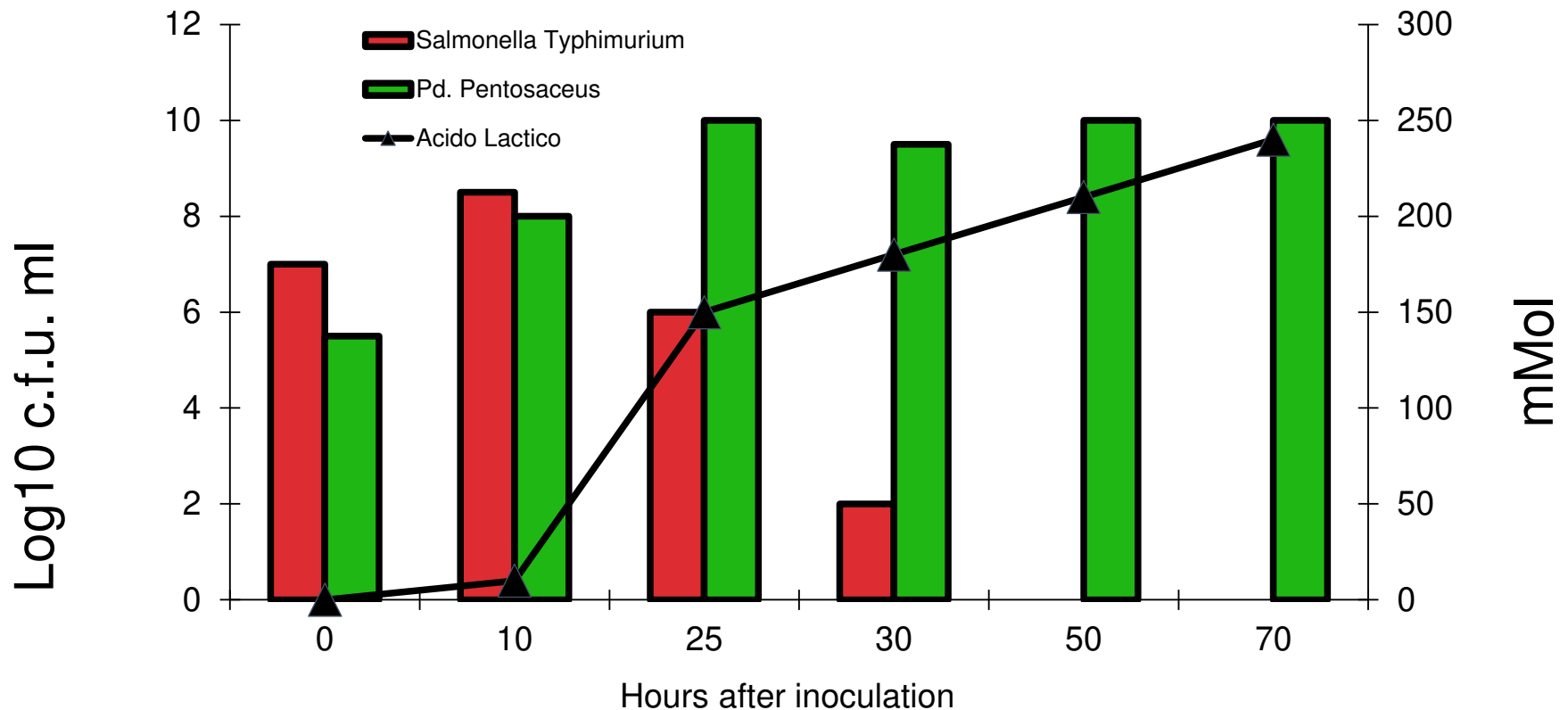
Alimentazione: Dimensione delle particelle



	2mm Exp. Gran.	4mm Exp. Gran.	4mm Gra.	4mm. Exp.	4mm
Salmonella Prevalence	12,9	5,6	8,6	4,6	2,8
FC	2,77	2,92	2,92	3,08	3,09
ADWG	812	793	794	764	797

