Pre-Harvest Technologies to Combat Salmonella in the Beef Lymphatic System

Brad Morgan

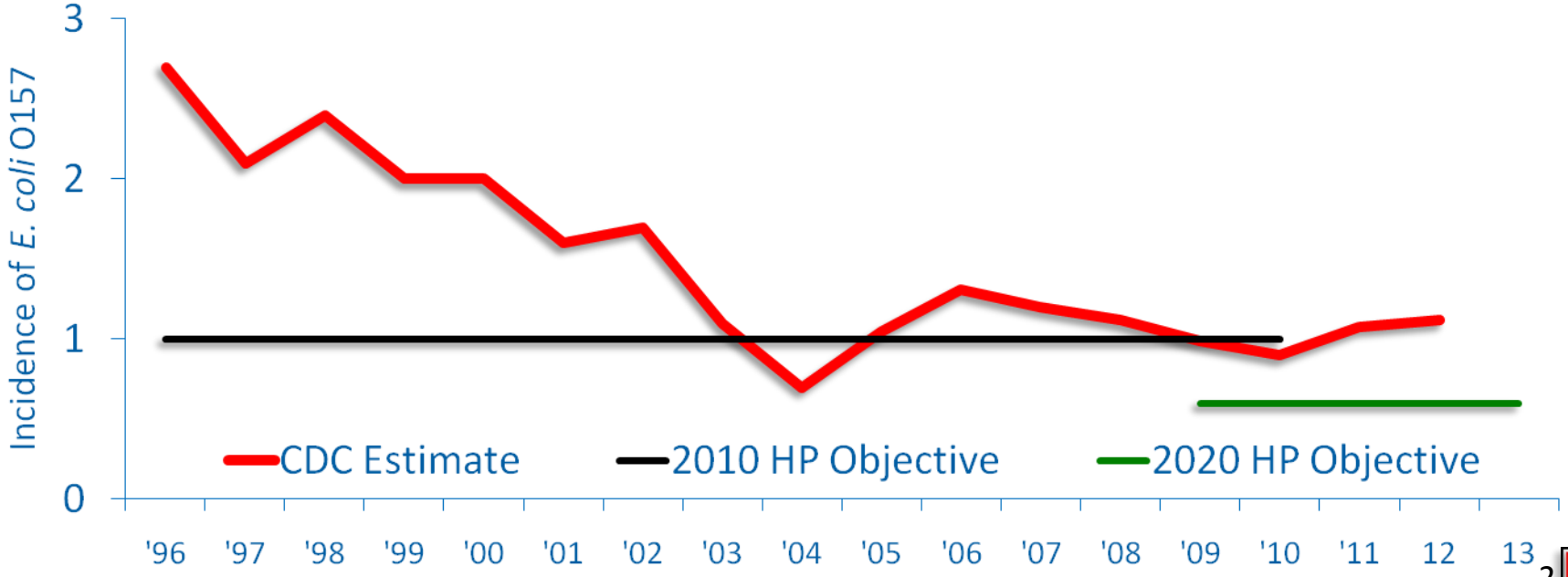
RMC 2013
RECIPROCAL MEAT CONFERENCE

June 16-19, 2013
AMSA 66th RMC – Meat Matters
Auburn University

zoetis

Background Information

- Many abattoirs excel at microbial process control
  - Some are achieving achievable control
- ***Impact*** observed across various metrics

