Can DuPont Impact *Salmonella* in Poultry?

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01JUL2014

Nine months ago I was asked the following question by Dr. Andrew Morgan.....

"With the extensive resources and capabilities of DuPont, do you (Greg) think that we, as Dupont, can do something to reduce the Salmonella carriage rate in poultry?"

My short answer is yes.

After much consideration, I think that we could certainly have impact on understanding the basis of the *Salmonella* organism's ecology in the avian gut in a way never before possible prior to the advent of genomic analytical capability.

Whether this knowledge can then be used to mitigate the issue and result in patents and products is a different question altogether.

Here I would like to describe my vision of how I view this problem and come to at least a more direct answer to the original question.

These are strictly my opinions.

I am reminded of a section from Jonathan's Gertner's book *"The Idea factory"* .
He describes how a commitment by Bell Laboratories to open-ended fundamental research led to the invention of the transistor ..... arguably one of the single the most transformational inventions to ever derive from basic theoretical and hypothesis-driven science.

"The formal purpose of the new 'solid-state' group was not so much to build something as to understand it.

Officially, Shockley's men were after a basic knowledge of the new materials; only in the back of their minds did a few believe they would soon find something useful for the Bell System."

Gertner, J. 2012. *The Idea Factory: Bell Labs and the Great Age of American Innovation. (pg 91)*

Our approach to *Salmonella* started off as many similar projects do, that is, to find a solution or 'build something'.
Unfortunately we still do not even really understand the problem.
Evidence of that lies in our inability to mitigate the problem outside of depopulating (destroying) positive flocks.

Therefore finding solutions to mitigate *Salmonella* now demands a less empirical approach but one stemming from hypotheses derived from understanding how *Salmonella* functions in nature.

Despite many decades of research, *Salmonella* carriage has remained a recalcitrant problem for the poultry industry.

- Many scientists started their careers on this problem and finished their careers studying the same problem. This is important because the literature would indicate ideas for mitigating *Salmonella* seem to be cyclical vs original.
  - *According to 7JUL14 PubMed, since 1960 there have been at least 5,383 peer-reviewed scientific papaers with the keywords 'salmonella AND poultry'*
  - *As with all transformational research, a break from the past and a commitment to the strong possibility of a negative outcome must be accepted.*

- The US-FDA has taken action to reduce and eventually withdraw usage of antimicrobial growth promotant and therapeutic antibiotics (*"FDA Announces Voluntary Withdrawal of 19 Antimicrobials for Use in Food-Producing Animals" FDA Center for Veterinary Medicine , April 2014)*
  - *This will likely increase Salmonella positive carcasses.*
  - *In addition, analytical tools are improving and are at a point where they are inexpensive enough to increase testing capacity greatly leading to a greater likelihood of finding positives.*

The US-FDA is moving ahead with its intention to remove antibiotic growth promoters from poultry production.

Final Rule is "New Animal Drugs for Use in Animal Feeds; **Withdrawal** of Approval of New Animal Drug Applications; Bambermycins; Hygromycin B; Lincomycin; Pyrantel; Tylosin; Tylosin and Sulfamethazine; Virginiamycin"

April 10, 2014 FDA Federal Register:

Update - Withdrawal of Antimicrobials Use for Food-Producing Animals - FDA is announcing that "... all 26 drug manufacturers affected by Guidance for Industry (GFI) #213 have now agreed to fully engage in the strategy by phasing out the use of medically important antimicrobials in food-producing animals for food production purposes and phasing in the oversight of a veterinarian for the remaining therapeutic uses of such drugs. While GFI #213 specified a three-year timeframe (until December 2016) for drug sponsors to complete the recommended changes to their antimicrobial products, some sponsors have already begun to implement them ..." - FDA intends to update the public "... on the progress that drug sponsors have made in aligning their products with GFI #213 ... on a six-month basis ..." - FDA notes that "... 31 approvals for affected products have been withdrawn to date, and there are no drug approval withdrawals currently pending. After an approval is voluntarily withdrawn, those product(s) can no longer be marketed or sold in the United States ..."

Food Industry Environmental Network, LLC --- http://www.fien.com Article #30300

What gaps preclude our understanding of how *Salmonella* inhabits this niche?

- We still do not understand the mechanisms by which *Salmonella* is able to live as a normal resident of the healthy avian.
- Some interventions show promise, but we do not even understand why they might work to even a limited extent: these situations are opportunities.
- We still do not measure *Salmonella* in a quantitative manner; we still use presence/absence testing. For any mitigation to be effective with that measure means it must either be 100% or 0% effective. We miss any reductions or increases in actual levels by not using a quantitative assay.
- We do not attempt large scale in vivo testing of treatments to the point where they are regular and routine.
- We have not mapped the microbiomes of a very large number of broilers/turkeys/layers in order to draw correlations with quantitative *Salmonella* carriage levels. Hundreds to thousands not dozens.
- We have not studied in more detail the relationships between host status (specifically stress hormone levels) and pathogen populations in the gut.

The following are long term approaches to understanding the bases of a biological phenomenon and how they can be manipulated.

Notes:

- Placing short-terms goals ahead of the results would not only indicate a lack of understanding of the whole approach, but, would be a profound waste of resources.

- Emphasizing our strengths in the areas of probiology, recombinant and fermentation-derived ingredient production chemical engineering and animal nutrition are all poised to move forward with the possible outcomes of the basic research.

- The Dupont analytical capability extends into microbiology, biochemistry as well as genomics. Missing are the animal biological sciences.

Areas of research possible within the large Dupont research enterprise to understand how *Salmonella* lives in the avian.

- Methodology Needed: ① Sal qPCR Assay. Redefine reductions/increases as being actual levels vs presence/absence. ② High througput in vivo Sal colonization model, ③ High throughput microbial community analysis.

- Microbial genetics: Identifying host and bacterial factors (genes or operons) which provide *Salmonella* an evolutionary advantage in the gut.

- Host Genetics: Identify global host responses (including immune status) to stress that modulate *Salmonella* levels in the ceca and gut.

- Microbial Ecology: Determine the microbiomes of several thousand broiler gut sections (DJI and ceca) across feeds, ages, geographies and disease state and their statistical relation to *Salmonella* levels..

- First Generation Antagonists: Single or <5 strains +/- enhancersBased on microbiome knowledge, identify antagonists using high-throughput in-vitro then in-situ inhibitory capability.

- Second Generation Antagonists: Using knowledge from high-throughput microbial community analysis to routinely check composition of mixtures of antagonists: this is a back-to-the-future approach (*e.g. MCE or Broilact*).

Objectives

Current rate of *Salmonella* positive broilers → Lower rate of *Salmonella* positive broilers → Below detection limits of *Salmonella* carriage

Whatever we do to consistently shift this continuum to the right is a positive outcome.

R & D

Methodology
① Sal qPCR Assay
② HT in vivo colonization model
③ HT microbial community analysis

1st-Gen Antagonists

2nd-Gen Antagonists

Microbial genetics

Host Genetics

Microbial Ecology

Notes

1. Clearly not an R&D activity suited for IB-Dupont Animal Nutrition; they might be able to market.
2. Would likely involve new hire(s) on the animal physiology and immunology areas.
3. Animal model could be contracted but only if in close proximity.
4. We already have community analysis in-house.
5. Microbial genetics is likely somewhere inside CR&D already.
6. Would demand real commitment vs short term business goal.
7. A solution is not necessarily in the form of a DFM. It might be a hybrid.
8. HT community analysis is also an analytical tool for routine testing of “old school” fecal mixtures or artificial mixtures of antagonistic DFMs like the former products *Broilact* and MCE {*Microbial Competitive Exclusion*}. This is a game-changer in my view.
9. Much further downstream and not to presume routes; but, vaccines do not seem to be a viable option at this time. This is still largely a commensal bacterium of no consequence to the host. Therefore it is not a disease, as was pullorum disease, and not really a vaccine candidate in my view.