Alimentazione: Acidificazione

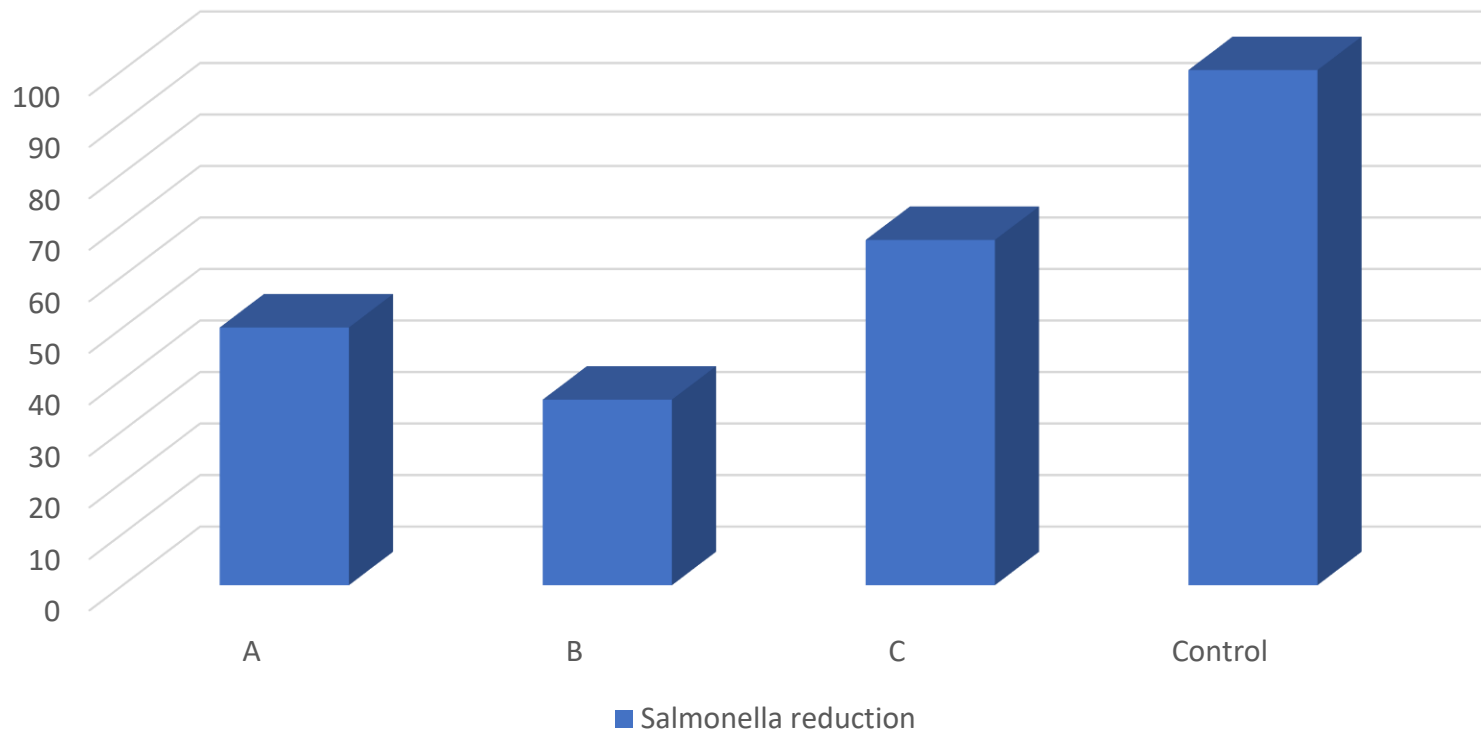
- Alimentazione liquida fermentata



Alimentazione: Integratori alimentari

Salmonella reduction

feces, cecal content and lymph-nodes at slaughterhouse



A: Butyric acid

B: Short Chain organic acids + natural extracts

C: MCFA + Lactic acid + oregano oil

Rasschaert G. et al. 2016. Effect of Organic Acids on Salmonella Shedding and Colonization in Pigs on a Farm with High Salmonella Prevalence. *Journal of food protection* 79(1):51-58