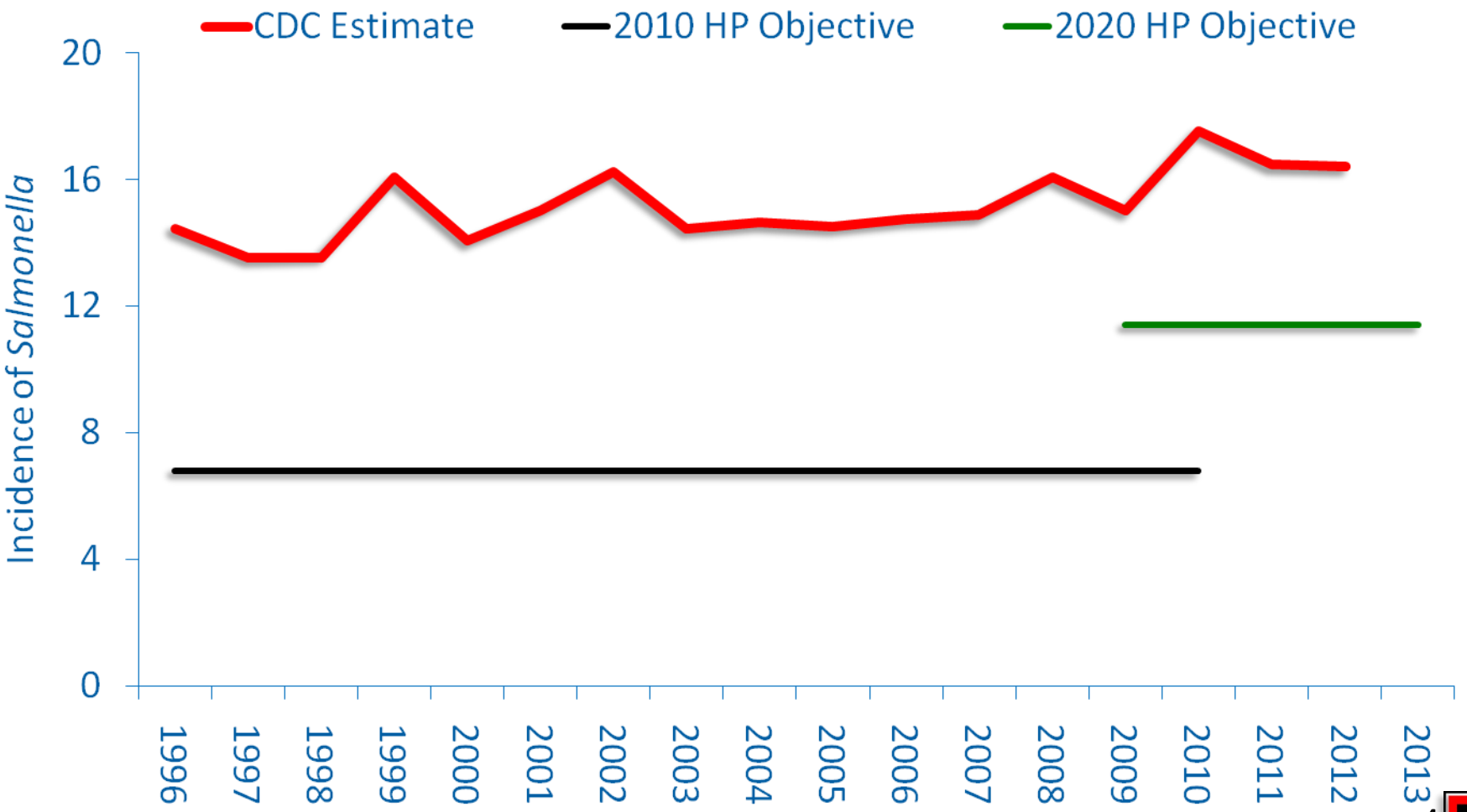


Reported Human cases / 100,000

Salmonella

- CDC reports U.S. incidence ~16 cases/100,000/yr
  - Only includes cases reported to the CDC
  - With under diagnosis/reporting, incidence estimated to be closer to 1 case/300 people/yr
    - Scallan *et al*. *Emerg Infect Dis*. 2011 17:7-15
- In terms of sources of carcass and beef contamination, our understanding comes from knowledge of *E. coli* O157:H7