Nine months ago I was asked the following question by Dr. Andrew Morgan.....

"With the extensive resources and capabilities of DuPont, do you (Greg) think that we, as Dupont, can do something to reduce the Salmonella carriage rate in poultry?"

My reply, if we commit to doing it correctly, then my response to your question is <u>YES</u>!

The miracles of science™

The following slides were presented to the Tyson Foods Poultry production team of their top nutritionists and veterinarians.

Food Safety, *Salmonella*, Chickens and DFMs

G.R. Siragusa, Ph.D.
Senior Principal Scientist – Microbiology
Food Protection
Waukesha, WI

Tyson Foods – DuPont
Research and Technical Meeting
SanFrancisco, CA
May 1, 2014

Why does Pre-harvest Food Safety Research continue to be important?

FSIS Posts *Salmonella Initiative Program*[*1]:

FSIS posted a Federal Register announcing the agency's intentions of moving forward with its Salmonella Initiative Program (SIP). The recent publication includes the details of how the agency will post an establishment's category on its Web site, as well as the requirements to participate in the SIP.

*To participate in the agency's SIP, an establishment must 1) ensure its Salmonella incidence continue to be 50 percent below the regulatory standard (Category 1), 2) be required to increase its testing program, and 3) incorporate Campylobacter[*2] protocols. The data collected by the establishment will be provided to the agency for verification of the plant's food safety program. Any positive samples will be shared with the agency for further analysis.*

*1 *Source*: National Turkey Federation Newsletter, January 31, 2008. http://members.eatturkey.com/ntf/files/February2008.pdf

*2 The USDA-FSIS Initiated its Nationwide Poultry Campylobacter Baseline Survey June 2007

Technical Appendix 1 Table 2. The number of foodborne disease outbreaks, with simple or complex implicated food vehicles, 1998–2008, and the estimated annual number of illnesses, hospitalizations, and deaths, by etiology

Etiologic Agent	Reported Outbreaks			Reported outbreak-associated illnesses			Estimated Annual Numbers*		
	Total	Simple food	Complex food	Total	Simple food	Complex food	Illnesses	Hosp.†	Deaths
Bacterial	2,469	1,301	1,168	72,890	40,107	32,783	3,645,773	35,797	662
Salmonella enterica	877	482	395	29,685	16,000	13,685	1,029,382	19,533	378
Ser. Enteritidis	284	149	135	8,627	4,629	3,998	168,041	3,162	62
Ser. Heidelberg	66	23	43	3,151	456	2,695	49,478	931	18
Ser. Javiana	17	11	6	1,279	916	363	40,337	759	15
Ser. Newport	58	40	18	2,280	1,903	377	95,119	1,790	35
Ser. Typhimurium	106	59	47	4,113	1,767	2,346	202,497	3,810	74
S. spp., other non-typhoidal	344	199	145	10,213	6,323	3,890	472,089	8,883	174
Ser. Typhi	2	1	1	22	6	16	1,821	197	0

	Estimated Annual Numbers*		
Etiologic Agent	Illnesses	Hosp.†	Deaths
Bacterial	3,645,773	35,797	662
Salmonella enterica	1,029,382	19,533	378
Ser. Enteritidis	168,041	3,162	62
Ser. Heidelberg	49,478	931	18
Ser. Javiana	40,337	759	15
Ser. Newport	95,119	1,790	35
Ser. Typhimurium	202,497	3,810	74
S. spp., other non-ty	472,089	8,883	174
Ser. Typhi	1,821	197	0

EID, CDC, 2014

Salmonella remains a recalcitrant problem for the US poultry producer

Figure 1. Contribution of different food categories to estimated domestically-acquired illnesses and deaths, 1998-2008*