Alimentazione: Integratori alimentari

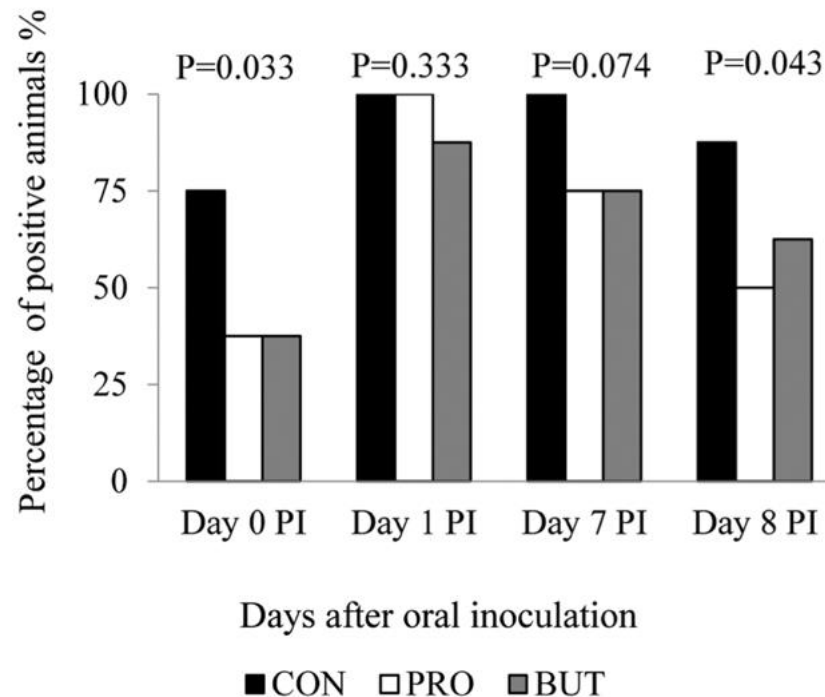


Figure 1. Percentage of *Salmonella* shedders along the post-inoculation (PI) period. Percentage of animals ($n = 8$) that showed *Salmonella* in feces at d 0, 1, 7 post-inoculation (PI) or colon digesta at d 8 PI. P-values obtained by Fisher's exact test. Treatments: CON, plain diet without additives; PRO, plain diet with 1 kg/t of Proporc (10^9 cfu/kg of feed of *Bacillus licheniformis*); BUT, plain diet with 3 kg/t of Gustor BP70 (2.1 g of partially protected sodium butyrate salt/kg of feed).

Vaccinazione

- Friendship *et al.* (2009) hanno riportato che da 15 studi valutati, 14 hanno presentato una riduzione della prevalenza di Salmonella, che varia dal 20-80% nei suini svezzati all'86% nelle scrofe.
- Un numero limitato di studi ha documentato vaccini suini che sono efficaci contro più sierotipi di Salmonella.
- Oggi sono disponibili vaccini spenti e attenuati.