Human Incidence of *Salmonella*

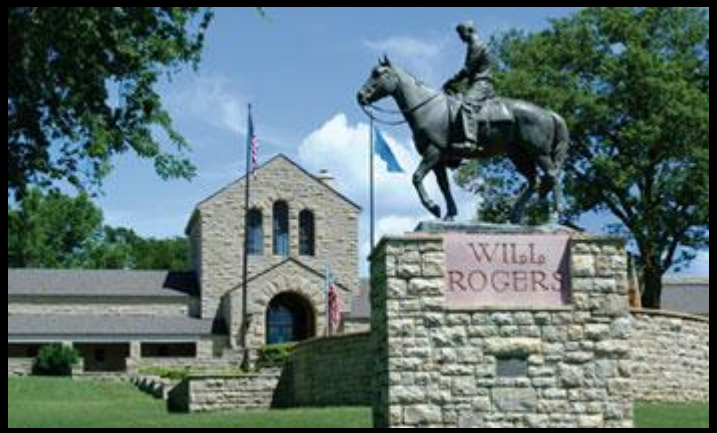


Reported Human cases / 100,000

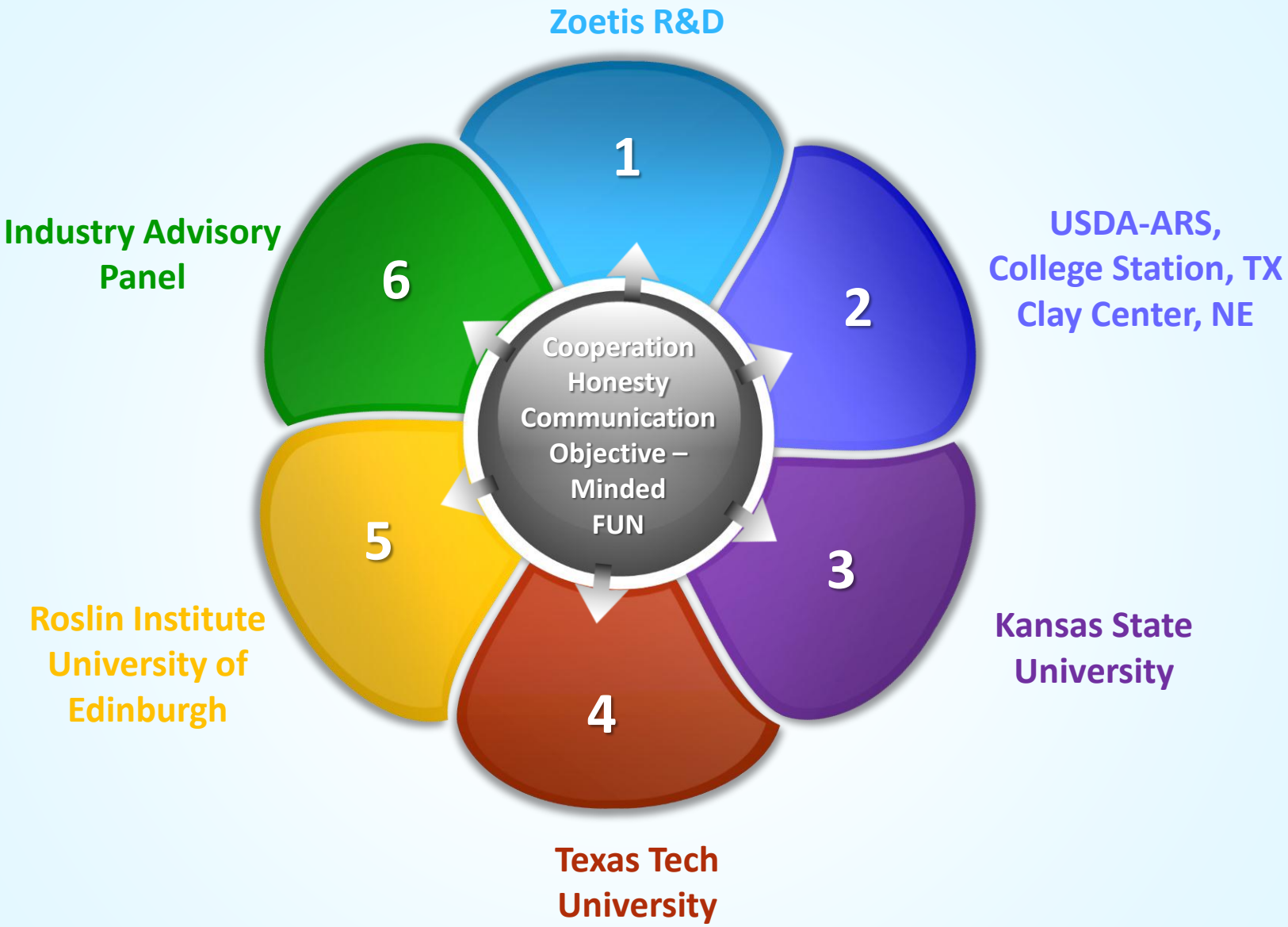
- Failure in public health mission of regulatory (competent) authorities
  - USDA/FSIS > meat, poultry, & egg products
- Competent authority has brand protection issues
  - Policies based on science (including social, etc.)
- Citizens (CSPI) have petitioned the 'competent authority' to declare certain *Salmonella* as adulterants in meat and poultry
  - Heidelberg, Newport, Typhimurium, Hadar

"A man can learn only two ways, one by reading, and the other by association with smarter people."

