An Update of Research at the National Animal Disease Center

Shawn Bearson
Heather Gerbyshak
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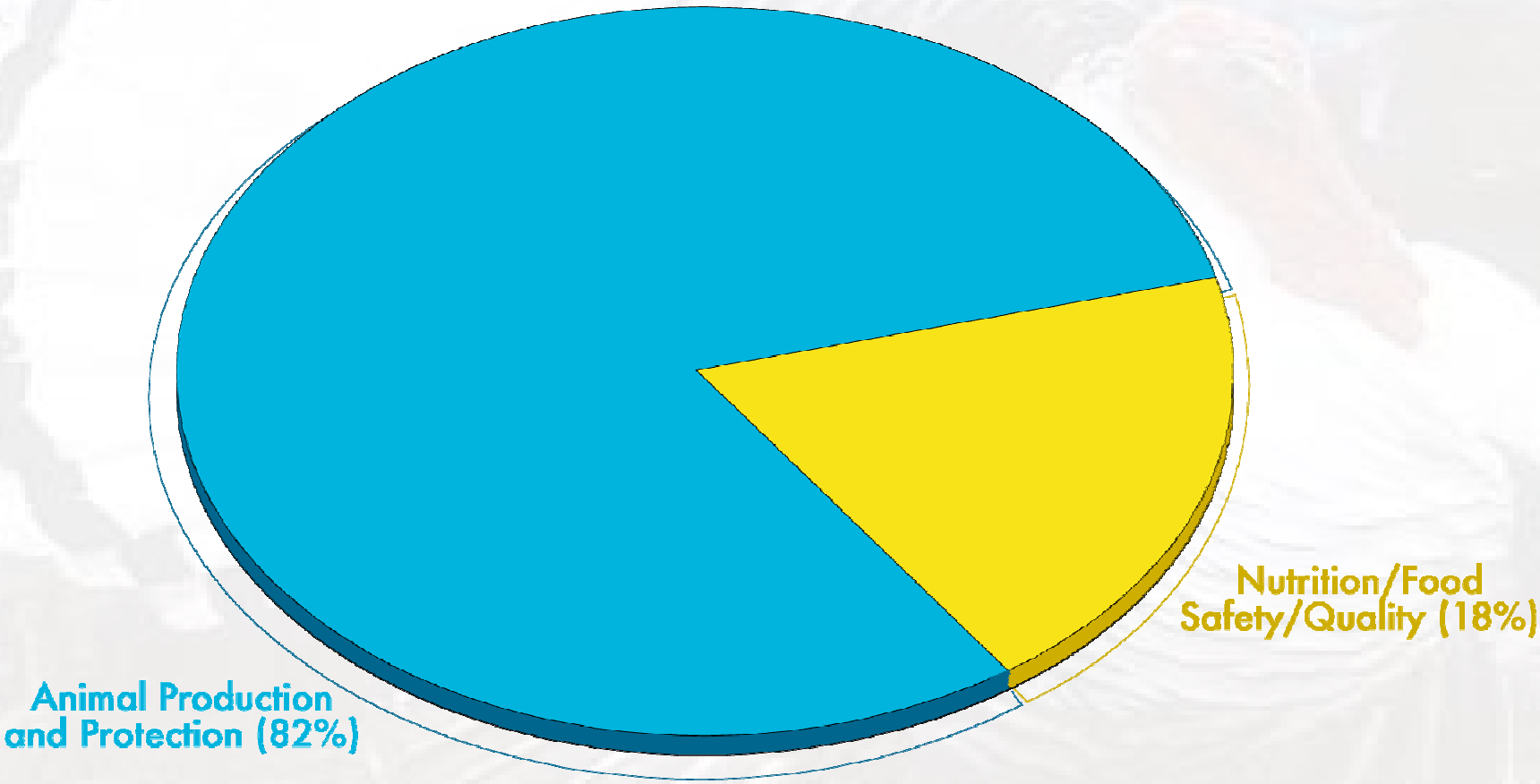
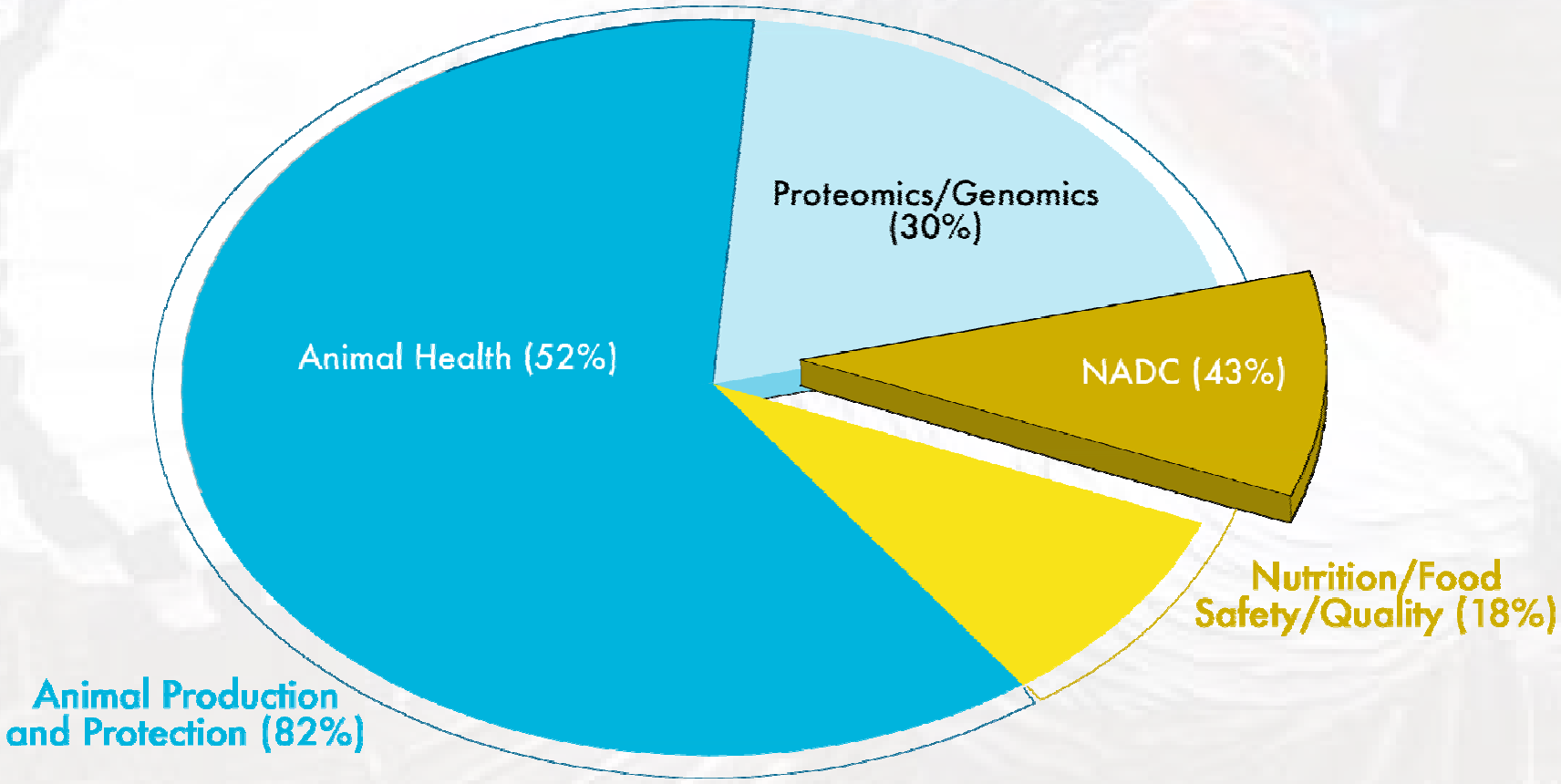
Midwest Turkey Producers and Processors

ARS Turkey Research



Food Safety


Proteomics/Genomics (30%)
Animal Health (52%)
NADC (43%)
Nutrition/Food Safety/Quality (18%)
Animal Production and Protection (82%)

Keeping an eye on food safety

U.S. plans testing at turkey plants to reduce cases of salmonella

By PHILIP BRASHER
REGISTER WASHINGTON BUREAU

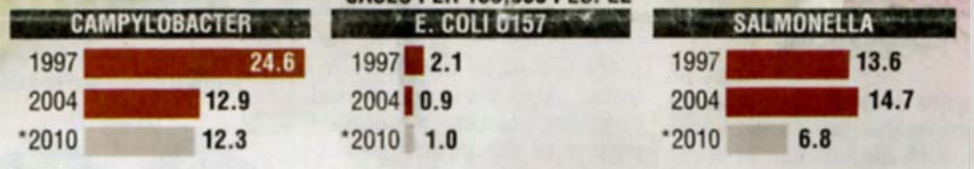
Washington, D.C. — The government wants to make that turkey drumstick a little safer.

The U.S. Agriculture Department plans to start testing for salmonella bacteria in plants that process turkeys.

Food safety

In 1997, the U.S. government set a goal to reduce the number of food-borne illness cases by 50 percent. A look at the progress made:

CASES PER 100,000 PEOPLE



* Goal

Source: U.S. Centers for Disease Control and Prevention

THE REGISTER

Turkey
FSIS Nationwide Baseline, 1997

	CARCASS	GROUND
C. perfringens	29.2%	28.1%
S. aureus	66.7%	57.3%
L. monocytogenes	5.9%	30.5%
C. jejuni/C. coli	90.3%	25.4%
E. coli 0157:H7	0%	0%
Salmonella	18.6%	49.9%

Salmonella associated human illnesses have remained above public health targets of Centers for Disease Control (CDC)… *E. coli* O157:H7, *Campylobacter* and *Listeria* associated enteritis … have been significantly reduced in recent years and are approaching Healthy People 2010 goals. FSIS and other regulatory agencies have … made reduction of *Salmonella* a priority. *Salmonella* outbreaks are primarily associated with poultry, in addition to meat animals and produce. There is a significant need for data to support hazard evaluation and risk assessment of bacterial pathogens from the complex poultry production and processing industry and to provide intervention technologies to help reduce prevalence of *Salmonella*.

Is holding of pigs at the abattoir a risk factor for *Salmonell*a infection?

8 studies, 1 message

- Studies
  - ✓ 1. Clean lairage study
  - ✓ 2. APEP - Accel. PRV Eradication Program
  - ✓ 3 & 4. Rapid infection experiments
  - ✓ 5. Environmental study
  - ✓ 6. Cull sow study
  - ✓ 7. Short hold and slatted floor study
  - ✓ 8. Truck hold study
- Message:
  - ✓ Holding pen is a significant risk for *Salmonella* infection in hogs prior to slaughter.

Where are critical control points within 24 hrs of turkey slaughter?

- ✓ On-farm after feed withdrawal?
- ✓ Loading on the truck?
- ✓Transport to abattoir (~3 hrs)?
- ✓Holding in the shed (~2 to 6 hrs)?

????

- ✓ Does prevalence increase in the final hours pre-slaughter?

- ✓ When?

FW/Transportation-*Salmonella*

BROILERS

1980- Increase in *Salmonella* in crated birds (Rigby et al)
1981- FW has no effect on carriage/shedding of *Salmonella* (Rigby et al)
1986- Stress increases peristalsis (Linton *et al*.)
1993- Increase in bacterial load with longer crating /holding (Renwick)
1995- Transport stress increases pathogens in hogs, poultry (Mulder)
1997- No change in *Salmonella* in ceca without yeast treatment (Line)
1997- Increase in *Salmonella* in crop not ceca (Ramirez)
1999- Increase in *Salmonella* in crop not ceca (Corrier)
2003- No change in *Salmonella* post-transport (Northcutt)

TURKEYS??

Do Feed Withdrawal, Livehaul, and Holding Impact the Prevalence of *Salmonella* and *Campylobacter* in Turkeys?