Vaccinazione

Table 2. Relevant references on vaccination strategies to control *Salmonella*

REFERENCE	STUDY DESIGN	EFFECT	REFERENCE	STUDY DESIGN	EFFECT
Roesler et al., 2006	Field Trial N=25 vac. sows, and N=37 control sows	Beneficial effect: no shedding in piglets from vaccinated sows vs 47.7% in piglets from antibiotic-treated controls	Groninga et al., 2000	Randomized challenge trial N = 24 pigs	Beneficial cross-protection effect: Overall, about 70% reduction in <i>Salmonella</i> prevalence in vaccinated (<i>Choleraesuis</i> vaccine) pigs (83% vs. 25%) at slaughter.
Roesler et al., 2004	Non-random challenge trial. N = 30 pigs	Beneficial effect: 20-80% reduction in the proportion of weaned pigs shedding <i>Salmonella</i> after vaccination with a Typhimurium-based vaccine.	Gibson et al., 1999	Randomized challenge trial N = 34 pigs	Beneficial cross-protection effect of Argus™ in reducing <i>S. Typhimurium</i> shedding load (not prevalence).
Springer et al., 2001	Non-random challenge trial N = 36 pigs	Beneficial effect: ileal/caecal mucosa & ileocecal lymph nodes (ICLN) of vaccinated (with a Typhimurium-based vaccine) grower-finisher pigs less colonized with <i>Salmonella</i> .	Maes et al., 2001	Randomized field trial N = 655 pigs	Beneficial cross-protection effect of Argus™ against ($p < 0.05$) <i>Salmonella</i> (over 90% reduction in ICLN prevalence).
Lindner et al., 2001	Field trial ² N = 575 sows + 16356 piglets	Beneficial effect: Vaccination (Typhimurium-based vaccine) was protective against <i>Salmonella</i> infections (over 86% reduction in the ICLN prevalence).	Charles et al., 2000	Randomized challenge trial N = 20 pigs per study	Beneficial cross-protection effect: (modified-live <i>Choleraesuis</i> vaccine via drinking water) No shedding vs 58% among controls. No beneficial cross-protection effect of a modified-live <i>S. Choleraesuis</i> vaccine (individually administered twice) against <i>S. Typhimurium</i> shedding.
Lumsden et al., 1991	Randomized challenge trial N = 17 pigs	Beneficial effect for the 1 st two weeks: 40% of shedders in the control group against 0% in the vaccinated (Typhimurium-based vaccine)			
Letellier et al., 1999b	Randomized challenge trial N = 20 pigs	Negative effect: Endovac™ - Vaccinated pigs were all shedding <i>Salmonella</i> compared to only 10% of the controls at necropsy.			
Roof & Doitchinoff, 1995	Randomized challenge trial N = 82 piglets	Beneficial effect: 44-75% reduction in the proportion of weaned pigs shedding <i>Salmonella</i> after vaccination with a <i>Choleraesuis</i> vaccine.			
Kramer et al., 1987	Randomized challenge trial N = 34	Beneficial effect: <i>Choleraesuis</i> vaccine (5.5×10^9 via conjunctival sac) responsible of 50-75% reduction of <i>Salmonella</i> -positive MLN in grower-finisher pigs. Second trial given IM had similar results.			
Kramer et al., 1992	Randomized challenge trial N = 67 pigs	Beneficial effect: No death and no cases of salmonellosis in SC-54 vaccinated weaned pigs, compared to 3 deaths & 9 cases of acute salmonellosis for the controls.			
Hanna et al., 1979	Randomized challenge trial N = 35 pigs	Beneficial (SUGGESTIVE³) effect: SC-54 vaccinated weaned pigs did not shed <i>Salmonella</i> , compared to non-vaccinated pigs.			
Kolb et al., 2003	Randomized field trial N _{study 1} = 420 pigs N _{study 2} = 420 pigs	Beneficial effect: 50-75% reduction in the proportion ICLN positive for <i>Salmonella</i> in vaccinated (Enterisorf®) grower-finisher pigs.			

Vaccinazione

Vaccini stabulogeni spenti

- *S. Typhimurium*
- Scrofe 75 + 100 giorni di gestazione
- Suinetti 28 + 56 giorni di vita

Ruggeri, J. *et al.* 2015. Inactivated *Salmonella enterica* serovar Typhimurium monophasic variant (*S. Typhimurium* 1,4,[5],12:i-) in sows is effective to control infection in piglets under field condition. *Veterinary Microbiology* 180 (2015) 82–89