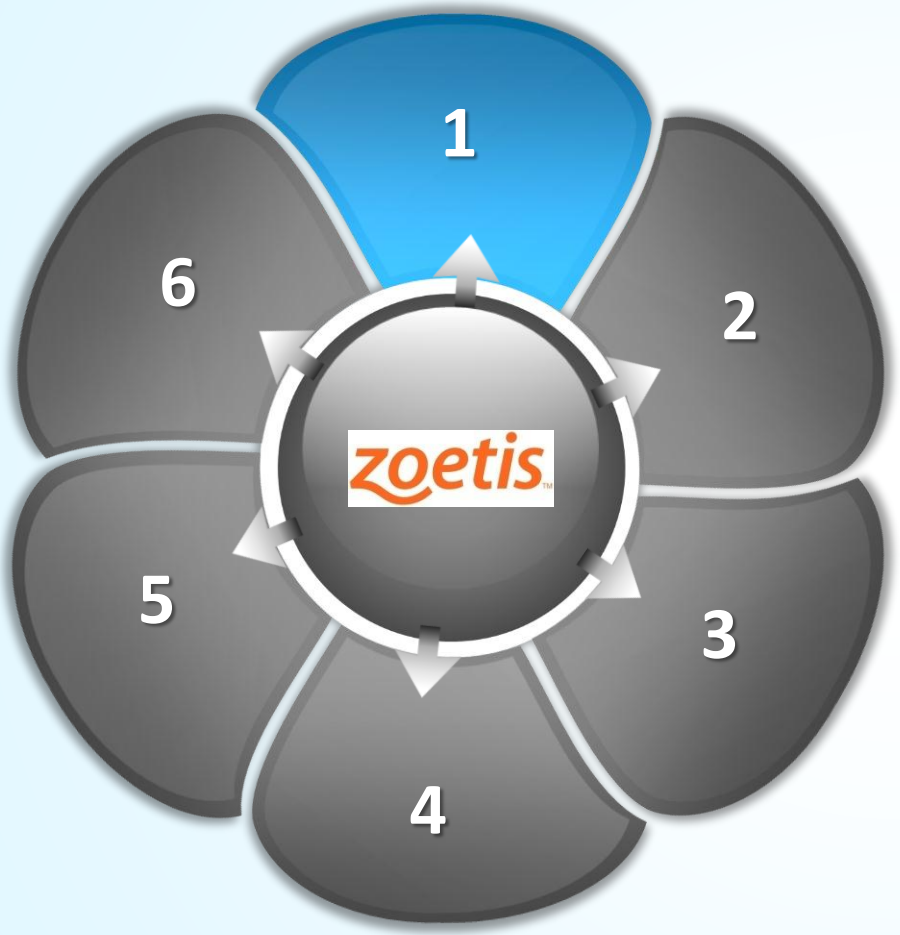
Will Rogers



Zoetis Salmonella Research Cluster



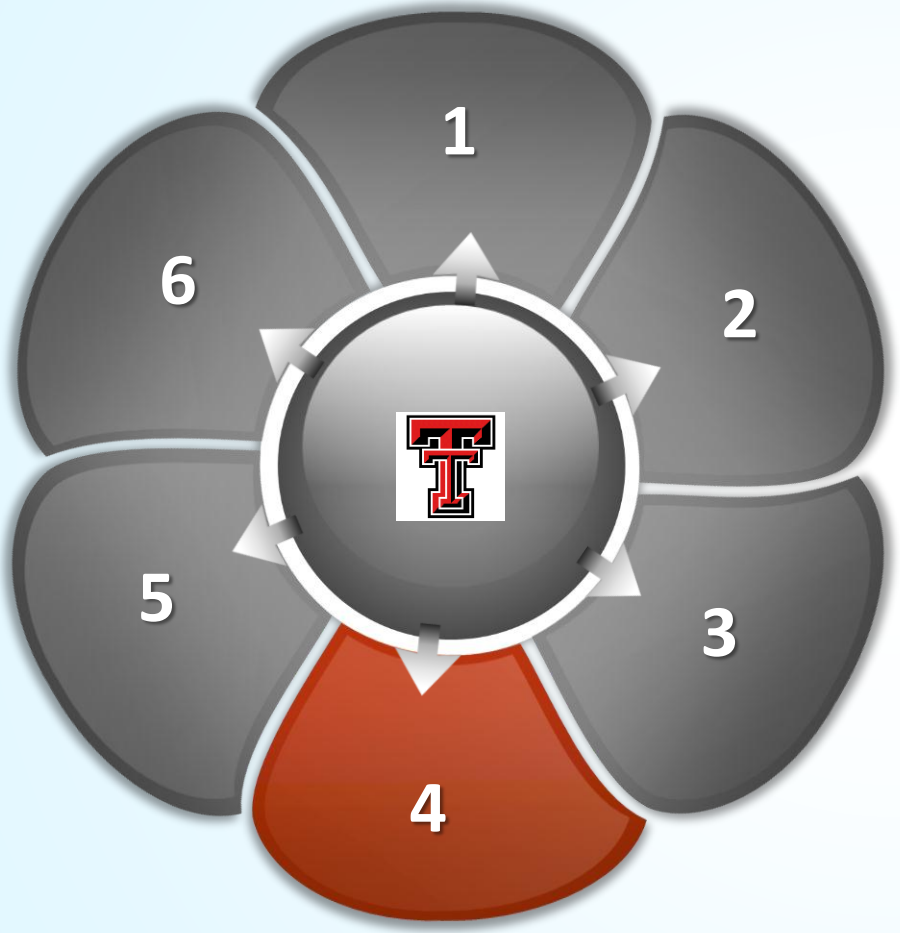
Zoetis R&D



Pete Cornell

- Kalamazoo, MI
- Developing research collaborations
- Utilize new research models
- Collaboration of food safety initiatives
- SRP Vaccine Offerings
- Large Field Study in TX Panhandle

Texas Tech University



Guy Loneragan

- National Research University
- Outstanding Food Safety Program
- In the Heart of “Cattle Country”
- Industry Relationships

Surveillance Studies

Funded by the Beef Checkoff

- Packing-plant surveillance populations
  1. Cattle from feedlots
  2. Cows culled from dairy and beef herds
  - Cattle that passed USDA inspections
- Sep 2010 to Oct 2011
  - Samples collected 6 times throughout year
  - ~75 nodes per abattoir per time
- Feb 2012 to Dec 2012
  - More plants, more sample periods