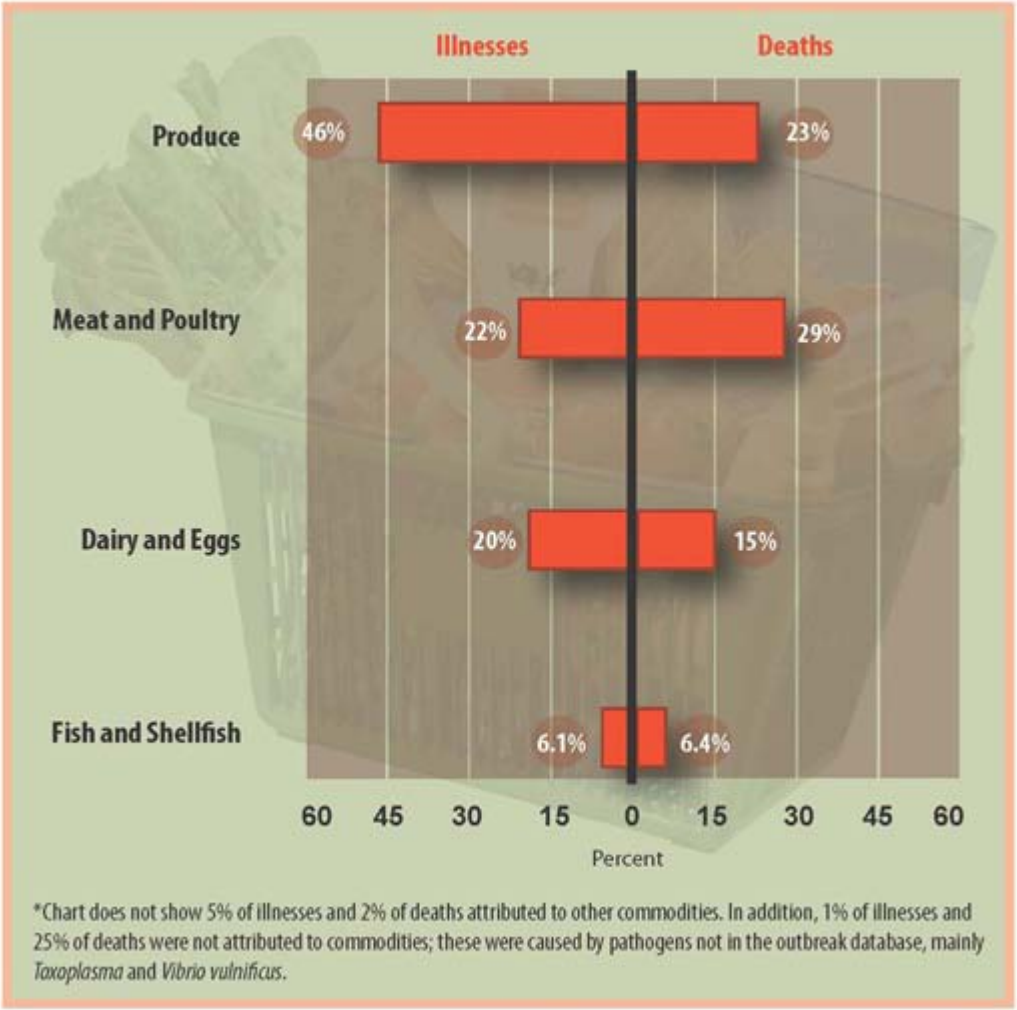
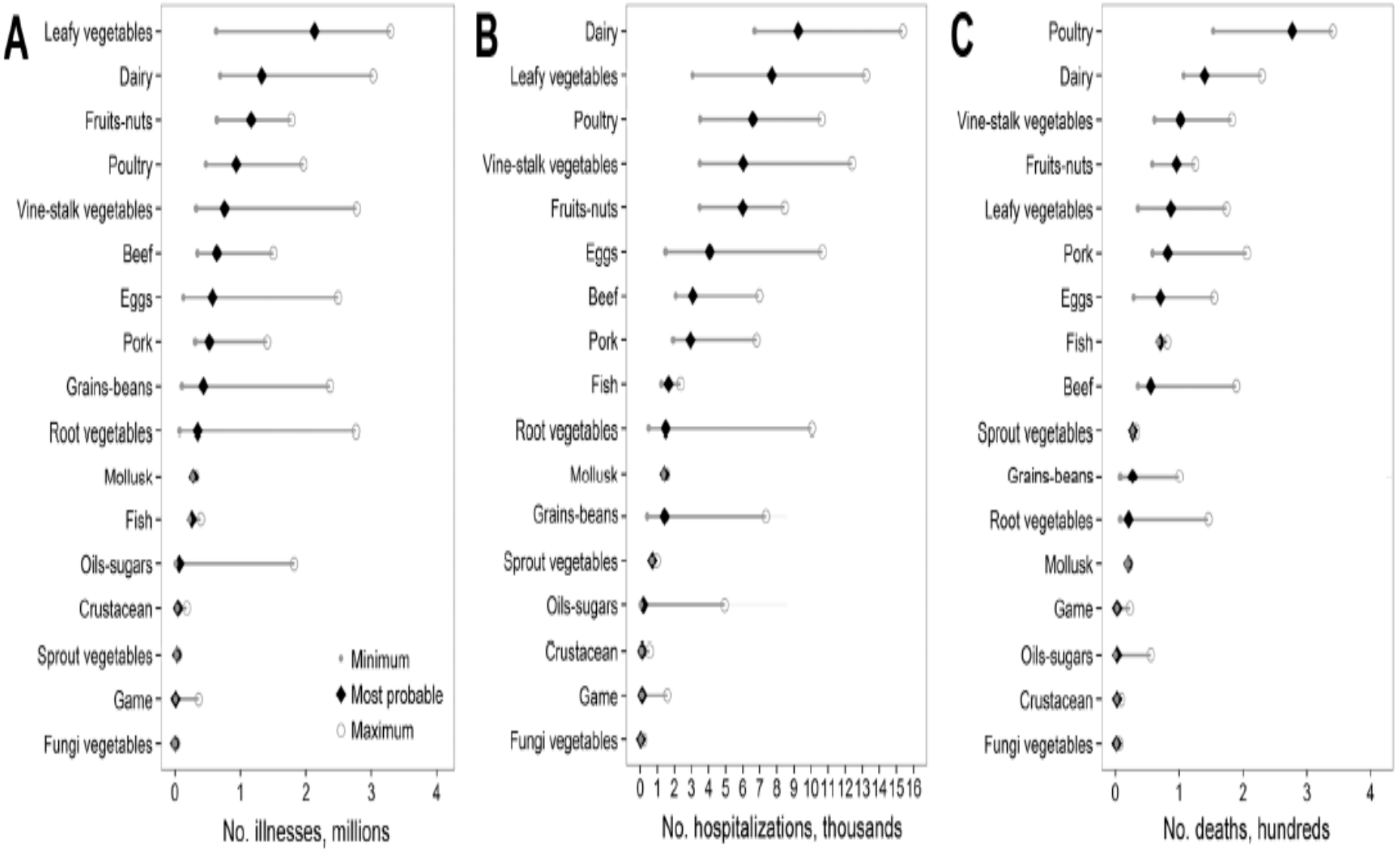


*Chart does not show 5% of illnesses and 2% of deaths attributed to other commodities. In addition, 1% of illnesses and 25% of deaths were not attributed to commodities; these were caused by pathogens not in the outbreak database, mainly *Toxoplasma* and *Vibrio vulnificus*.

Source: Painter JA, Hoekstra RM, Ayers T, Tauxe RV, Braden CR, Angulo FJ, Griffin PM. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg Infect Dis [Internet]. 2013 Mar [date cited]. http://dx.doi.org/10.3201/eid1903.111866

EID, CDC, 2014

Salmonella remains a recalcitrant problem for the US poultry producer



EID, CDC, 2014

Salmonella remains a recalcitrant problem for the US poultry producer

Table 1. Estimates of annual domestically acquired foodborne illnesses attr
by pathogen type, United States, 1998–2008*

Commodity or commodity group	All agents	Bacterial
Aquatic animals†	589,310 (6.1)	142,415 (3.9)
Fish	258,314 (2.7)	15,362 (0.4)
Shellfish†	330,997 (3.4)	127,053 (3.5)
Crustaceans	46,528 (0.5)	32,626 (0.9)
Mollusks	284,469 (3.0)	94,427 (2.6)
Land animals†	4,021,839 (41.7)	2,334,000 (64.0)
Dairy	1,330,098 (13.8)	656,951 (18.0)
Eggs	574,298 (6.0)	179,421 (4.9)
Meat-poultry†	2,117,442 (22.0)	1,497,628 (41.1)
Meat†	1,174,257 (12.2)	844,006 (23.2)
Beef	639,640 (6.6)	482,199 (13.2)
Game	9,934 (0.1)	5,111 (0.1)
Pork	524,684 (5.4)	356,697 (9.8)
Poultry	943,185 (9.8)	653,622 (17.9)