Salmonella shedding in pigs faeces

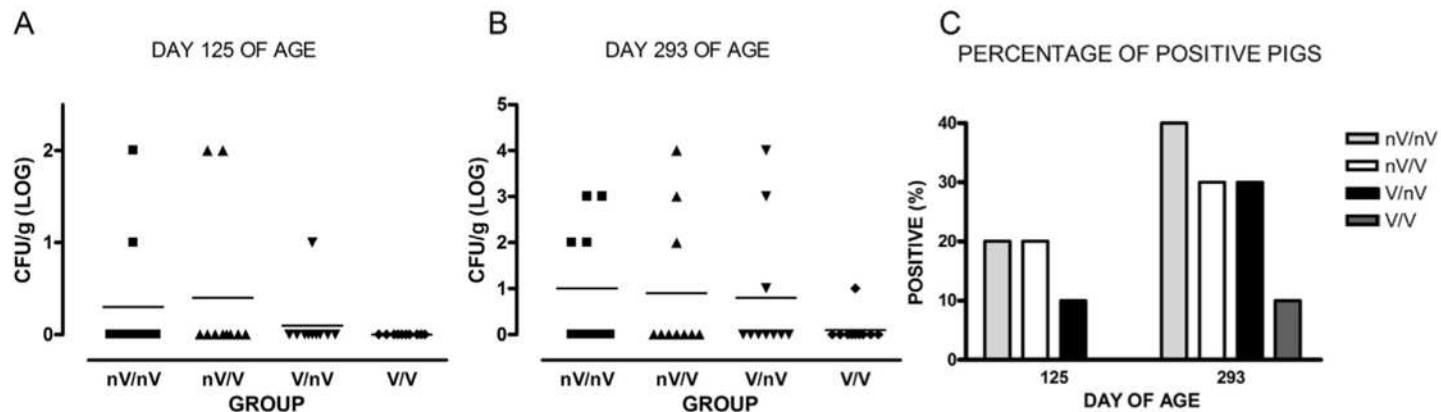
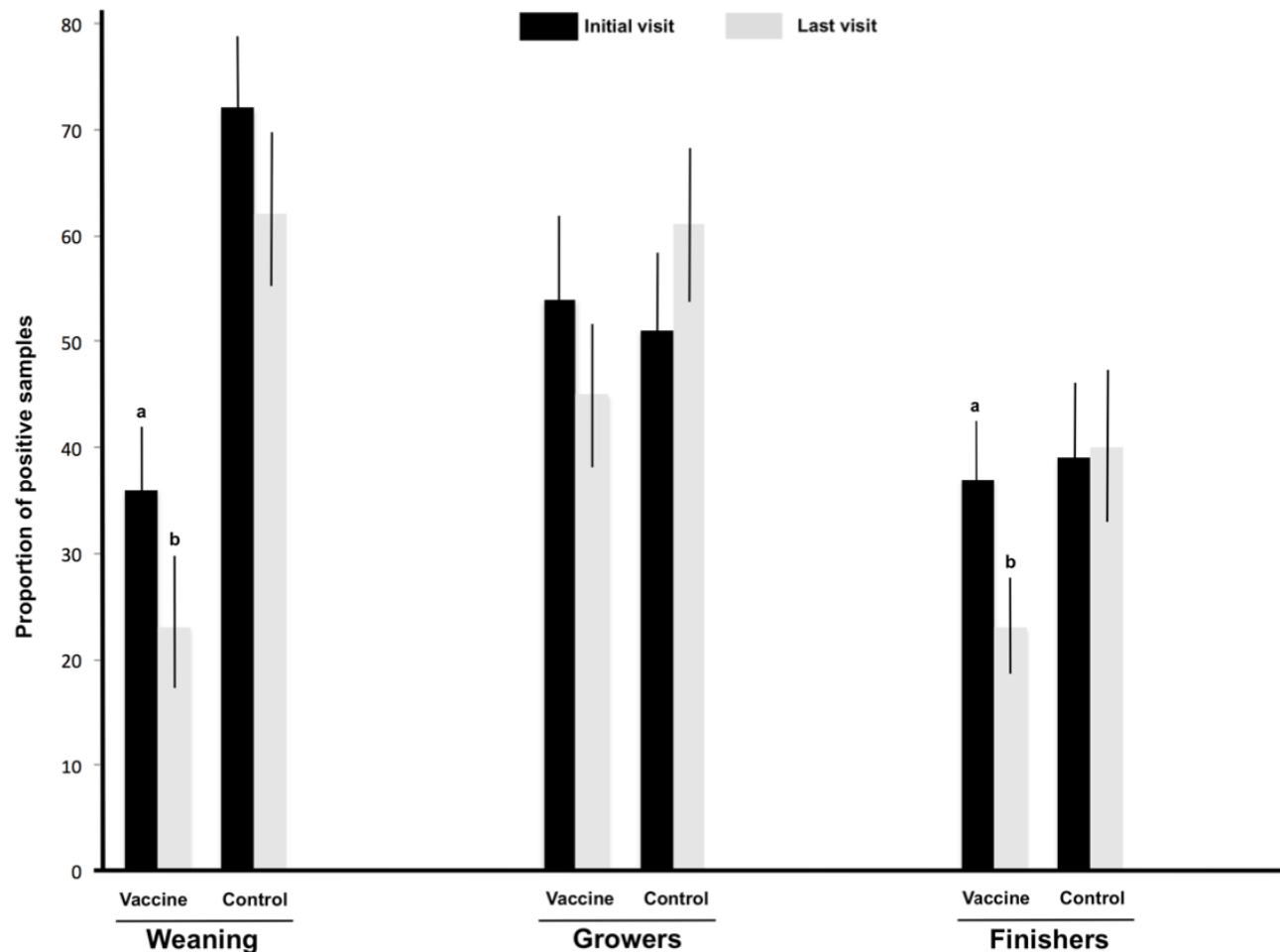


Fig. 5. Vaccination of sows and of their piglets tends to reduce number of shedder pigs during the growing-fattening stage. In (A and B), each symbol represents a pool of faeces collected from three pigs of the same litter belonging to the four groups (nV/nV, nV/V, V/nV, V/V) at day 125 and 293 of age and the amount of bacteria in faeces. In (C), columns represent the percentage of positive faecal samples of the four groups of pigs. No significant differences are recorded among groups in *Salmonella* contamination at each time point.