IV Wesley, WT Muraoka, E Harbaugh, D Trampel, M Rostagno, HS Hurd, F Rivera

National Animal Disease Center and Iowa State University

Materials & Methods

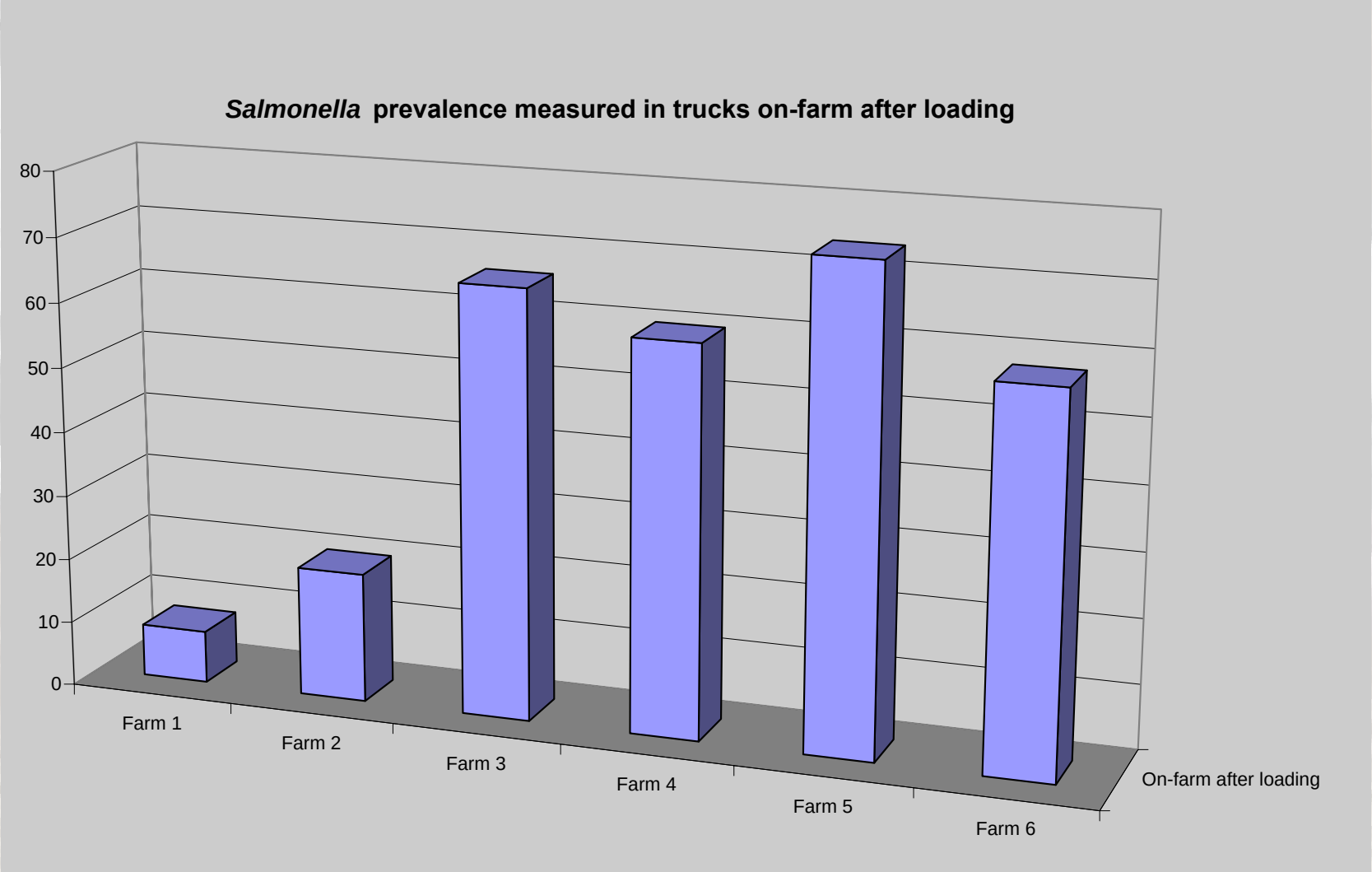
- 6 commercial turkey operations
- Samples cultured:
  - Barn environment (n=25 swabs)
  - Cage floors after loading (n=100)
  - Cage floors after transport & holding (n=100)
  - Cecum, crop, spleen (n=50 each)

Results

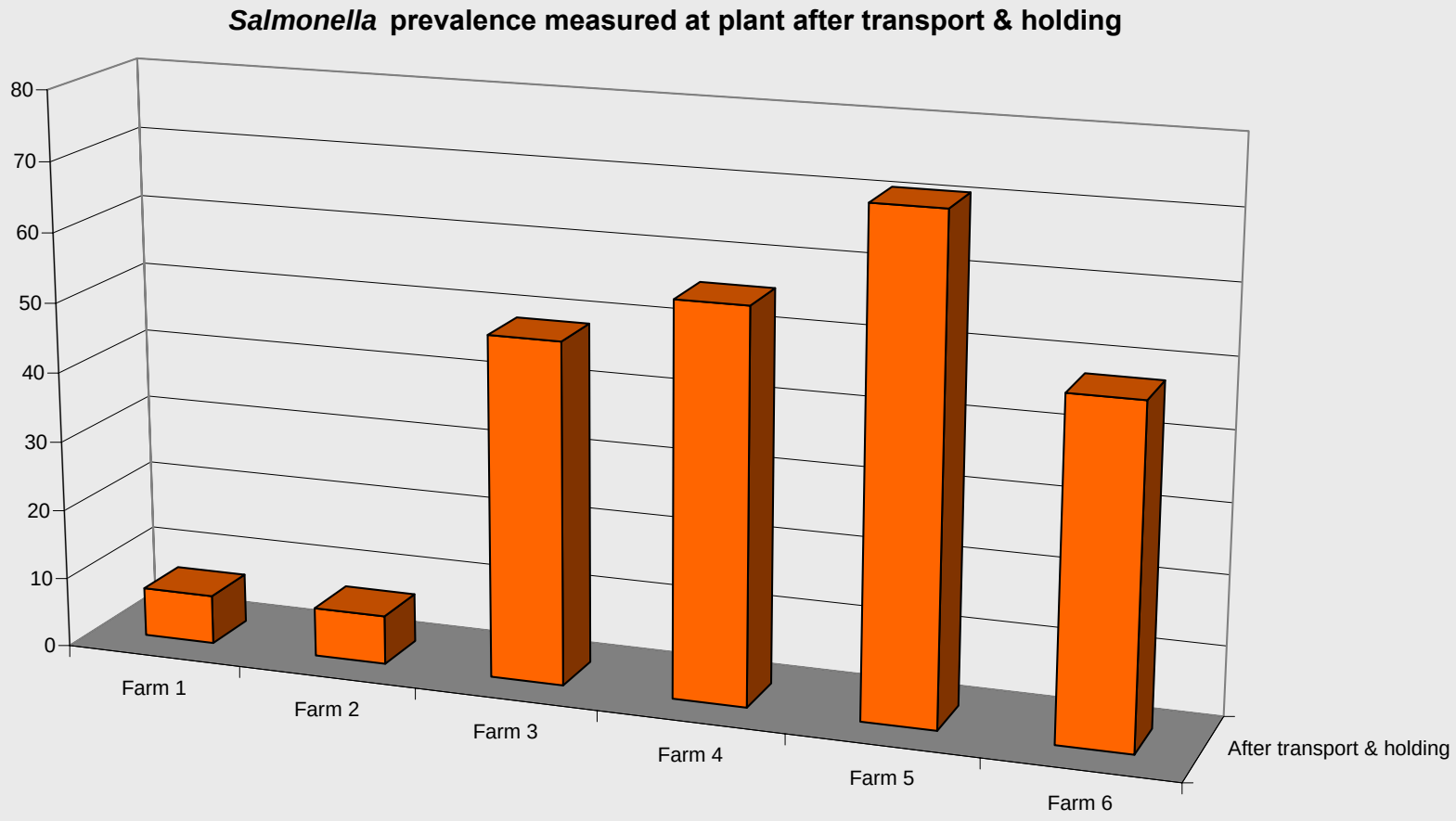
Summary: time off-feed (hrs)

Flock #	Loading	Transit	Holding	**TOTAL**
1	1.0	2.75	4.0	**6.75**
2	0.76	3.0	4.38	**8.14**
3	0.78	0.265	9.75	**10.78**
4	0.75	0.75	5.1	**6.6**
5	0.73	1.0	4.87	**6.6**
6	0.92	0.92	5.7	**7.62**
Mean	8.2 ± 0.11	1.45 ± 1.1	5.6 ±2.1	**7.8 ±1.6**
Range	7.3 – 1.0	0.3 -3.0	4.0 - 9.8	**5.6 - 10.8**

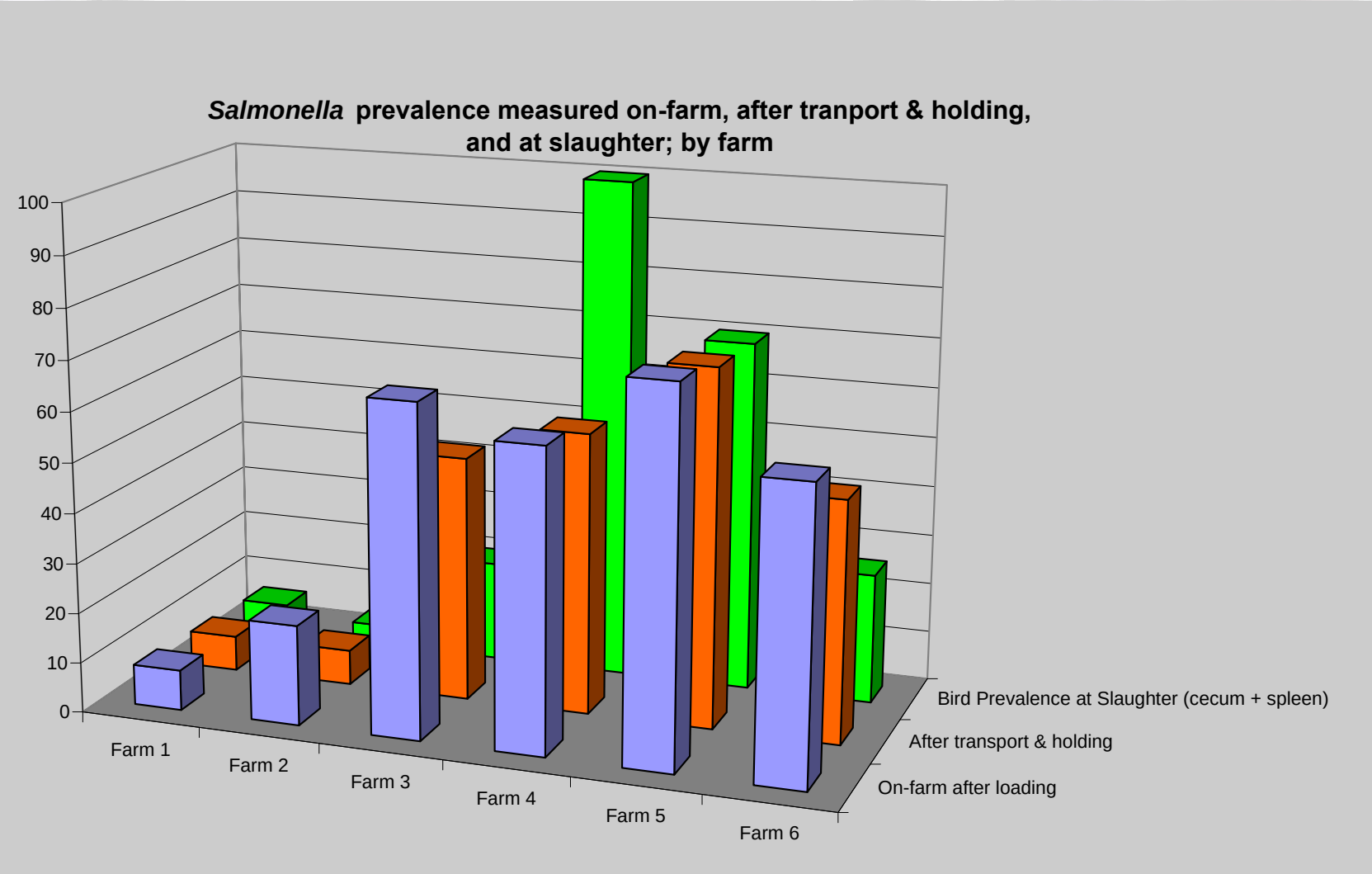
Cage floors pre- transport



Cage floors: Post-transport, holding



Summary



Wesley et al. 2006. J of Food Protection (in press)