United States Department of Agriculture

National Institute of Food and Agriculture

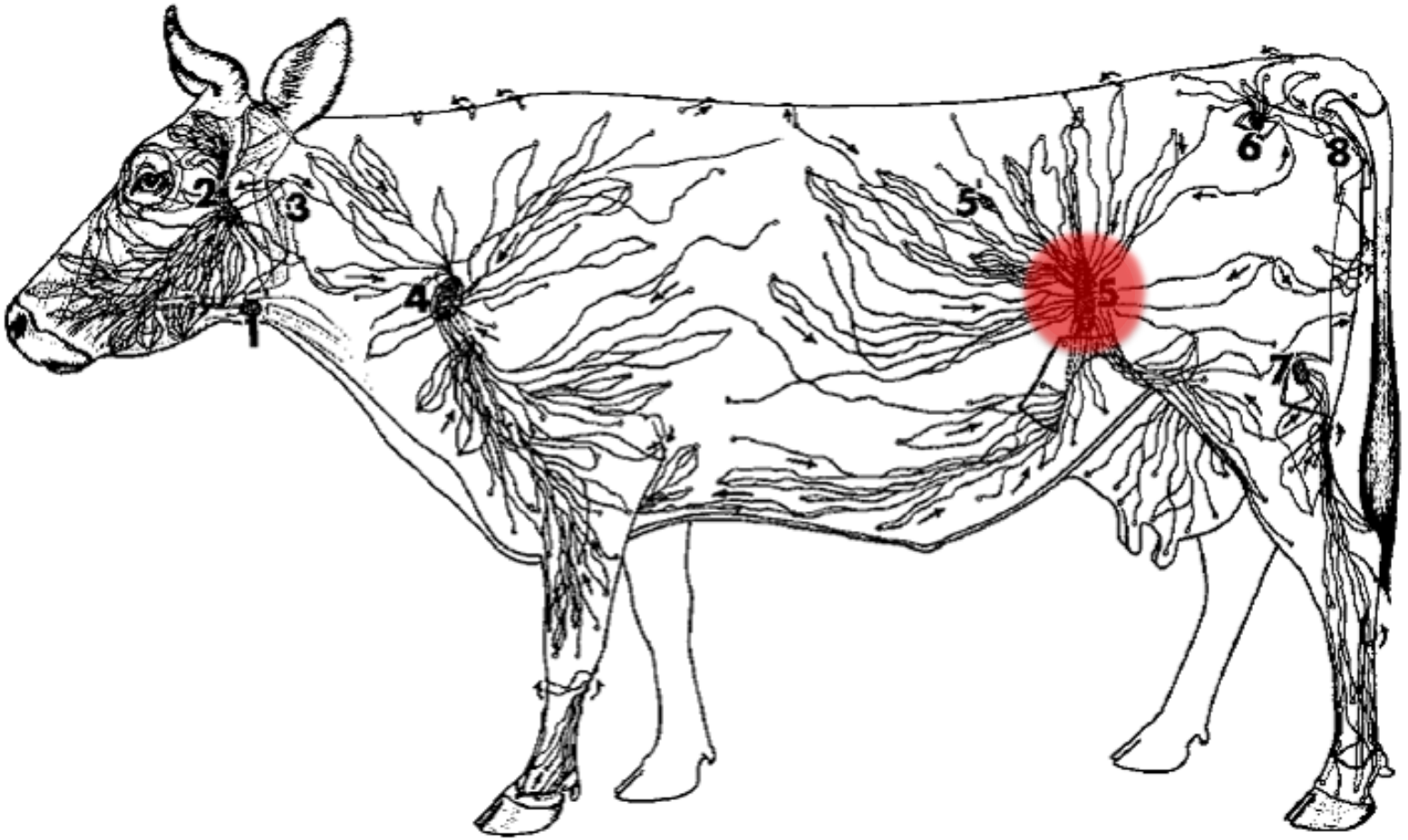
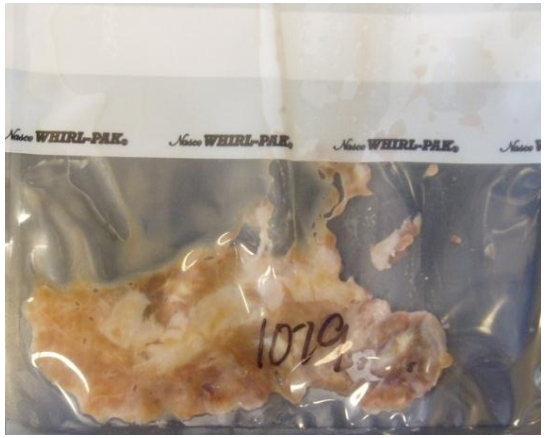


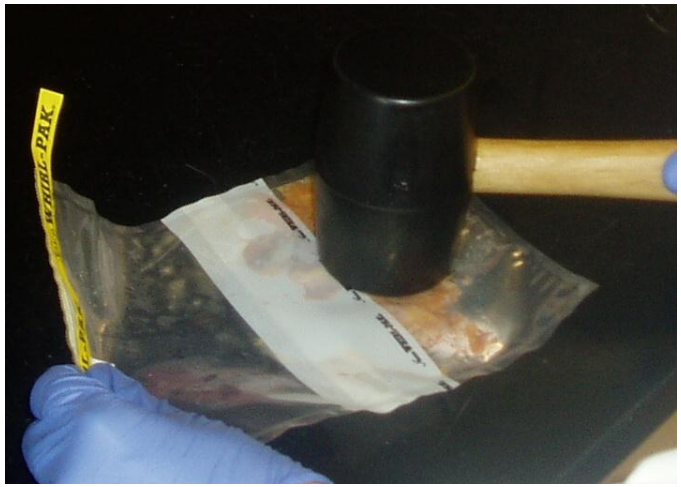
FIGURE 34–1. Superficial lymph flow of the cow.

1, Mandibular ln.; 2, parotid ln.; 3, lateral retropharyngeal ln.; 4, superficial cervical ln.; 5, subiliac ln.; 5′, lnn. of paralumbar fossa; 6, gluteal ln.; 7, popliteal ln.; 8, tuberal ln. (After Baum, 1912.)