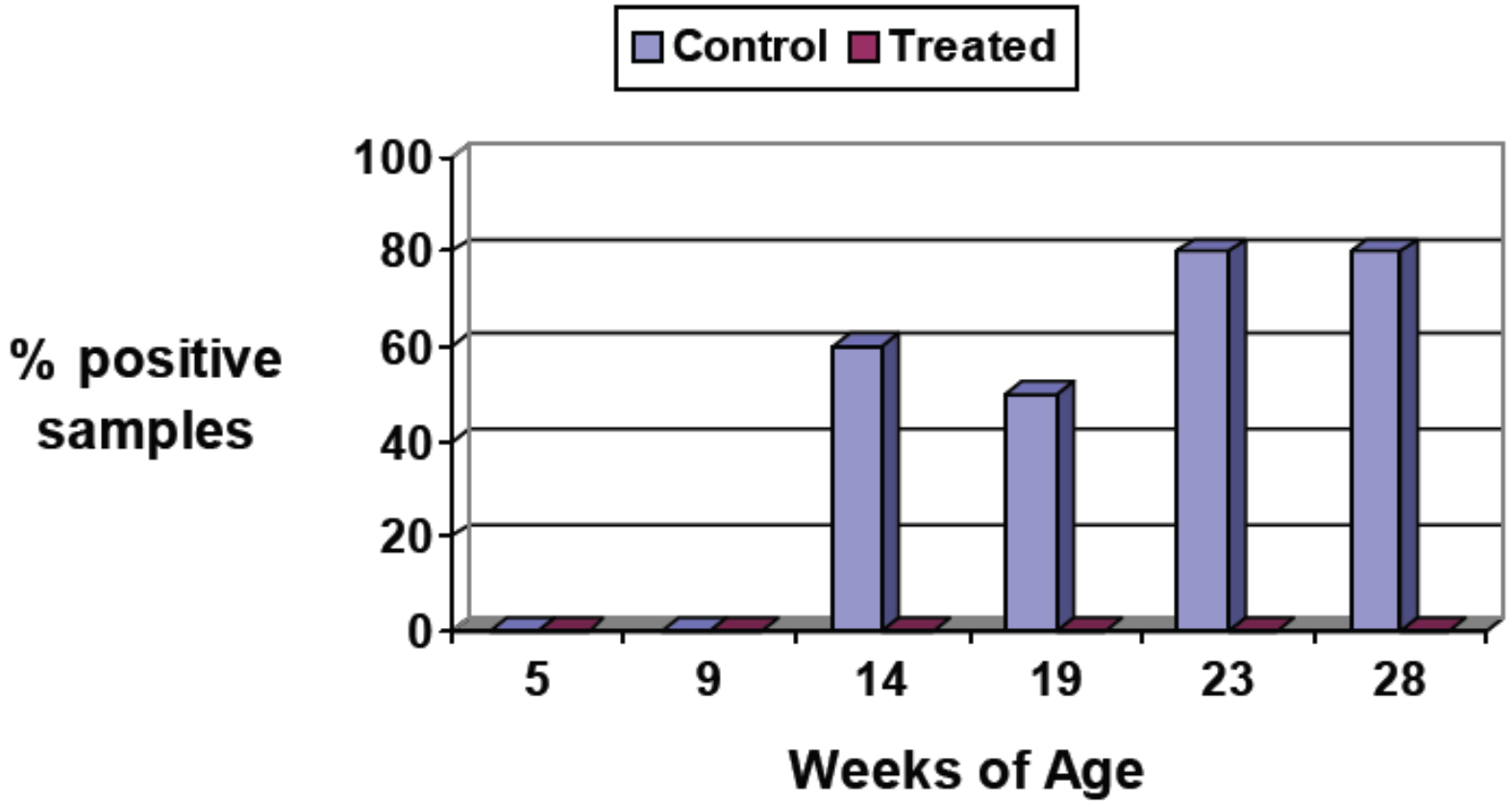
EID, CDC, 2014

Campylobacter and Salmonella reductions in Poultry Production

- An accepted premise of *Salmonella or Campylobacter* transmission from the bird to the carcass is that the final whole carcass rinse *Salmonella* status is predominantly determined by the carriage status of the incoming flock.

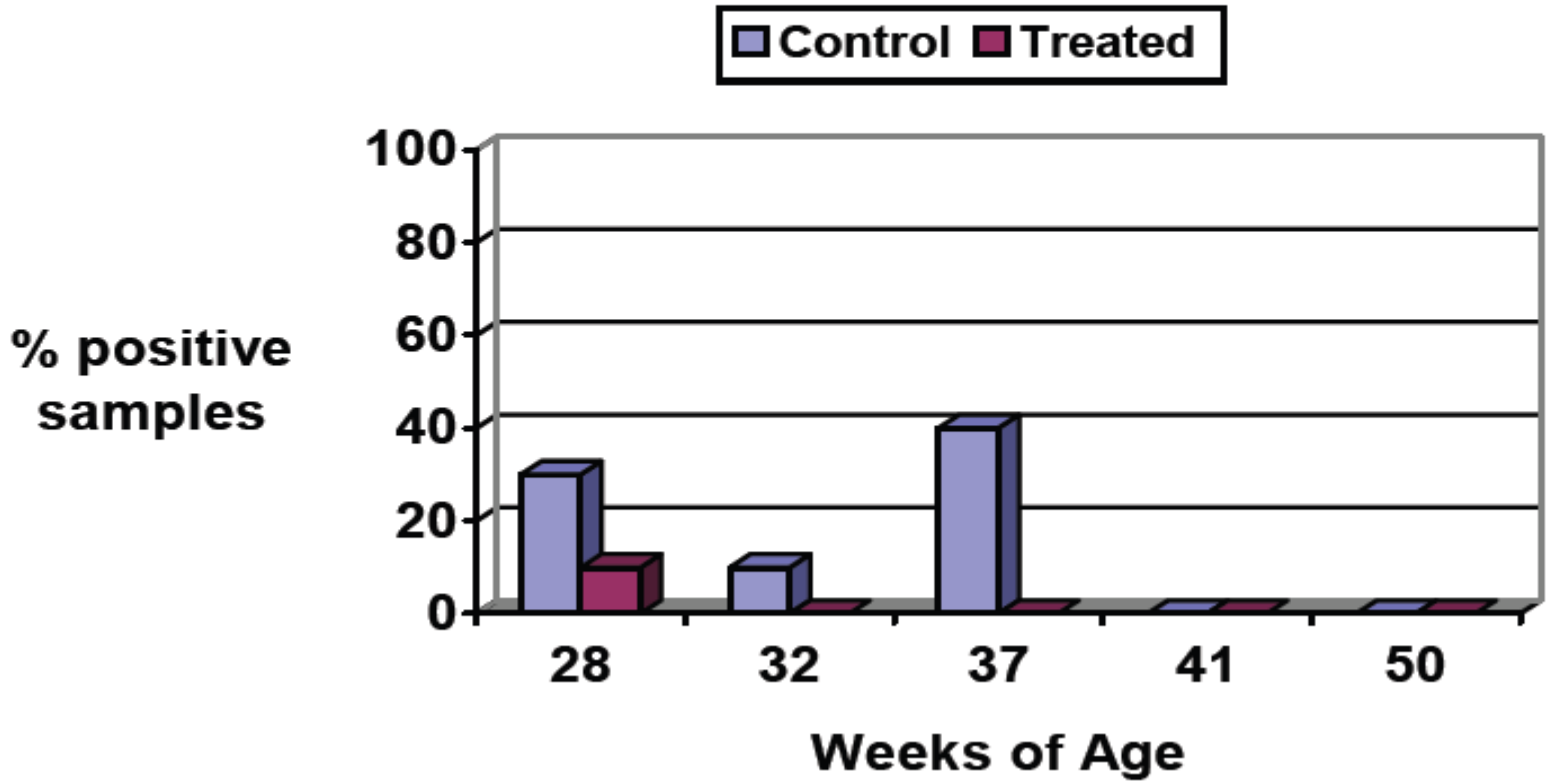
MicroTreat P litter treatment.

Figure 1. *Salmonella* positive litter samples collected from each turkey breeder grow house.



MicroTreat P litter treatment.

Figure 2. Mean *Salmonella* positive litter samples collected from each of two turkey breeder laying houses.



MicroTreat P litter treatment.