Salmonella: Study Design

On-farm (n=30 birds)

Crop
Ceca
Spleen
Liver/Gall bladder
Cloacal loop
Colon

Abattoir (n=30 birds)

Crop
Ceca
Spleen
Liver/Gall bladder

S. enterica prevalence: On-farm vs. Abattoir

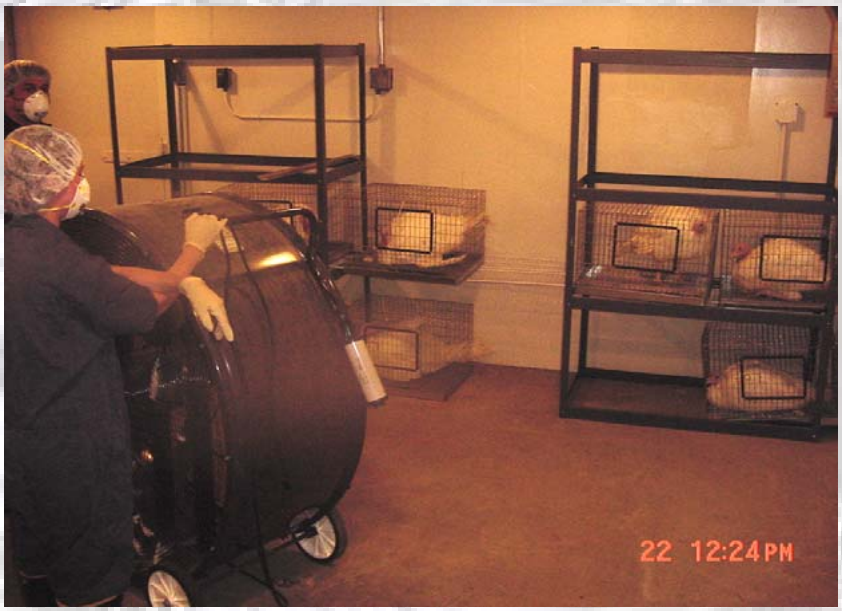
	On-farm							Abattoir				
Flock	Cloaca	L Int	Ceca	Crop	Spleen	Liver	Any	Ceca	Crop	Spleen	Liver	Any
A	3.3%	0%	6.7%	3.3%	0%	ND*	13.3%	10%	0%	0%	ND*	10%
B	33.3%	53.3%	36.7%	10%	46.7%	10%	83.3%	36.7%	14.3%	33.3%	45.5%	73.3%
C	37.9%	65.5%	96.7%	43.3%	33.3%	6.7%	96.7%	66.7%	10%	20%	13.3%	80%
D	0%	0%	0%	0%	0%	0%	0%	10%	0%	0%	16.7%	23.3%
E	0%	0%	6.7%	0%	0%	0%	6.7%	6.7%	3.3%	0%	3.3%	6.7%
F	0%	0%	0%	0%	0%	0%	0%	6.7%	0%	0%	0%	6.7%
Overall	22/179	35/179	44/180	17/180	27/180	5/150	60/180	41/180	8/178	16/178	20/142	60/180
	12.3%	19.6%	24.4%	9.4%	15%	3.3%	33.3%	22.8%	4.5%	9%	14.1%	33.3%

*ND = Not Done.

Rostagno et al. 2006. Poultry Science (in press)

Is the TURKEY shed = HOG holding pen?

Materials and Methods

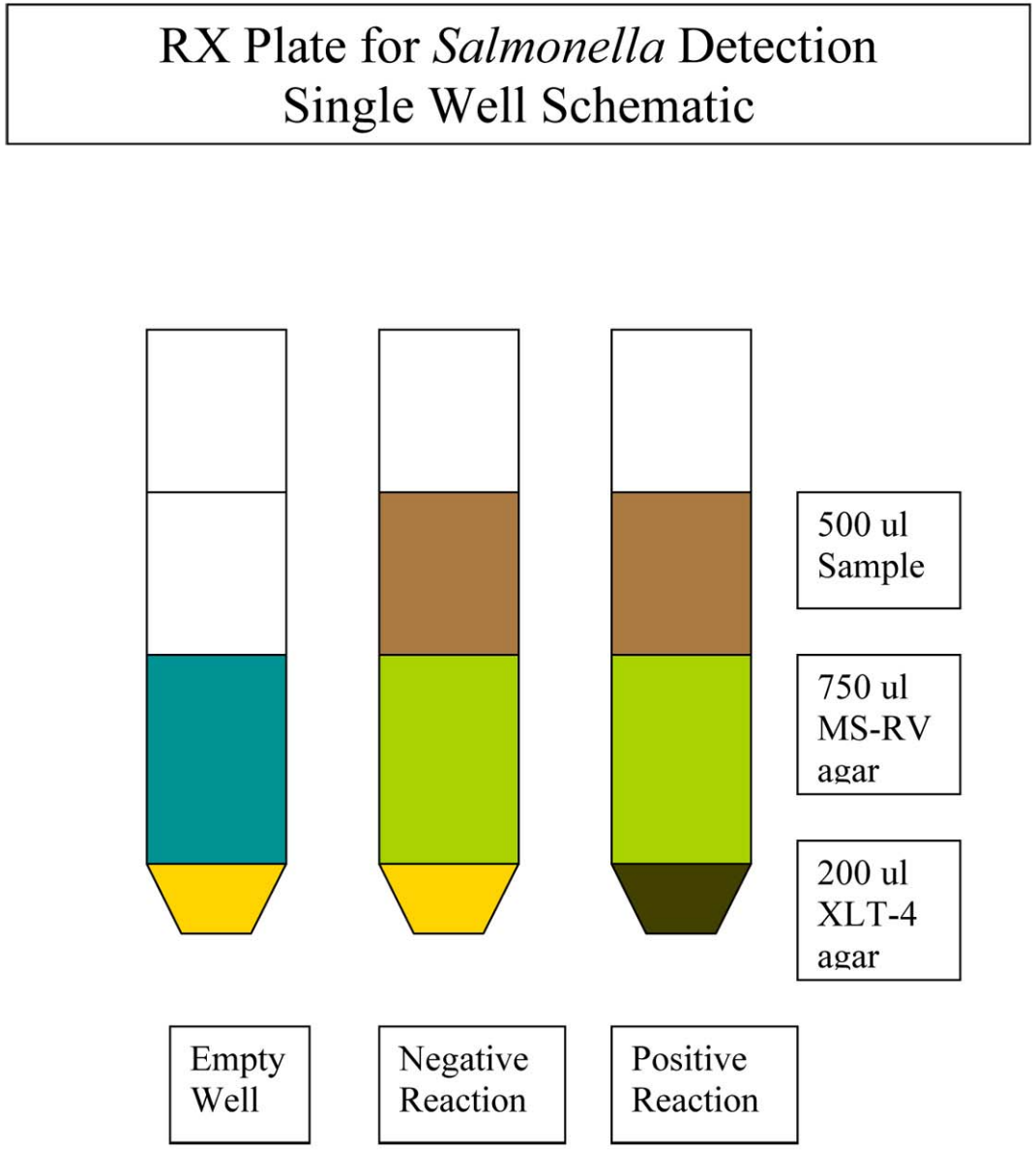


- ✓ Inoculate 15 birds with “marked” *Salmonella*
- ✓ 24 hours later negative birds into room
- ✓ Place groups 6-8 feet apart
- ✓ Large fan (56”) between
- ✓ Necropsy at 2 and 4 hours after exposure

Salmonella (+) samples 4 hrs post exposure to contaminated fecal dust

Exposure Dose	Nasal Passages	Infra-Orbital Sinuses	Trachea	Lungs	Air Sacs	Ceca	Small Intestine	Spleen	# Of Birds With Any Positve Sample
$1.2X10^9$ cfu/gm	4/4	4/4	2/4	4/4	0/4	3/4	4/4	0/4	4/4
$2.6X10^7$ cfu/gm	2/4	1/4	2/4	4/4	0/4	1/4	3/4	0/4	4/4
$2.6X10^5$ cfu/gm	0/8	0/8	1/8	3/8	1/8	2/8	4/8	0/8	6/8

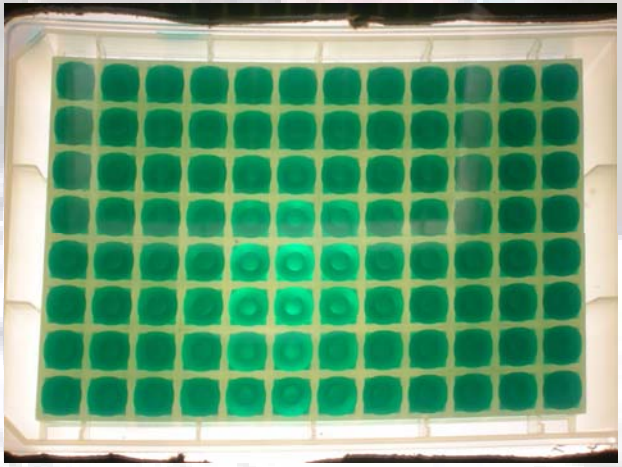
Harbaugh et al. 2006. Poultry Science (in press)



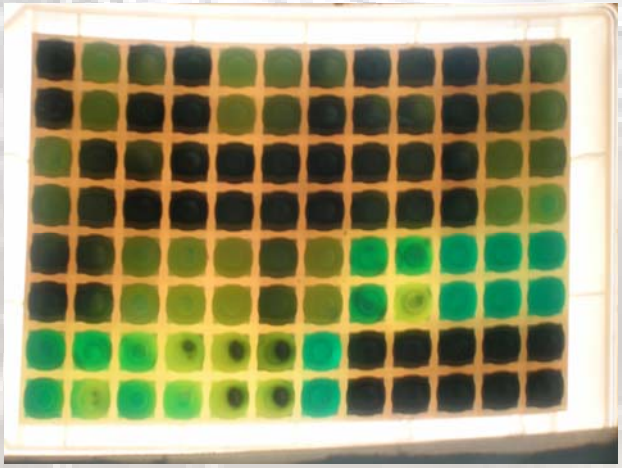
Gailey et al. 2006. (submitted)

Microtiter detection: *Salmonellla*

- Uninoculated 96-deep well plate



- Inoculated with caecal contents (48 hrs)



Transport-*Campylobacter*

BROILERS

1986- Stress increases peristalsis (Linton)

1995- Fewer *Campylobacter* when slaughtered on-farm (12.1%) vs. abattoir (56.7%) (Stern)

2001-Increase in *Campylobacter*/gram post-transport (Whyte)

2001-Crates contaminate broilers (Newell)

2002- Catching and crating increase contamination (Slader)

2003- Feed withdrawal increases *Campylobacter* on older carcasses (56 days; Northcutt)