Vaccinazione



Smith, R. *et al.* (2018). Maternal Vaccination as a Salmonella Typhimurium reduction strategy on pig farms. *Journal of Applied Microbiology*. 124(1):274-285.

Vaccinazione

Vaccini vivo attenuato

- *S. Typhimurium*
- Scrofe 6 + 3 settimane prima del parto
- Suinetti 3 + 25 giorni di vita
- Ingrasso 11-12 + 14-15 settimane di età

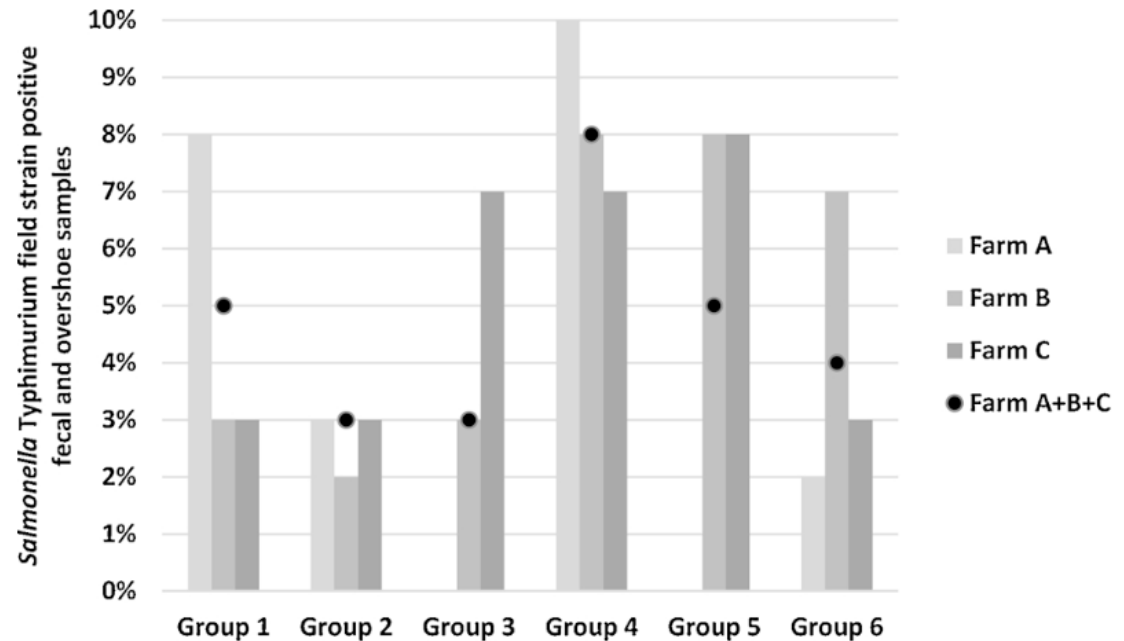


Fig. 1. Percentage of *Salmonella* Typhimurium field strain positive fecal and overshoe samples collected from the growing and fattening pigs in the different experimental groups¹ on farm A, B and C in production cycle 1 and 2.

¹Group 1: vaccination of sows, group 2: vaccination of sows and piglets, group 3: vaccination of sows and fattening pigs, group 4: vaccination of piglets, group 5: vaccination of fattening pigs, group 6: non-vaccinated control group. Sows, piglets and fattening pigs were vaccinated against *Salmonella* Typhimurium with an attenuated histidine-adenine auxotrophic vaccine (Salmoporc®, IDT Biologika).