From: Ruminant Lymphatic System (Saar and Getty) In Anatomy of the Domestic Animals. Eds Sisson and Grossman



Brichta-Harhay methods developed for this project and described in *JFP* 2008;71:1685-8



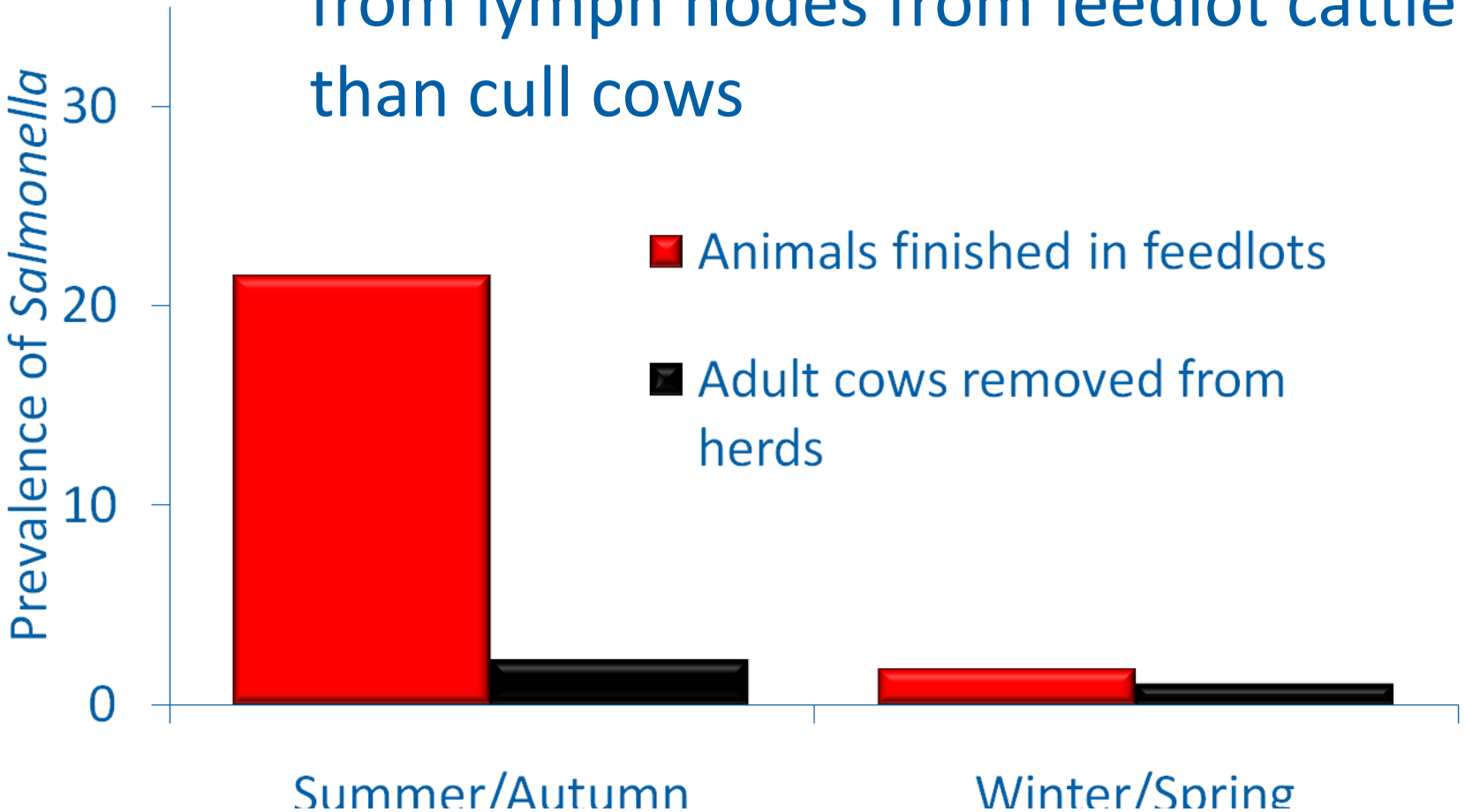
2010-2011 Surveillance

- Plants from which samples were received:
  - 5 in Texas (categorized as southern)
  - 2 in Nebraska (categorized as northern)
  - 1 in California (included in northern category)
    - Most plants have 6 collection windows; 2 each in:
    - Sep-Nov, Feb-Mar, Jul-Sep
- 3,327 lymph nodes assayed
  - 8.0% positive
  - Accepted for publication