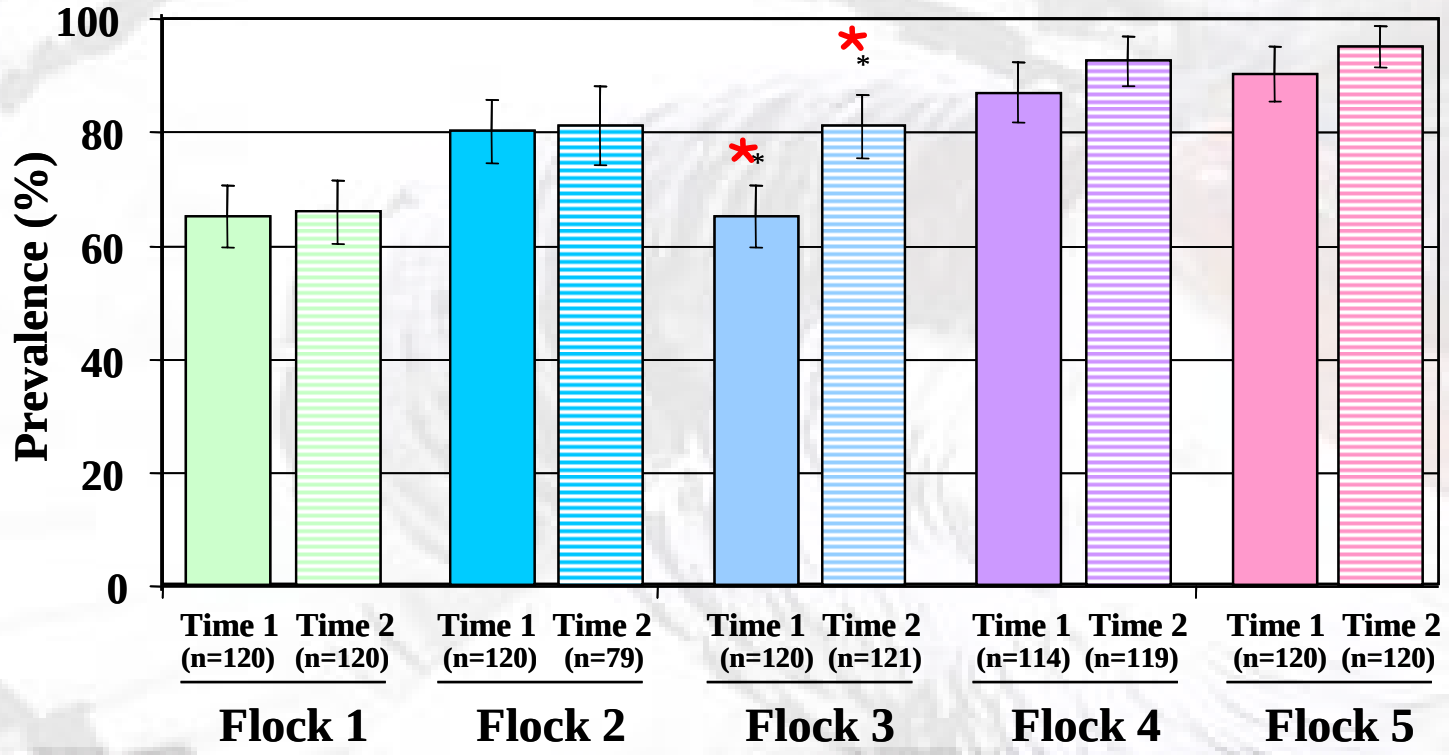
TURKEYS

2005-*C. jejuni* and *C. coli* shifts associated with transport/holding (Wesley *et al* 2005)

Materials and Methods

Cloacal swabs on farm (T_1, n~120/trial)

Cloacal swabs at abattoir (T_2, n~120/trial)



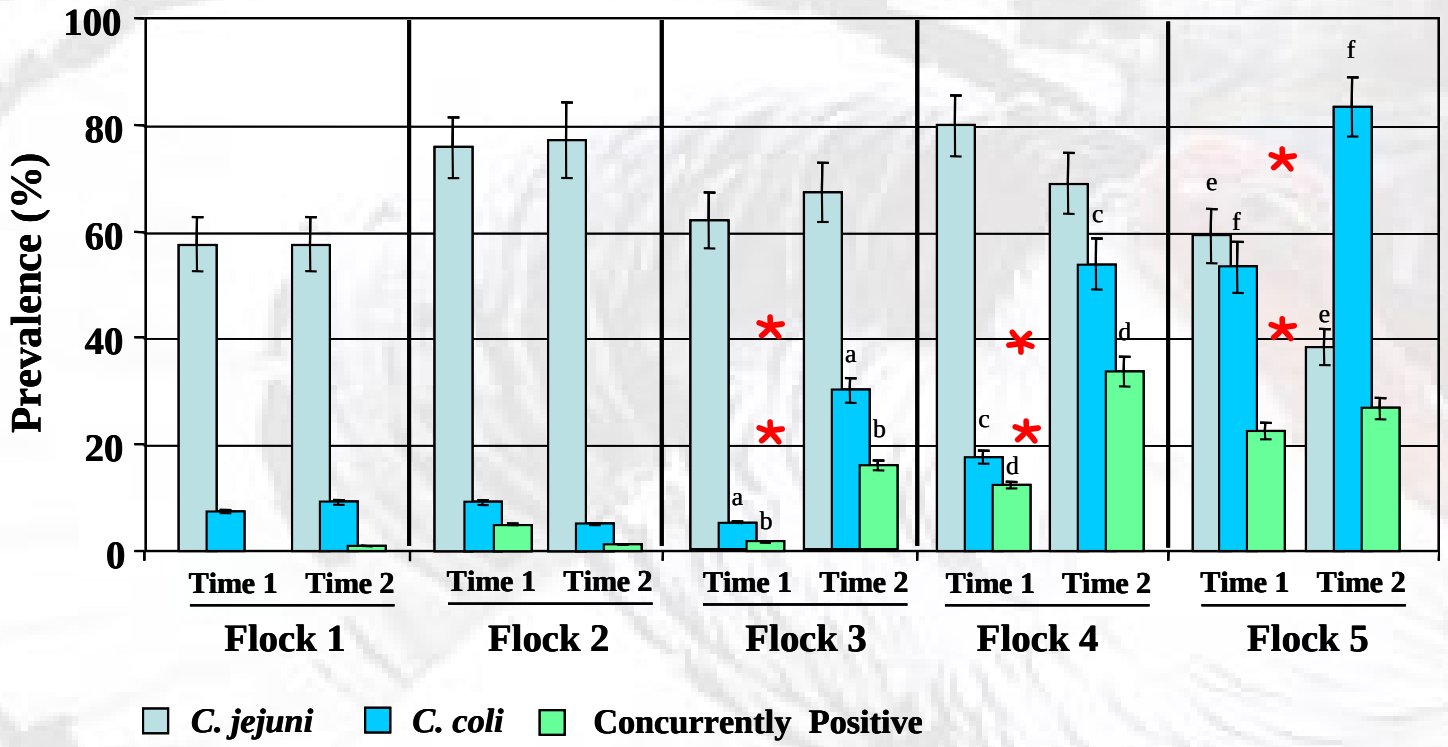
Prevalence of *Campylobacter spp*. in five Midwestern turkey flocks as determined by cloacal swabs.

Time 1 = prevalence prior to loading into transport cages (on- farm)

Time 2 = prevalence after transport and holding (at the abattoir)

Error bars indicate 95% Confidence Interval (precision of prevalence estimate).

* Indicates a significant difference ($P < 0.01$) between Time 1 and Time 2.



Prevalence of *C. jejuni*, *C. coli*, and concurrently positive turkeys

Time 1=at the farm
Time 2 after transport and holding at the abattoir

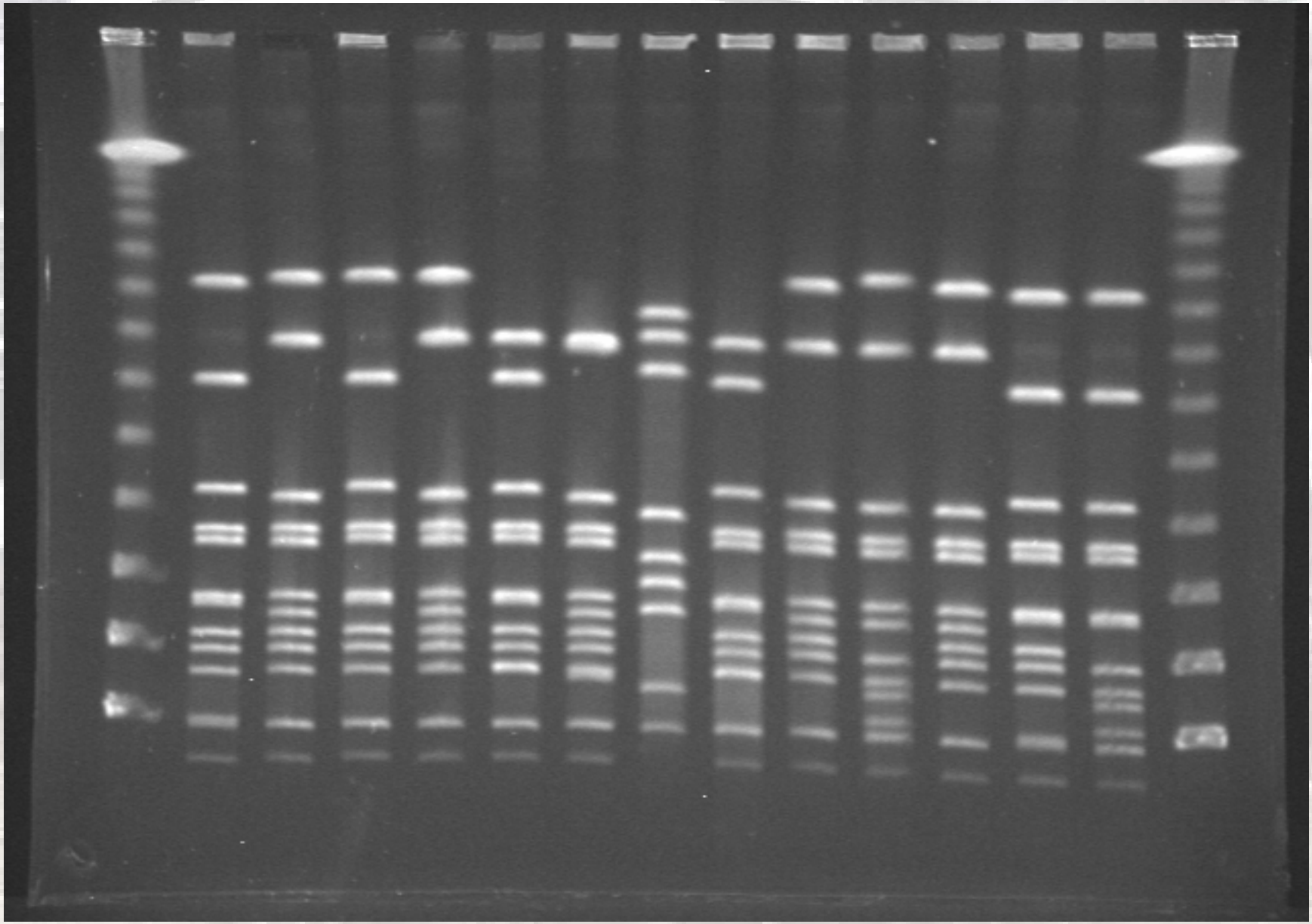
Error bars indicate 95% Confidence Interval (precision of prevalence estimate). Error bars with the same letter indicate a significant difference
* ($P < 0.01$) in *Campylobacter* prevalence between T1 and T2.