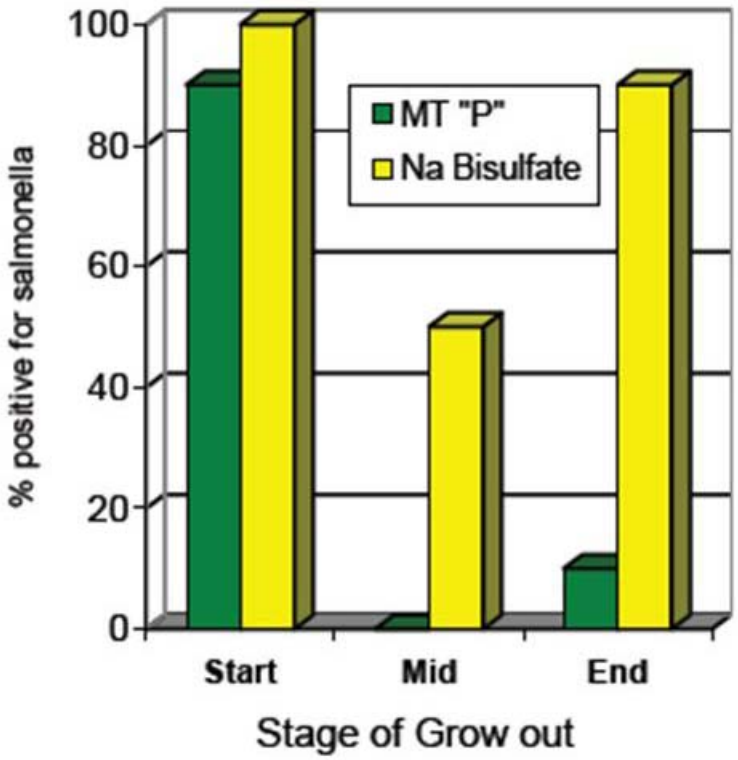


Figure 1. The effect of MicroTreat "P" on salmonella incidence over grow out phase.

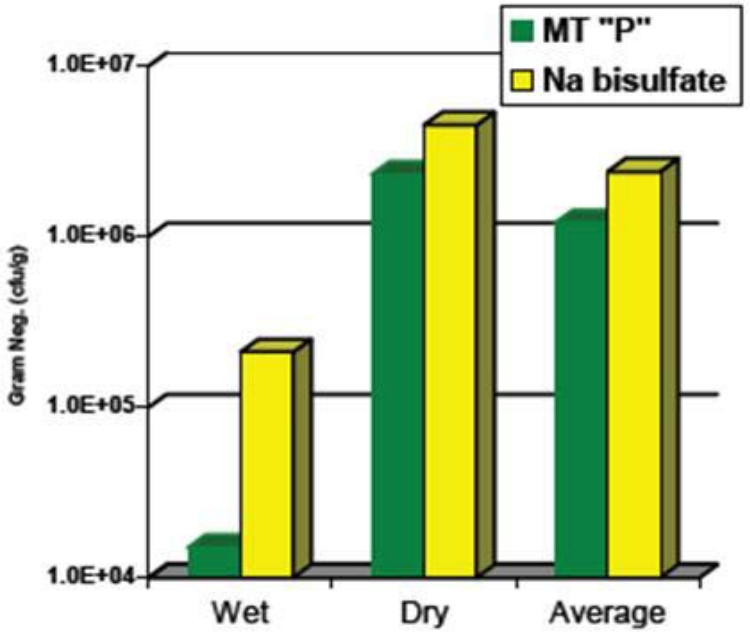
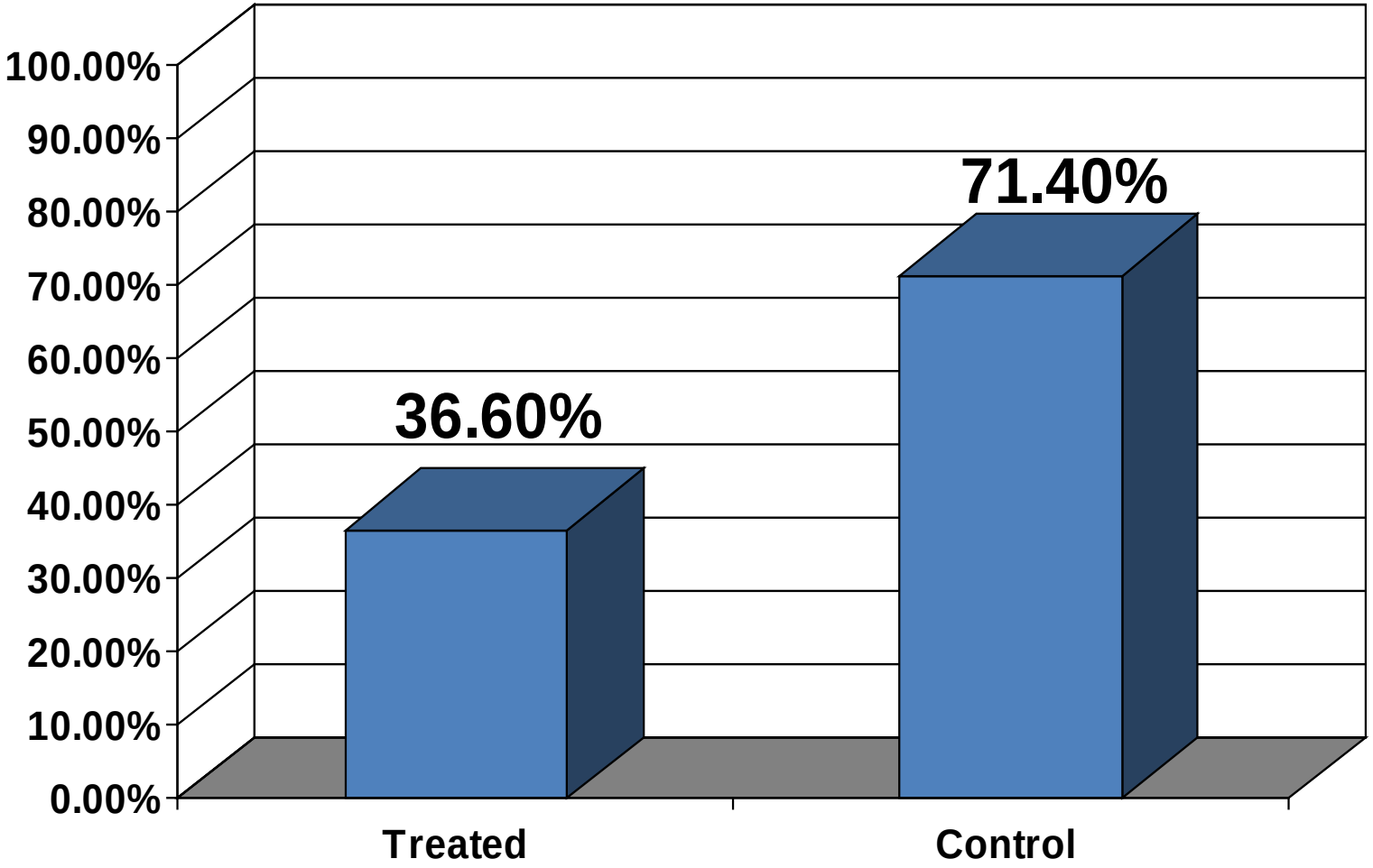


Figure 2. The effect of MicroTreat "P" on total gram negative population

MicroTreat "P" was applied to litter in a 500 x 76' finishing house containing 10,000 toms to determine its effect on the presence of salmonella. A second house also containing 10,000 was treated with a sodium bisulfate product. Both products were applied at manufacturer's instructions. The field trial was

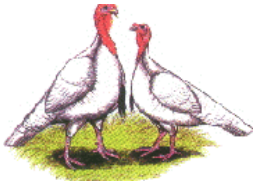
Salmonella Boot Swab Results

Average of all five sites



A total of five sites, two houses per site per treatment and six drag swabs per house.