Vaccinazione

- Protocollo combinato
 - *Salmonella* Typhimurium attenuato orale
 - *Salmonella Choleraesuis* spento intramuscolare
- A: Protocollo combinato
- B: Doppio intervento vaccino *S. Choleraesuis*
- C: Controllo

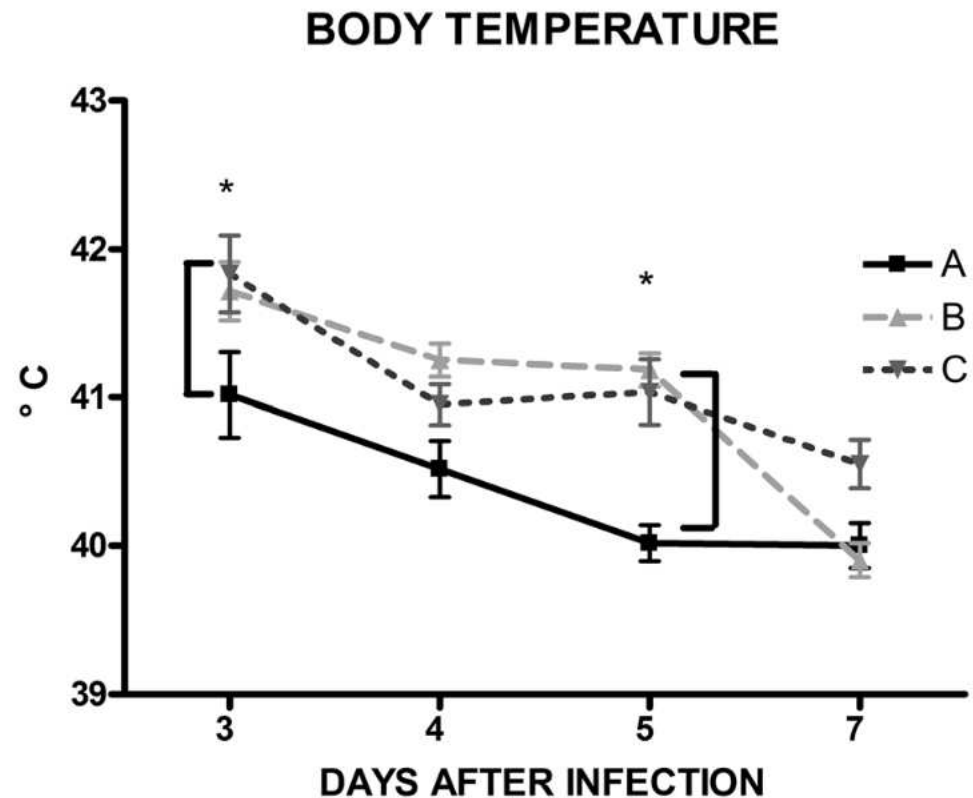


Fig. 2 Vaccination with attenuated *S. Typhimurium* $\Delta znuABC$ prevents fever. Body temperature of groups A, B and C is shown at different time points (day 3, 4, 5 and 7 after challenge). Symbols represent mean and bars standard deviation. Symbols (*) represent differences statistically significant among groups with $p < 0.01$

Vaccinazione

SCIENTIFIC REPORTS



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**Recombinant attenuated
Salmonella Typhimurium with
heterologous expression of
the *Salmonella* Choleraesuis
O-polysaccharide: high
immunogenicity and protection**

Received: 10 February 2017

Accepted: 3 July 2017

Published: 28 July 2017

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Vaccinazione

JOURNAL
OF MEDICAL
MICROBIOLOGY

RESEARCH ARTICLE

Bearson *et al.*, *Journal of Medical Microbiology* 2017;66:651–661
DOI 10.1099/jmm.0.000482



OPEN
MICROBIOLOGY

Salmonella DIVA vaccine reduces disease, colonization and shedding due to virulent *S. Typhimurium* infection in swine

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Gil *et al. Vet Res* (2020) 51:3
<https://doi.org/10.1186/s13567-019-0730-3>



RESEARCH ARTICLE

Open Access

A DIVA vaccine strain lacking RpoS and the secondary messenger c-di-GMP for protection against salmonellosis in pigs



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Conclusione

- La salmonellosi continua a essere una delle zoonosi più preoccupanti per la salute umana
- Il suo controllo e la sua prevenzione devono essere basate su molteplici strategie:
 - Biosicurezza
 - Esterna
 - Interna
 - Igiene e disinfezione
 - Buone pratiche di management
 - Controllo di altre malattie
 - Nutrizione
 - Vaccinazione
 - Vaccinazione dalle scrofe in cicli chiusi
 - Vaccinazione suinetti acquistati

Grazie