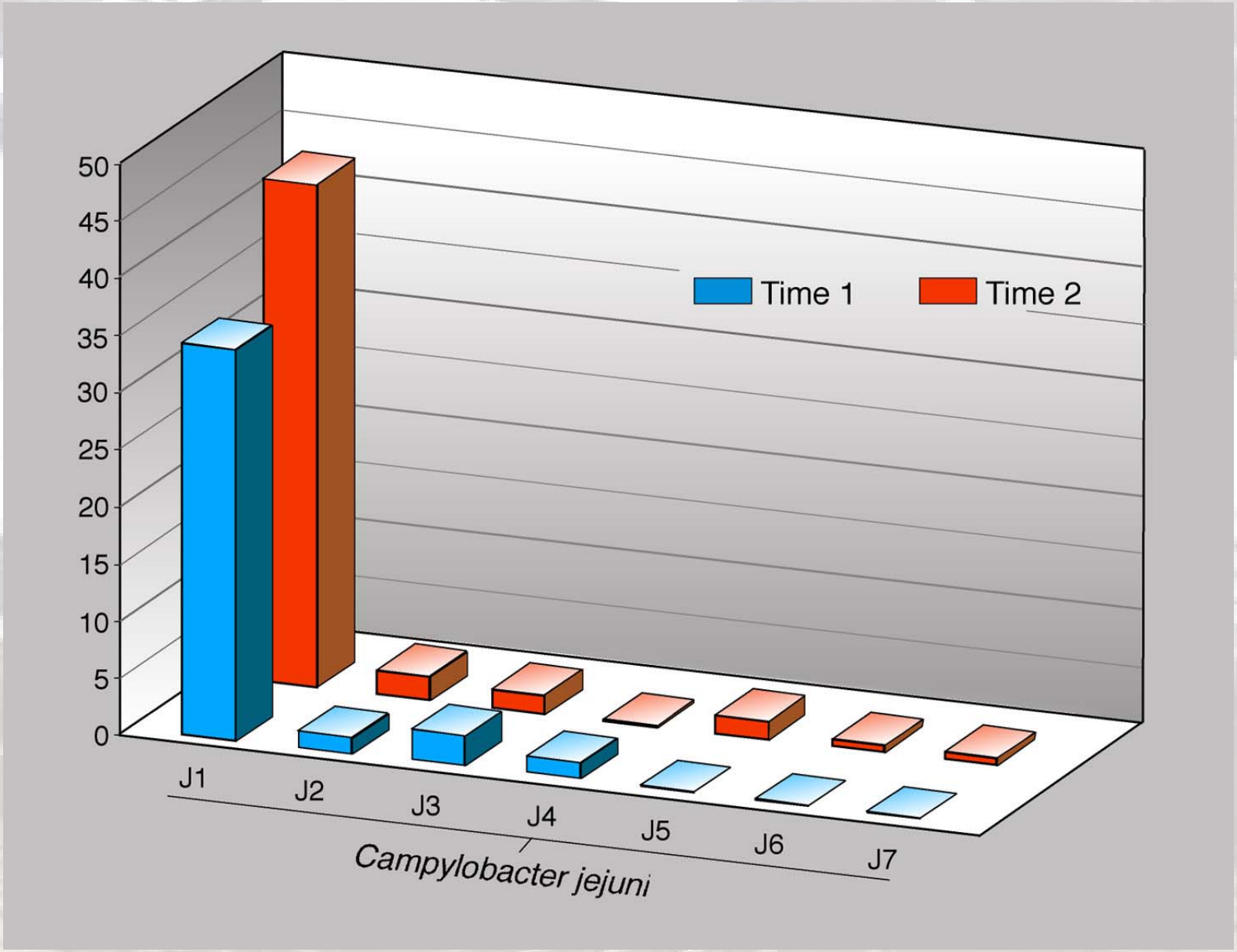
Are all *C. coli* alike?

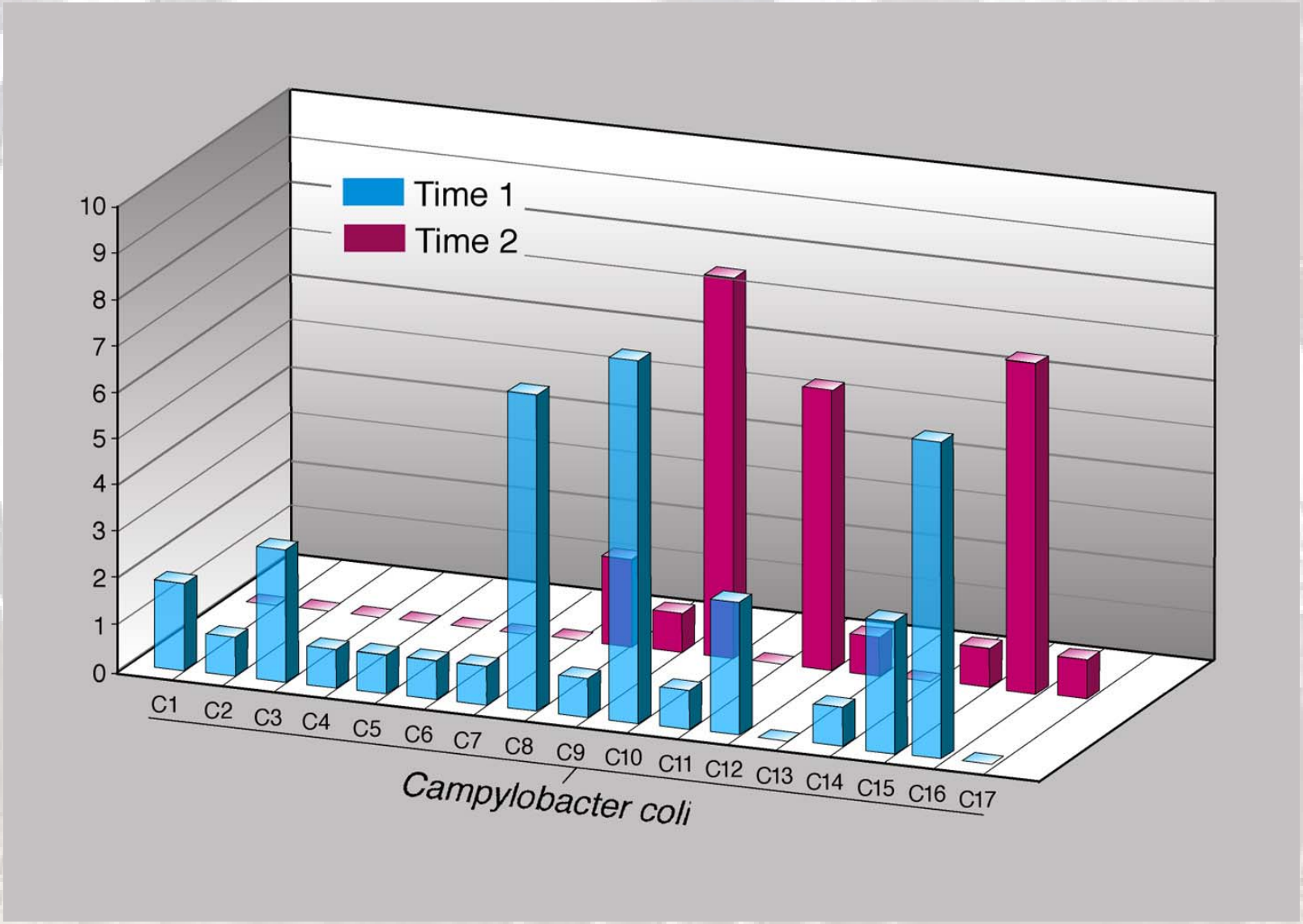
- Differentiate by Pulsed field gel electrophoresis (PFGE)

- *Sma*I

Sma I PFGE of *C. coli*




50
45
40
35
30
25
20
15
10
5
0
Time 1
Time 2
J1
J2
J3
J4
J5
J6
J7
Campylobacter jejuni



Wesley et al. 2006. Applied Environmental Microbiology

Campylobacter Diversity

- *C. coli* =16 patterns/30 isolates
- *C. jejuni* =9 patterns/50 isolates
- Therefore, *C. coli* more diverse. Why?

Campylobacter: Study Design

On-farm (n=30 birds)

Crop
Ceca
Spleen
Liver/Gall bladder
Cloacal loop
Colon

Abattoir (n=30 birds)

Crop
Ceca
Spleen
Liver/Gall bladder

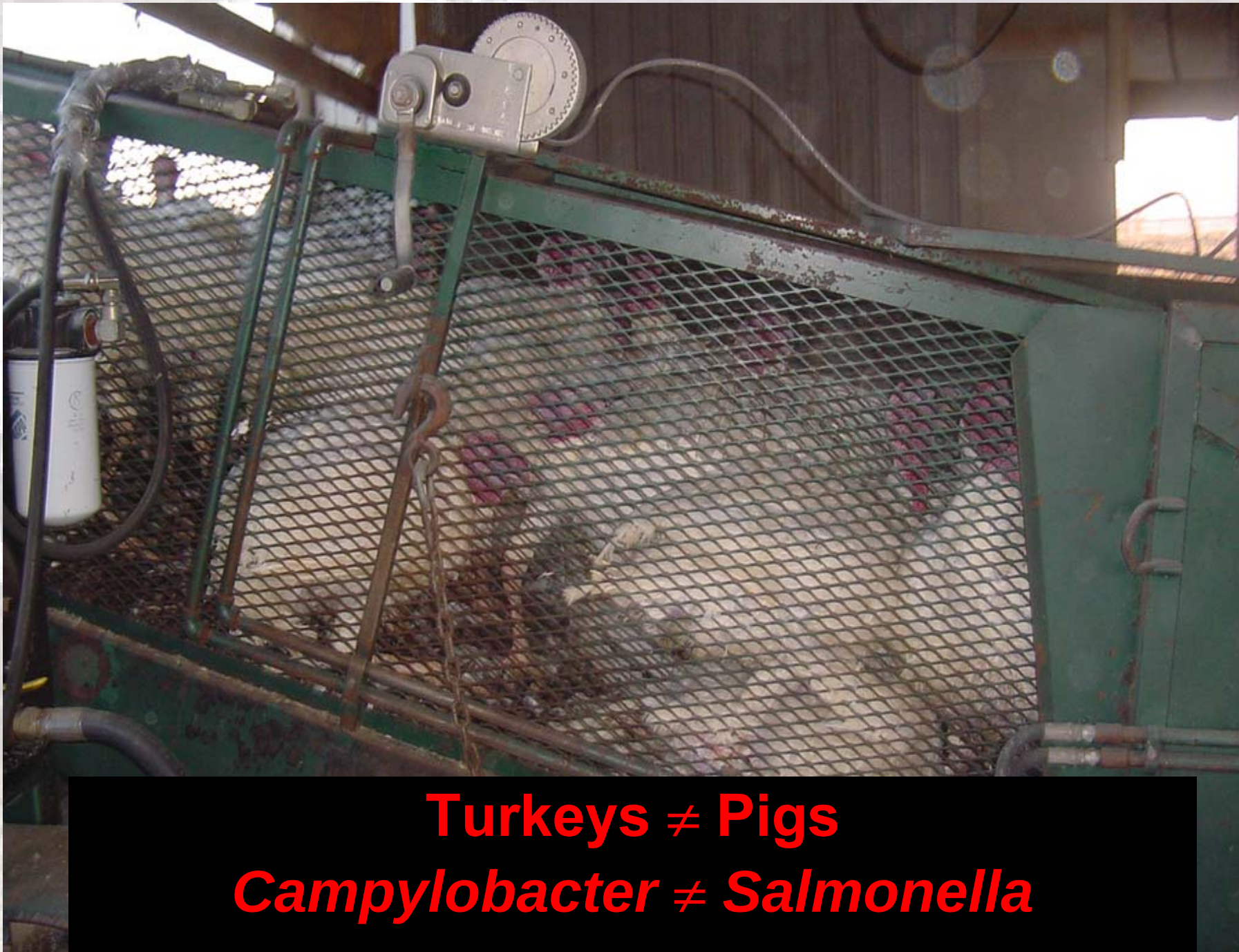
Summary of 6 flocks (n=360 tukeys)

	CROP	DUODENUM	GALL BLD	SPLEEN	ILEUM	CECA	COLON
FARM							
Campylobacter	**3***	74.6	**0***	0	87.3	64	86.7
C.coli	1.1	10.67	0	0	18.67	43.89	23.33
C. jejuni	2.2	72	**0***	0	84.7	27.78	78.89
ABATTOIR							
Campylobacter	**24***	74.67	14.67*	2.67	92.67	57	80
C. coli	16.7	14.67	4	0	42	40	41
C. jejuni	12.7	67.33	11.33*	2.67	66.67	24.44	55.56

Conclusions

- ↑ *Campylobacter* in crop
- ↑ *Campylobacter* in gall bladder
- *C. coli* in cecum
- *C. jejuni* in intestinal tract

Need to speciate and enumerate



Turkeys ≠ Pigs

Campylobacter ≠ Salmonella

Do Feed Withdrawal, Livehaul, and Holding Impact the Prevalence of *Salmonella* and *Campylobacter* in Turkeys?

Salmonella---NO!

Quantitate?

Genotype?

Campylobacter--YES!

Quantitate?

Genotype?