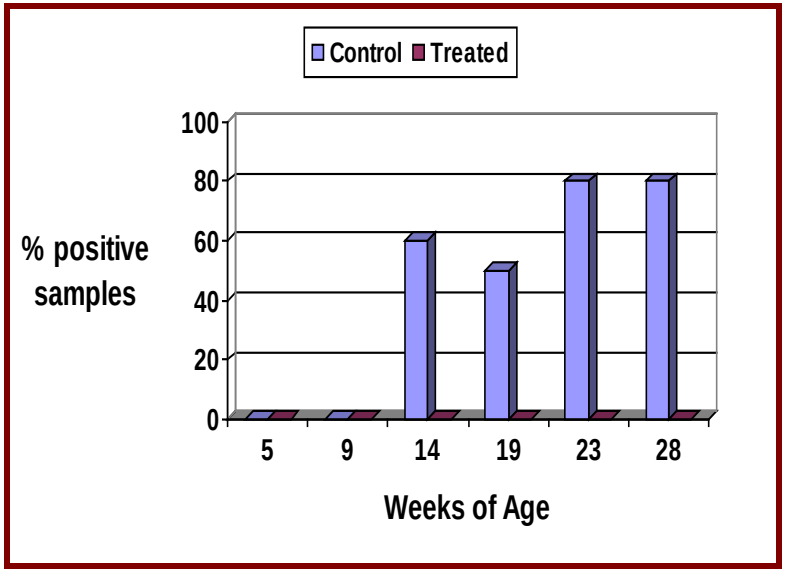
Salmonella frequency in *MicroTreat P* treated turkey breeder farm litter.



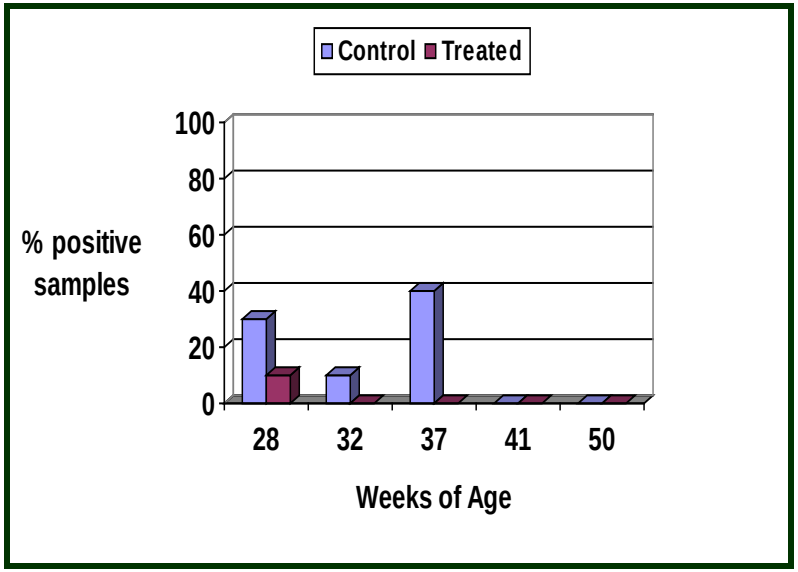
Turkey Breeders

Control — *5-28 wks.* — **Treated**

Control Breeder House 1	Control Layer House 2	*50 wks.*	Treated Breeder House 1	Treated Layer House 2

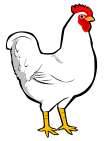


Breeder



Layer

Reduced *Salmonella* carriage in CSI-DFM
(3-strain *Bacillus* spp.) treated broiler growout farms.



Broiler Farms

3 West Farms

2 Consecutive Flocks
~7-8 wks/flock

2 East Farms

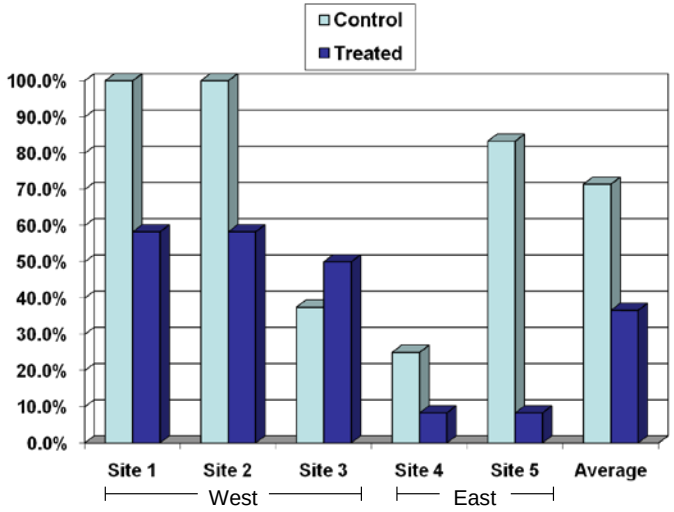
2 CSI Trt Flocks

2 Control Flocks

2 CSI Trt Flocks

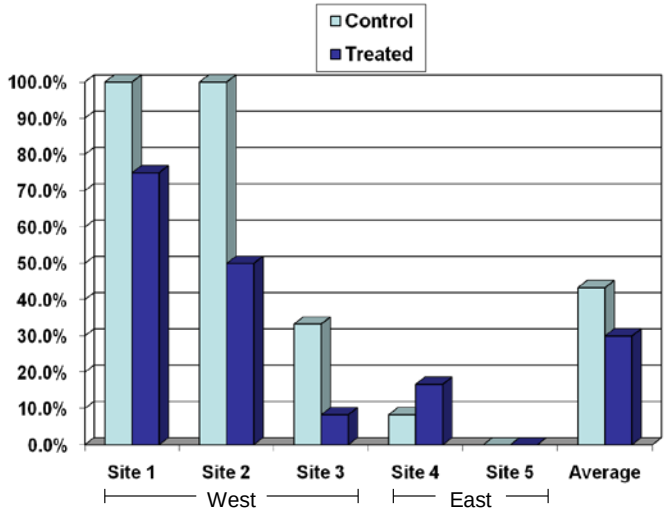
2 Control Flocks

Figure 1. Feeding trial Flock 1, percent of confirmed *Salmonella.*



Growout Flock 1

Figure 2. Feeding trial flock 2, percent of confirmed *Salmonella.*



Growout Flock 2

Campylobacter in turkeys

Percent *Campylobacter* Positive Samples by Treatment					
		End of Finishing (1 Flock)			
Regimen	Pre-placement Cecal contents	Pre-transport Cloacal Swab	Post-transport Cloacal Swab	Carcass Rinse	Total Positives
Antibiotic fed	15.7% (17/108)	35.0% (7/20)	15.0% (3/20)	16.7% (10/60)	17.8% (37/208)
On-Avicorr	9.2% (11/120)	30.0% (6/20)	40.0% (8/20)	8.6% (6/70)	13.5% (31/230)
P-value	NS (0.16)	NS (1.00)	NS (0.16)	NS 0.19)	NS (0.24)

Fisher's exact test (Statistically significant-alpha<0.05)

	Farm	Pre-Treatment + (out of 8 swabs)	Post-Treatment + (out of 8 swabs)	Pre-Treatment Infection Rate (%)	Post-Treatment Infection Rate (%)	Percent Decrease
FMB 11™	Farm A	8	7	100	87.5	12.5
	Farm B	8	8	100	100	0
	Farm C	8	8	100	100	0
	Farm D	6	2	75	25	50
	Farm E	8	4	100	50	50
	Average	7.600	5.800	95.000	72.500	22.500
	Farm	Pre-Treatment + (out of 8 swabs)	Post-Treatment + (out of 8 swabs)	Pre-Treatment Infection Rate (%)	Post-Treatment Infection Rate (%)	Percent Decrease
Avicorr	Farm A	6	1	75	12.5	62.5
	Farm B	8	8	100	100	0
	Farm C	8	3	100	37.5	62.5
	Farm D	8	3	100	37.5	62.5
	Farm E	8	8	100	100	0
	Average	7.600	4.600	95.000	57.500	37.500

Figure 2. Treatment chart indicating pre- and post-treatment results. Highlighted rows designate flocks that required antibiotic treatment during the trial.