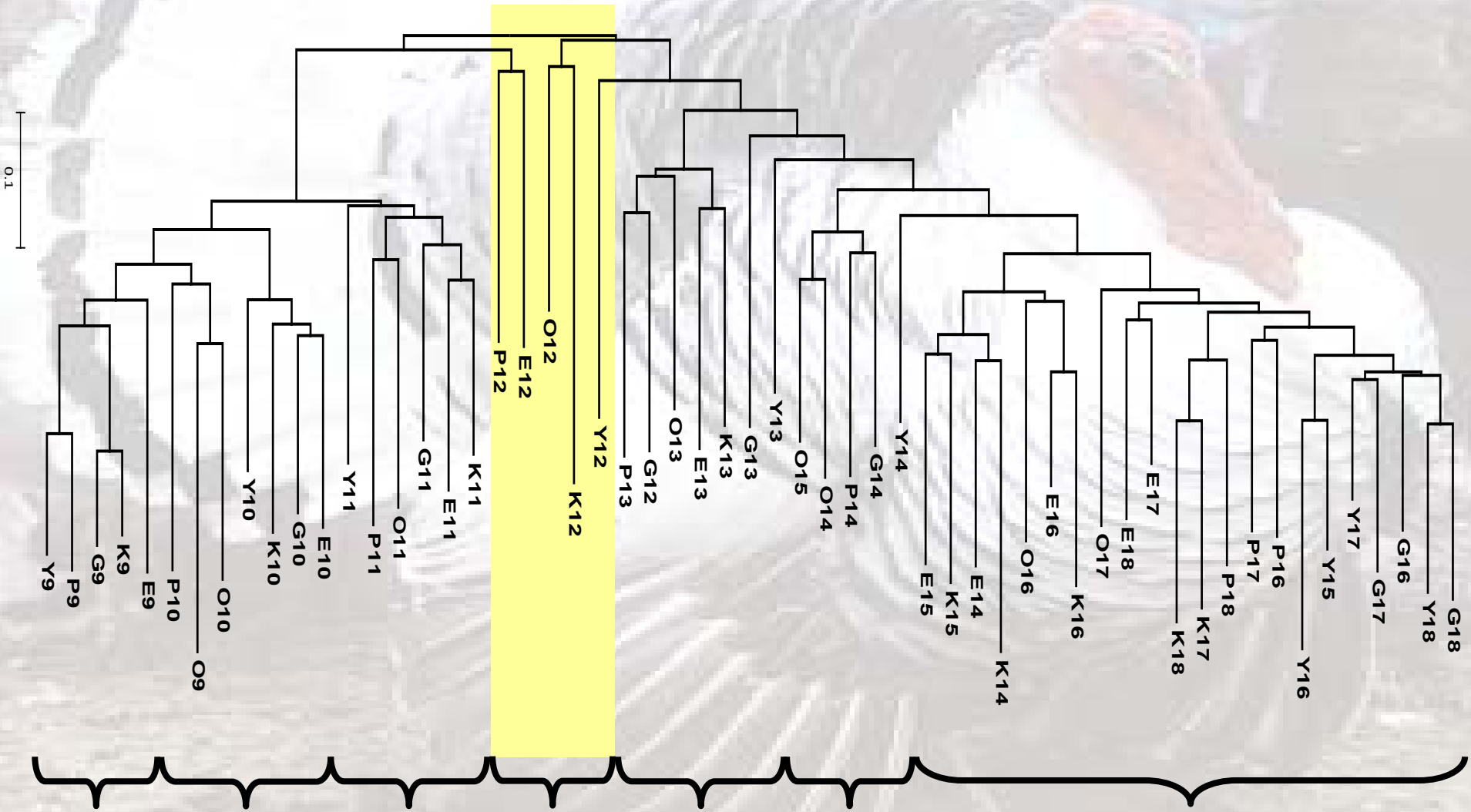
Substantial microbial community changes in the intestine of the pre-adolescent turkey

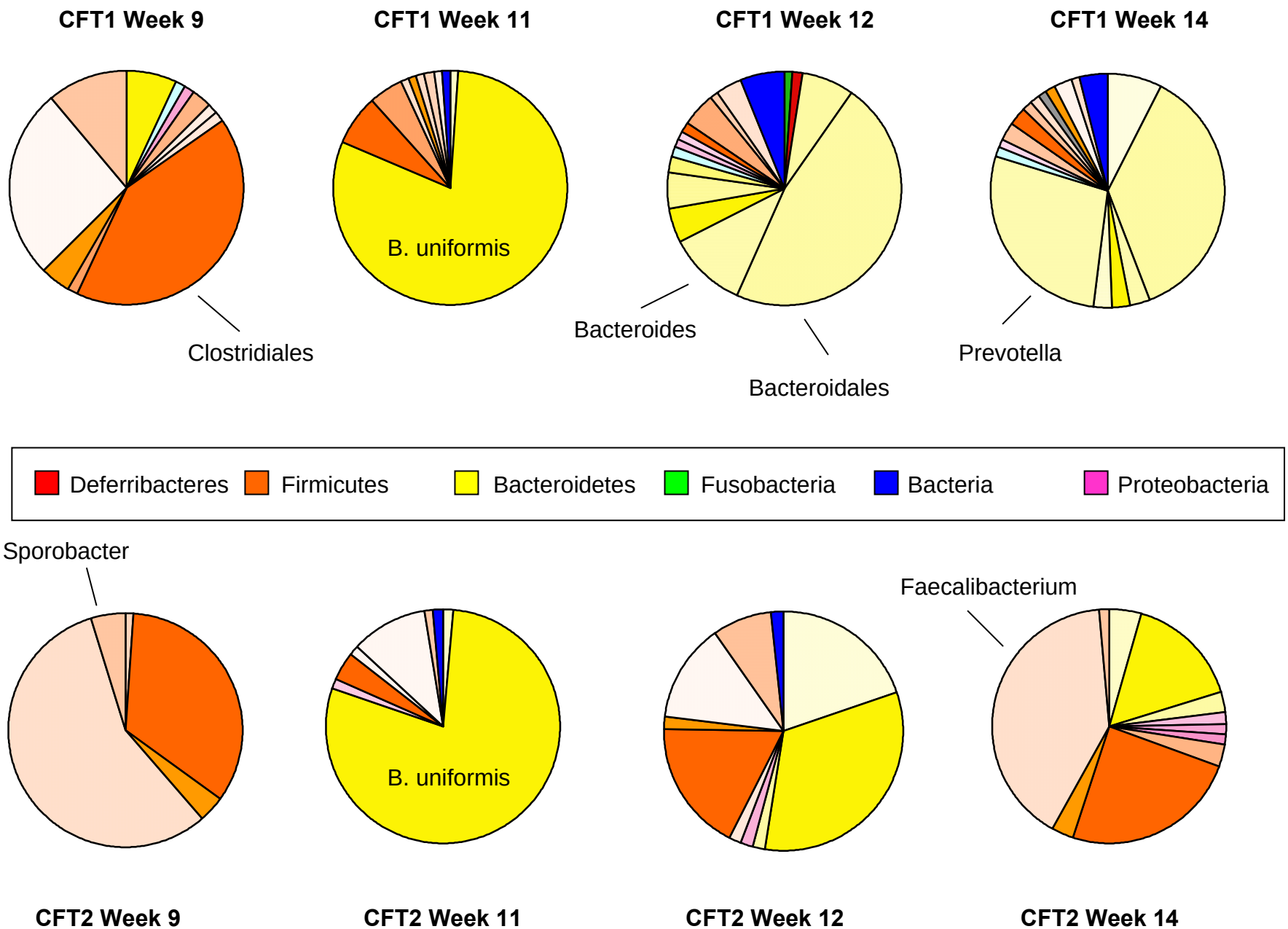
Alexandra Scupham

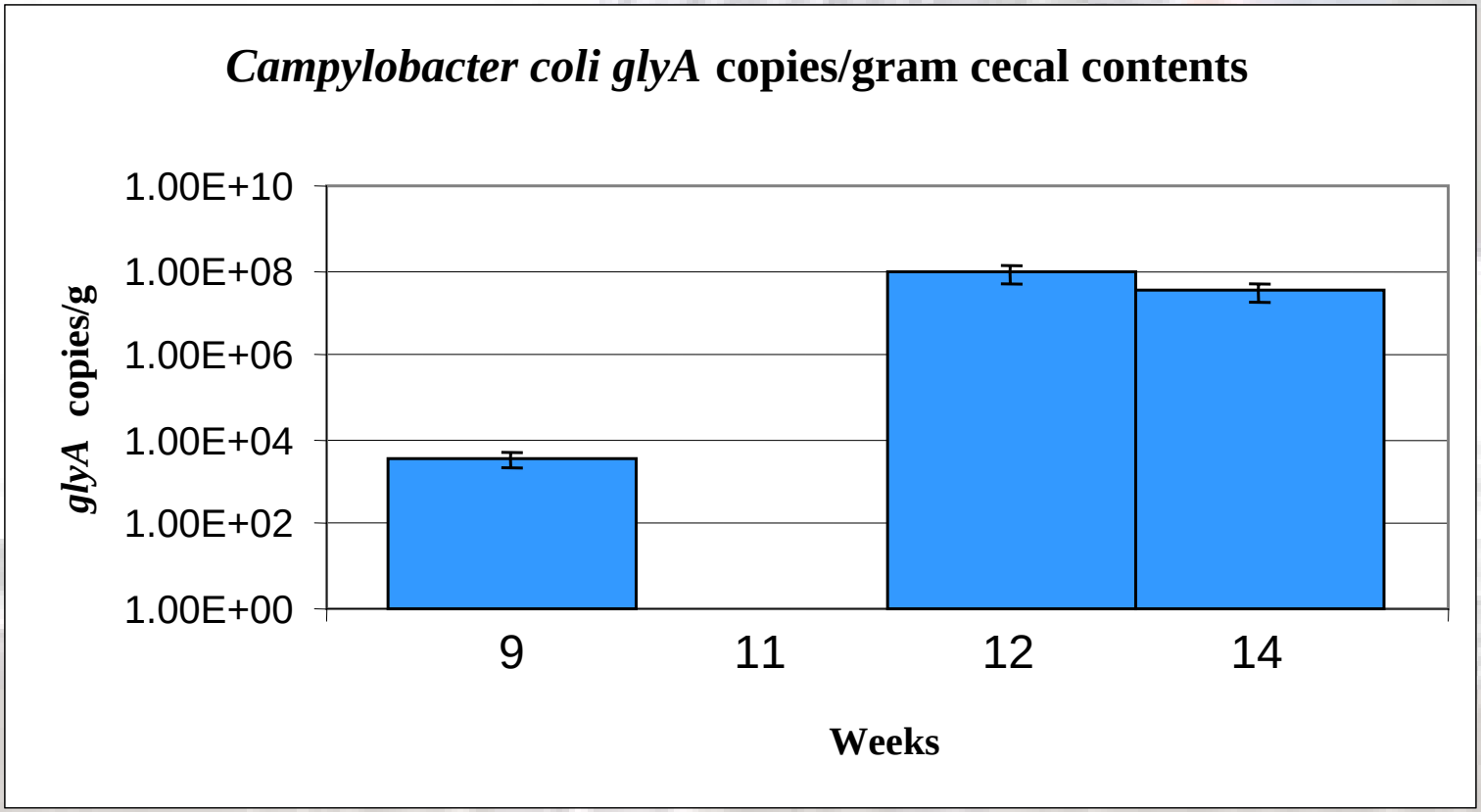
Cecal Feces Time-courses (CFT)

- Two time-courses (2004, 2005)
- Day-of-hatch males
- Diurnal lighting (12L:12D)
- Weekly sampling of cecal feces (CF)
- Total DNA analysis
  - ARISA
  - Library sequencing
  - Real time PCR

Bacterial ARISA




CFT1 Week 9
CFT1 Week 11
CFT1 Week 12
CFT1 Week 14
B. uniformis
Clostridiales
Bacteroides
Bacteroidales
Prevotella
Deferribacteres
Firmicutes
Bacteroidetes
Fusobacteria
Bacteria
Proteobacteria
Sporobacter
B. uniformis
Faecalibacterium
CFT2 Week 9
CFT2 Week 11
CFT2 Week 12
CFT2 Week 14


Campylobacter coli glyA copies/gram cecal contents
1.00E+10
1.00E+08
1.00E+06
1.00E+04
1.00E+02
1.00E+00
glyA copies/g
9
11
12
14
Weeks

Time-course Conclusions

- Cecal microbiota change throughout life; stabilize towards puberty
- Bacterial species richness does not change significantly over the last 10 weeks
- Microbial communities respond to host signals
- Perturbation of non-climax communities allows colonization by pathogens
- *B. uniformis* dominate the microbiota after an environmental perturbation at week 11; may drive establishment of the adult climax community.