Farm	Pre-Treatment + (out of 8 swabs)	Post-Treatment + (out of 8 swabs)	Pre-Treatment Infection Rate	Post-Treatment Infection Rate	Percent Decrease
Farm A	8	7	100	87.5	12.5
Farm B	8	8	100	100	0
Farm C	8	8	100	100	0
Farm D	6	2	75	25	50
Farm E	8	4	100	50	50
Farm A	6	1	75	12.5	62.5
Farm B	8	8	100	100	0
Farm C	8	3	100	37.5	62.5
Farm D	8	3	100	37.5	62.5
Farm E	8	8	100	100	0
Average	**7.600**	**5.200**	**95.000**	**65.000**	**30.000**
Farm	**Pre-Treatment + (out of 8 swabs)**	**Post-Treatment + (out of 8 swabs)**	**Pre-Treatment Infection Rate**	**Post-Treatment Infection Rate**	**Percent Decrease**
Farm A	8	7	100	87.5	12.5
Farm B	8	8	100	100	0
Farm D	6	2	75	25	50
Farm E	8	4	100	50	50
Farm A	6	1	75	12.5	62.5
Farm B	8	8	100	100	0
Farm D	8	3	100	37.5	62.5
Average	**7.429**	**4.714**	**92.857**	**58.929**	**33.929**

Figure 3. Treatment chart indicating use of either probiotic. Highlighted rows designate flocks that required antibiotic treatment. The second set of numbers compiles all the flocks that did not require antibiotic treatment.

Effect of xylanase and a blend of essential oils on performance and *Salmonella* colonization of broiler chickens challenged with *Salmonella* Heidelberg

A. M. Amerah,*[1] G. Mathis,† and C. L. Hofacre‡

Table 3. Effect of essential oils (100 g/t) and xylanase supplementation (2,000 U/kg of feed) on *Salmonella* prevalence

Item	Unchallenged control	Challenged control	Essential oils	Xylanase	Essential oils + xylanase
Salmonella-positive cecal samples on d 42 (%)[1]	0	32.5[a]	7.5[b]	12.5[b]	7.5[b]
Positive drag swab samples at 14 d (%)[2]	0	100[a]	87.5[ab]	87.5[ab]	62.5[b]
Positive drag swab samples at 42 d (%)[2]	0	100[a]	62.5[b]	100[a]	62.5[b]

[a,b]Values in a row not sharing a common superscript are significantly different ($P < 0.05$).

[1]Each value represents the percentage of *Salmonella*-positive cecal samples from 40 replicates (5 unchallenged birds/replicate pen).

[2]Each value represents the percentage of positive drag swabs of 8 replicates.

Table 2. Weight gain, feed intake, and feed conversion ratio (FCR) of broilers as influenced by xylanase supplementation (2,000 U/kg of feed) and essential oils (100 g/t)[1]

Item	Control	Challenged control	Essential oils	Xylanase	Essential oils + xylanase	SEM[2]
1–21 d						
Weight gain (g)	422[c]	419[c]	442[b]	469[a]	461[a]	5.7
Feed intake (g)	658	659	649	659	644	7.1
FCR	1.56[a]	1.58[a]	1.47[b]	1.41[c]	1.40[c]	0.01
21–42 d						
Weight gain (g)	1,360[c]	1,381[c]	1,481[b]	1,615[a]	1,681[a]	29
Feed intake (g)	2,994	3,047	3,072	3,088	3,109	77
FCR	2.20[a]	2.21[a]	2.07[b]	1.91[c]	1.85[c]	0.03
1–42 d						
Weight gain (g)	1,781[c]	1,800[c]	1,924[b]	2,084[a]	2,142[a]	27
Feed intake (g)	3,652	3,706	3,721	3,747	3,753	70
FCR	2.01[a]	2.01[a]	1.90[b]	1.78[c]	1.73[c]	0.02

Amerah et al Poultry Sci 2012

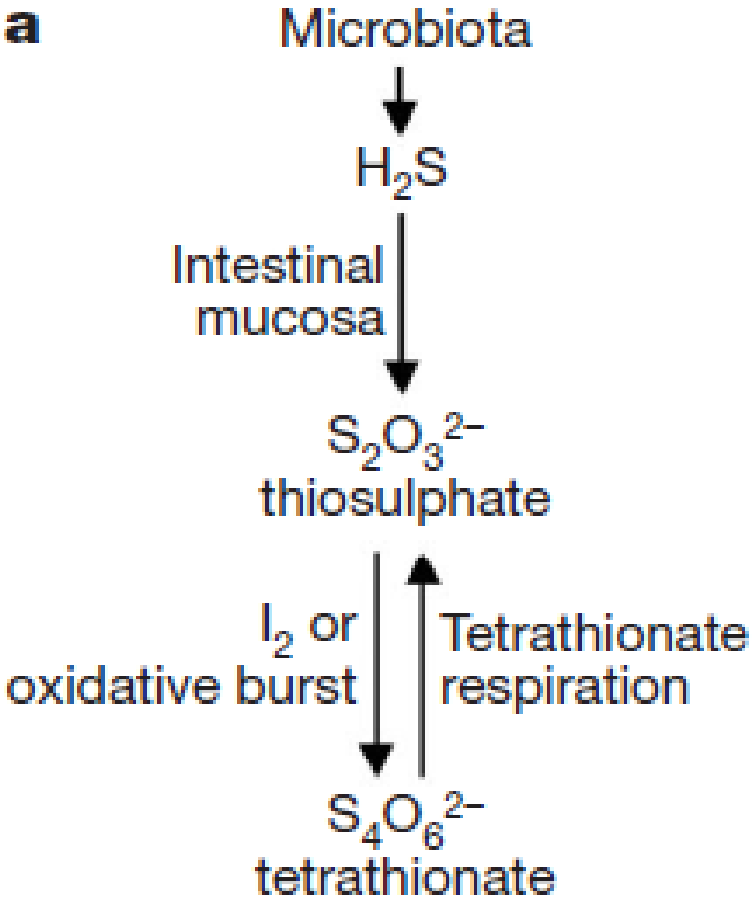
Promising areas of pursuit for DFM and Potential Nutritional Reductions of *Salmonella* in the avian GI tract pre-processing.