Transcriptional profiling of the porcine response to *Salmonella*

National Animal Disease Center
Shawn Bearson PhD, Jolita Uthe DVM

Beltsville Agricultural Research Center
Joan Lunney PhD, Atabak Royaee PhD
Dan Kuhar

Iowa State University
Christopher Tuggle PhD, YanFang Wang PhD,
Dan Nettleton PhD, Jack Dekkers PhD,
Long Qu

Objective

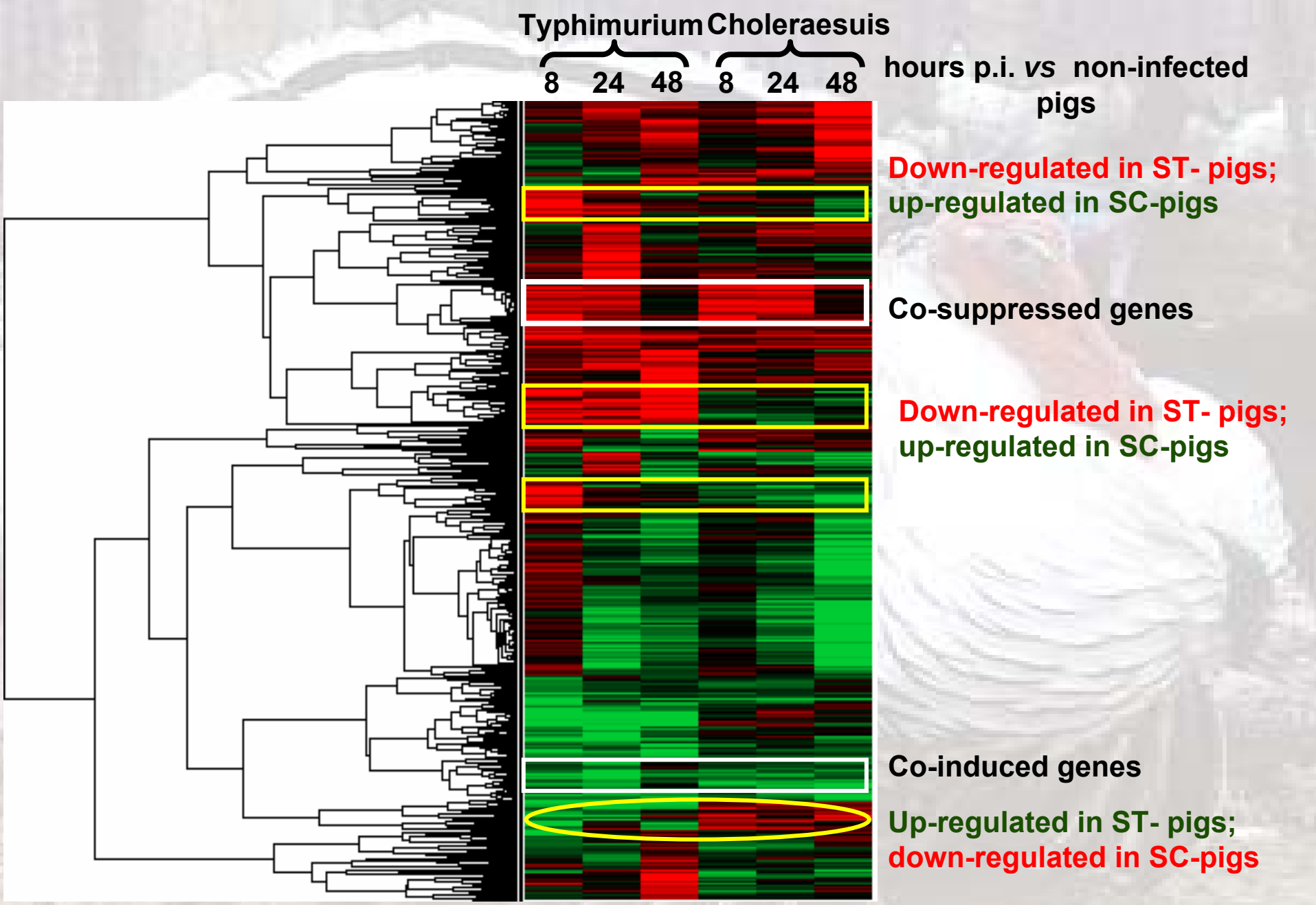
Identify transcriptional differences in the porcine response to experimental inoculation with *Salmonella enterica* serovars Choleraesuis (narrow host range) and Typhimurium (broad host range)

Three Functional Genomic Analyses

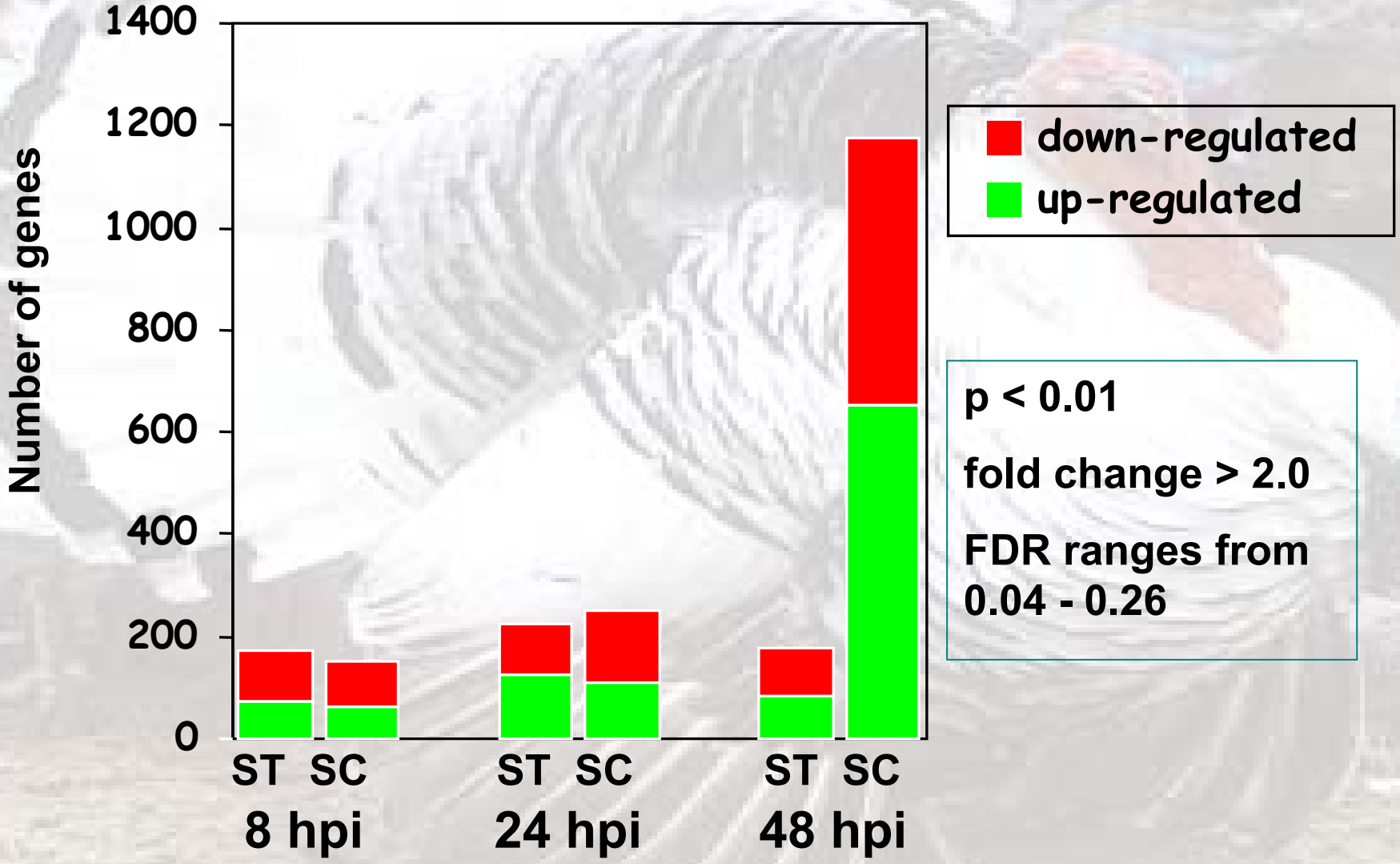
Suppression Subtractive Hybridization

Real-time PCR of a panel of immune-related genes

DNA microarray analysis

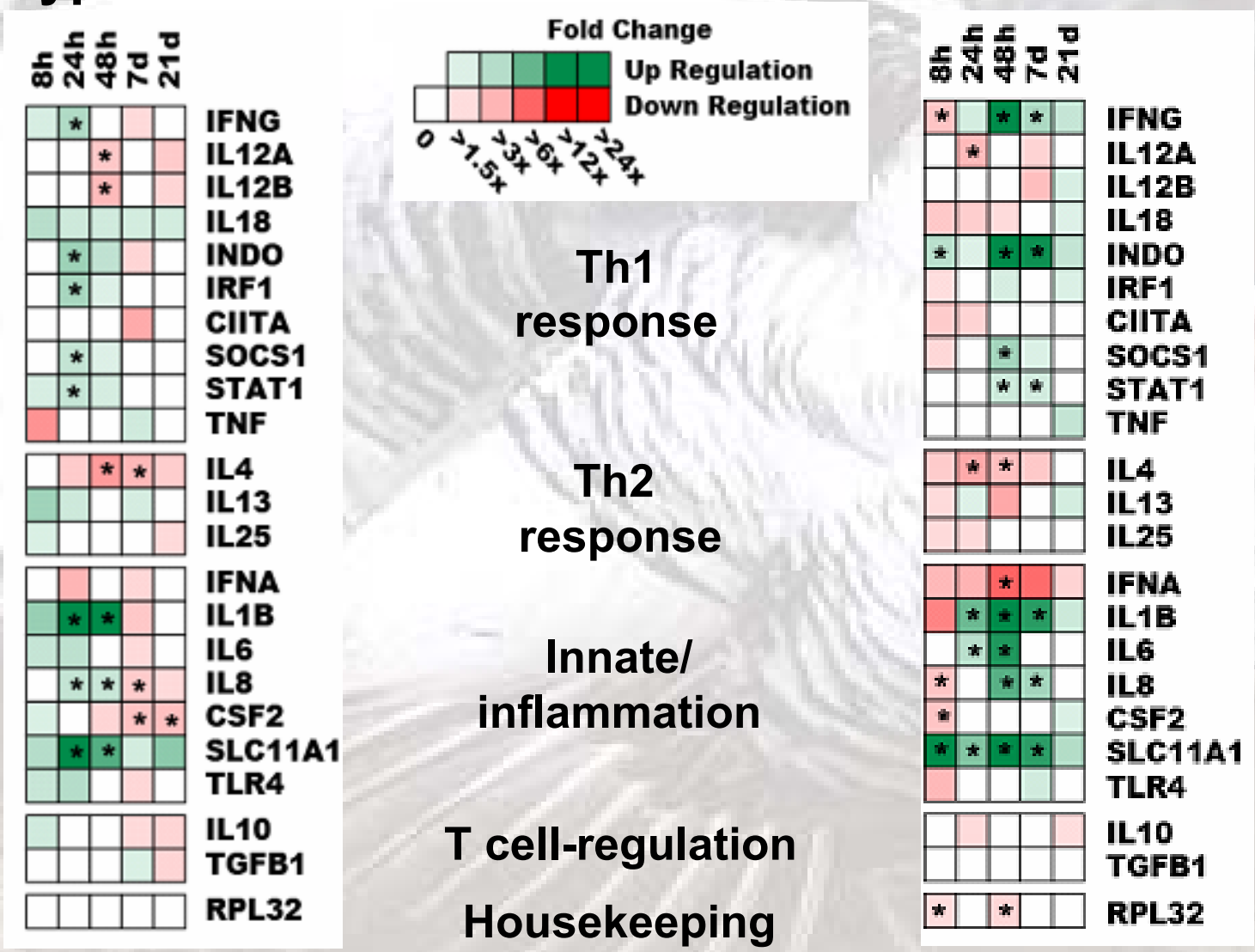

Typhimurium
Choleraesuis
8 24 48
8 24 48
hours p.i. *vs* non-infected pigs
Down-regulated in ST- pigs; up-regulated in SC-pigs
Co-suppressed genes
Down-regulated in ST- pigs; up-regulated in SC-pigs
Co-induced genes
Up-regulated in ST- pigs; down-regulated in SC-pigs

Number of genes differentially-expressed during *Salmonella* infections



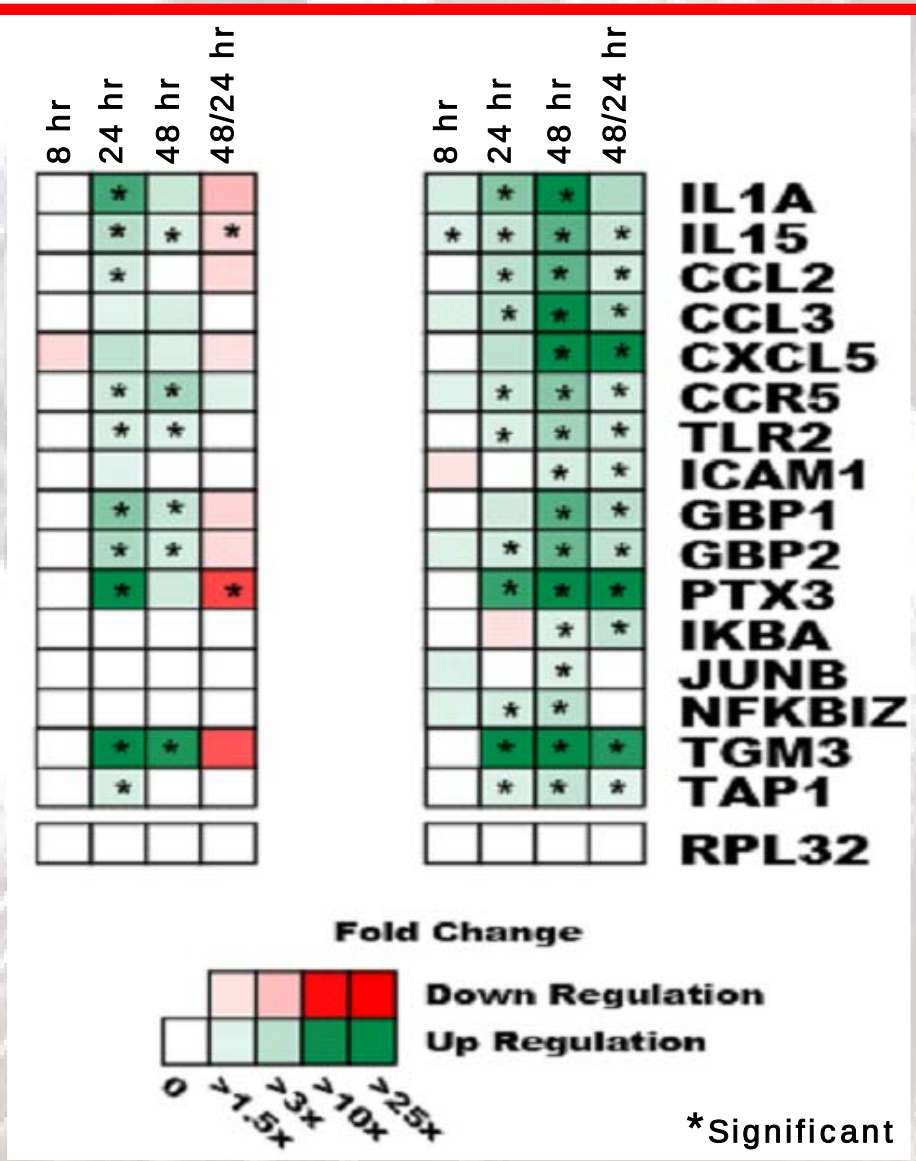
*Compared to the non-infected pigs

Real-time PCR of 22 Immune-related Genes



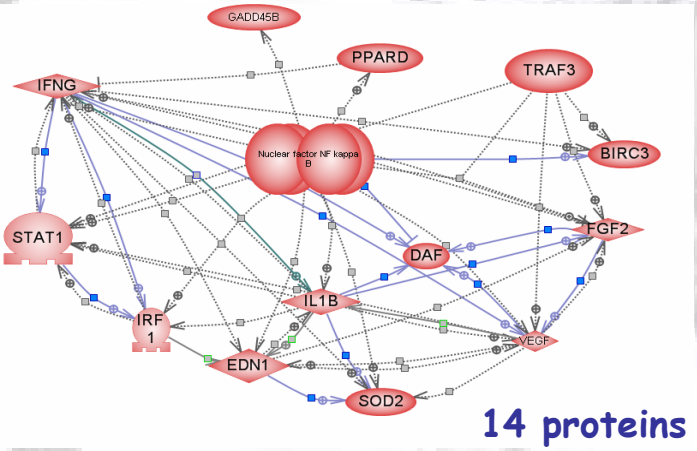
*Significant compared to non-infected control pigs

Real-time PCR analysis of several NFκB-regulated genes confirms the differences in transcriptional induction between the *Salmonella*-infected swine observed in the microarray study

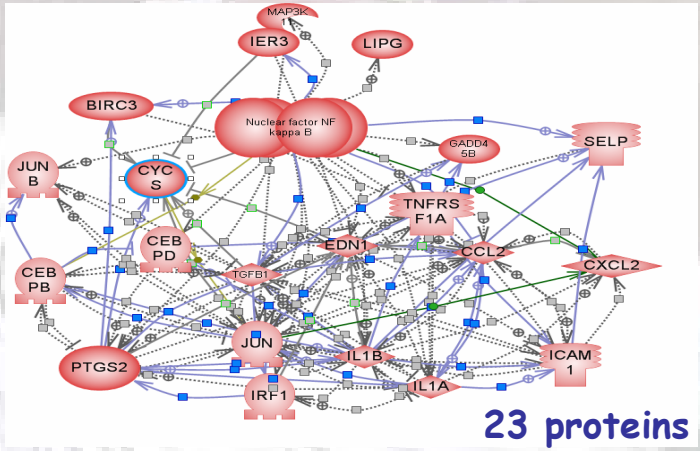


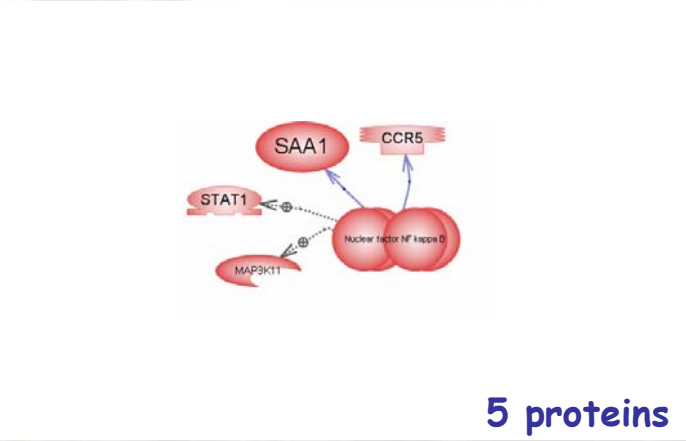
Fewer NFκB-regulated genes identified as transcriptionally activated in Typhimurium- *versus* Choleraesuis-infected pigs

Typhimurium-infected pigs | **Choleraesuis-infected pigs**



24 hpi





48 hpi

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Are similar studies possible in turkeys in response to

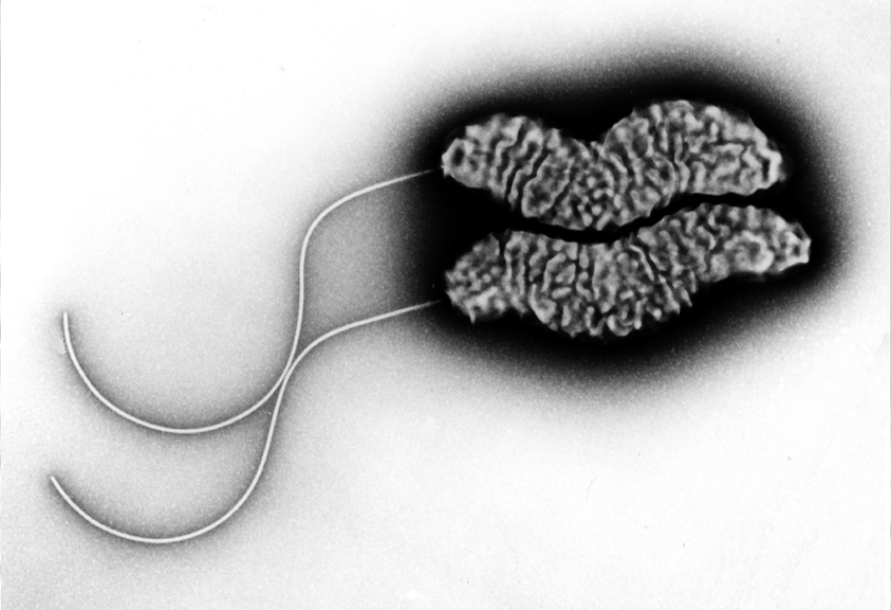
Salmonella????

Campylobacter???

Help Wanted!

Materials and Methods

Campylobacter jejuni



CASES:
1,963,141

HOSPITALIZATIONS:
10,539

DEATHS:
99

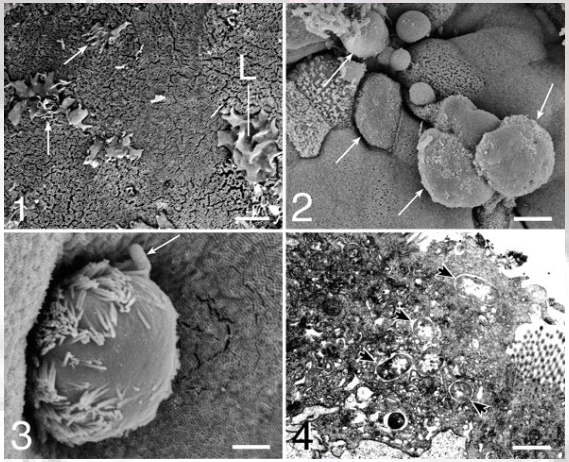
Mead et al. 1999. Emerging Infectious Diseases 5:607-625

Typhimurium vs Choleraesuis (swine)

Clinical

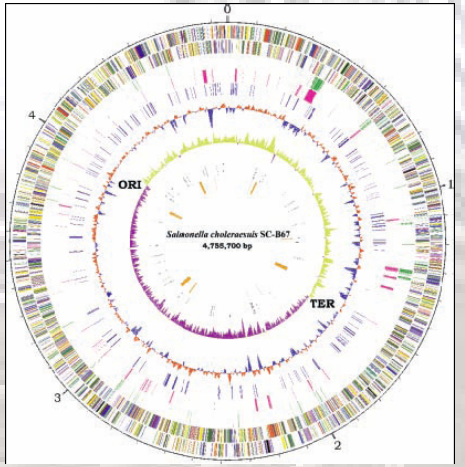
Woods et al. 1999. Am J Vet Res

Histological



Meyerholz et al. 2003. Vet Pathol.

Genetics



Nucleic Acids Res 2005.