- Identify antagonists with in-situ inhibitory capability
- Identifying factors which provide *Salmonella* an evolutionary advantage in the gut. (2 examples)
- Redefine reductions as being actual levels vs presence/absence. (qPCR Assays)

Siragusa, 2014, Opinion

Gut inflammation provides a respiratory electron acceptor for *Salmonella*

Sebastian E. Winter[1], Parameth Thiennimitr[1,2], Maria G. Winter[1], Brian P. Butler[1], Douglas L. Huseby[3], Robert W. Crawford[1], Joseph M. Russell[1], Charles L. Bevins[1], L. Garry Adams[4], Renée M. Tsolis[1], John R. Roth[3] & Andreas J. Bäumler[1]



Winter et al NATURE 2010

Gut inflammation provides a respiratory electron acceptor for *Salmonella*

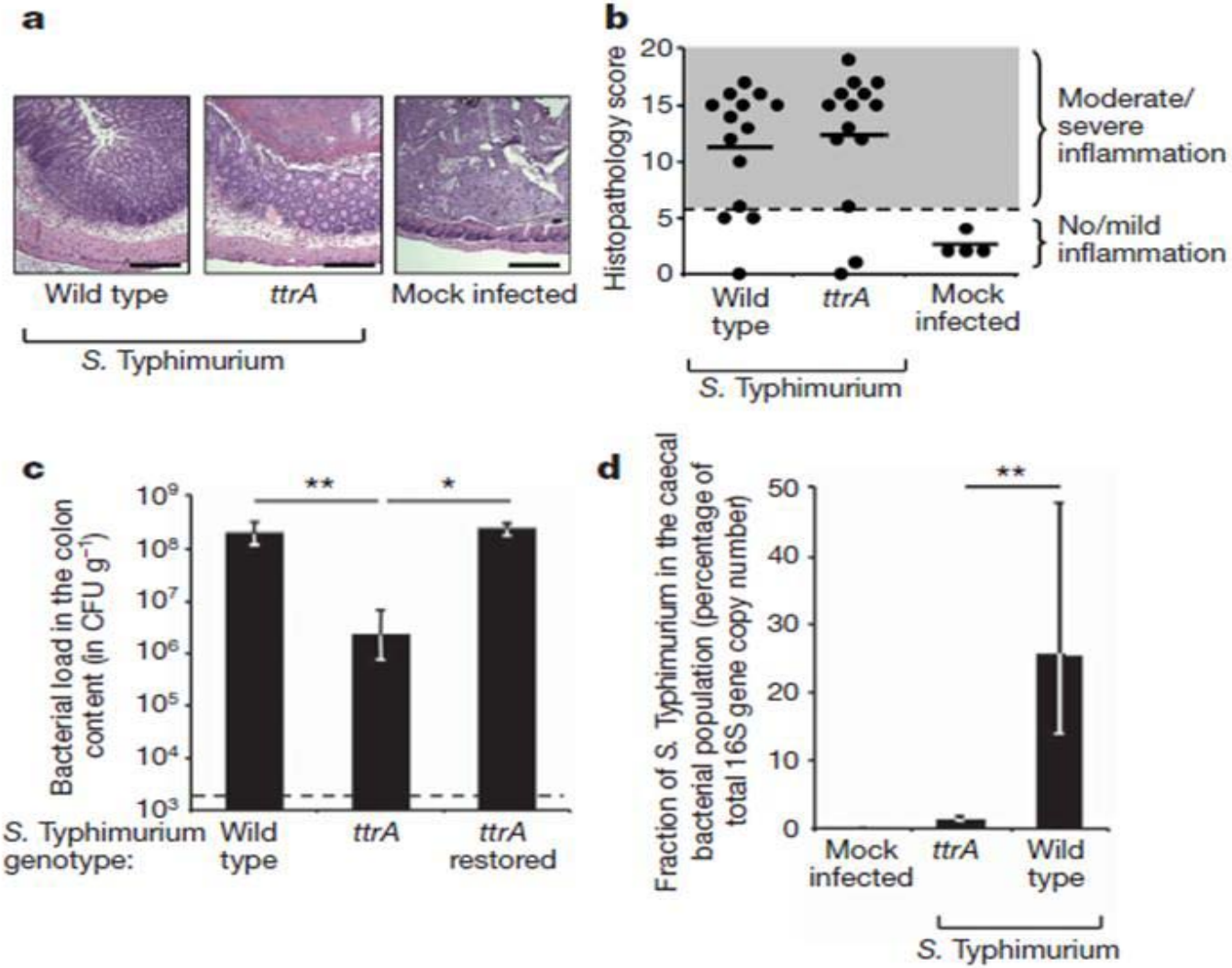


Figure 4 | Tetrathionate respiration increases the abundance of *S.* Typhimurium in the intestinal lumen.

Winter et al NATURE 2010

Salmonella Typhimurium's Transthyretin-Like Protein Is a Host-Specific Factor Important in Fecal Survival in Chickens

Sarah C. Hennebry[1,3]*, Leanne C. Sait[2], Raju Mantena[3], Thomas J. Humphrey[2], Ji Yang[3], Timothy Scott[3], Andreas Kupz[3], Samantha J. Richardson[1,4], Richard A. Strugnell[3]

These data demonstrate that the TLP gene is required for survival of S. Typhimurium in a high uric acid environment such as chicken faeces, and that metabolic traits of Salmonellae in natural and contrived hosts may be fundamentally different.

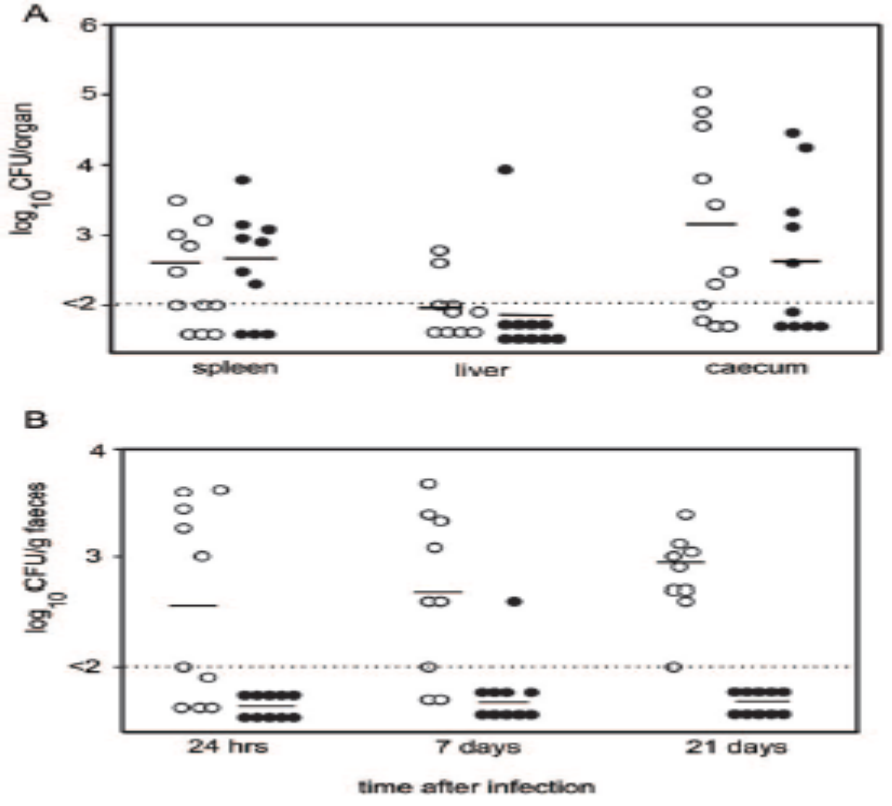


Figure 6. Survival of SL1344 and SL1344 Δ*yedX* in poultry organs and faeces following oral inoculation. A. Numbers of

Hennebry et al PLOSOne, 2012

Promising areas of pursuit for DFM and Potential Nutritional approaches to reduce *Salmonella* in the avian GI tract pre-processing.

- Redefine reductions/increases as being actual levels vs presence/absence. (qPCR Assays)
- Determine the microbiomes of several thousand broiler gut sections (DJI and ceca) across feeds, ages, geographies and disease state.
- Identify antagonists using high-throughput in-situ inhibitory capability.
- Identifying factors which provide *Salmonella* an evolutionary advantage in the gut. (2 examples)

Siragusa, 2014, Opinion

Thank you.

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