2010-2011 Surveillance

Funded by the Beef Checkoff

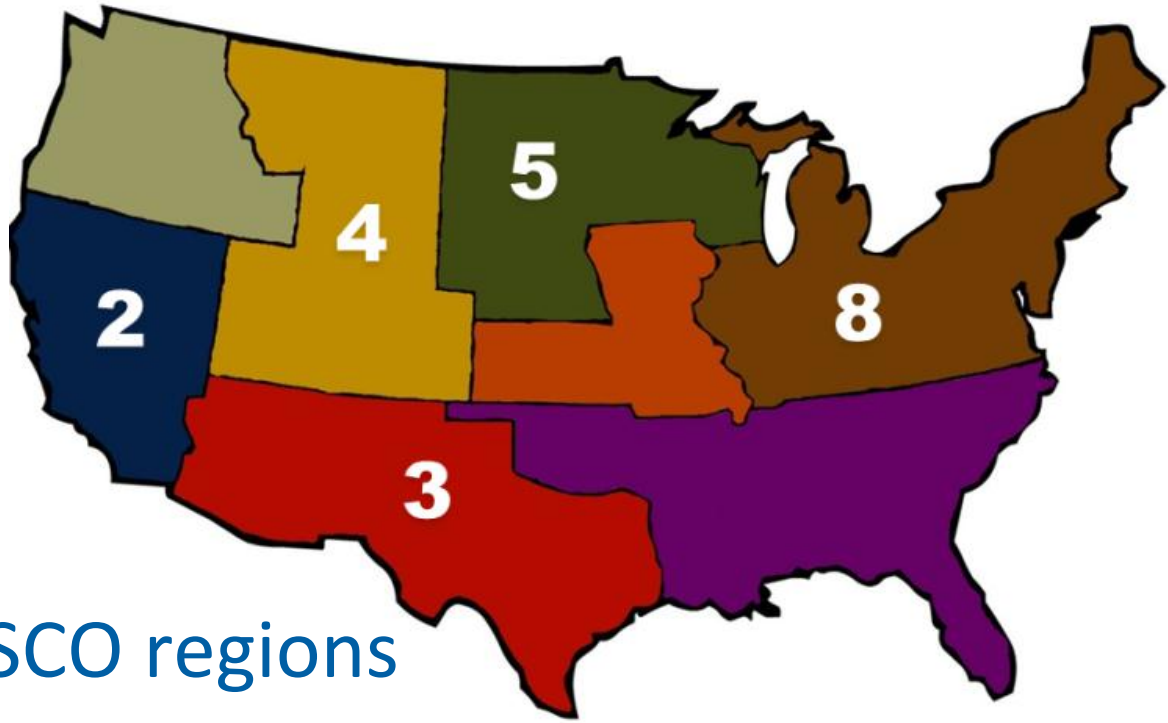
- 9 times more likely to be recovered from lymph nodes from feedlot cattle than cull cows



Serotype	Feedlot (n=233)	Adult cows (33)
Montevideo	**48.5**	**12.1**
Anatum	**27.5**	**6.1**
Reading*	5.2	3.0
Thompson	3.9	3.0
Meleagridis	3.4	
Kentucky	1.7	12.1
C07 NT	2.6	
Mbandaka	1.3	9.1
Muenchen	1.7	
Bredeney		9.1
Infantis	1.3	
Newport*	0.9	3.0
Braenderup		6.1
Brandenburg		6.1
Cerro	0.9	
Dublin		6.1
Muenster	0.9	
Panama		6.1
Saint Paul	0.4	3.0
Cubana		3.0
Give		3.0
Kiambu		3.0
Typhimurium*		3.0
Uganda		3.0

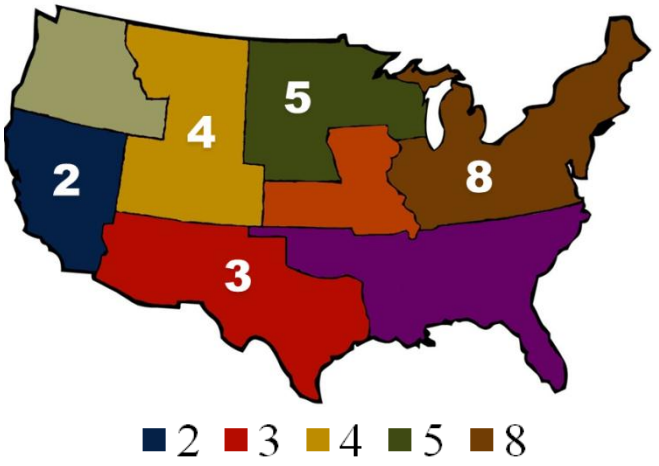
- 24 serotypes identified
- 14 serotypes from 233 positive nodes from feedlot animals
- 18 serotypes from 33 positive nodes in cull cows

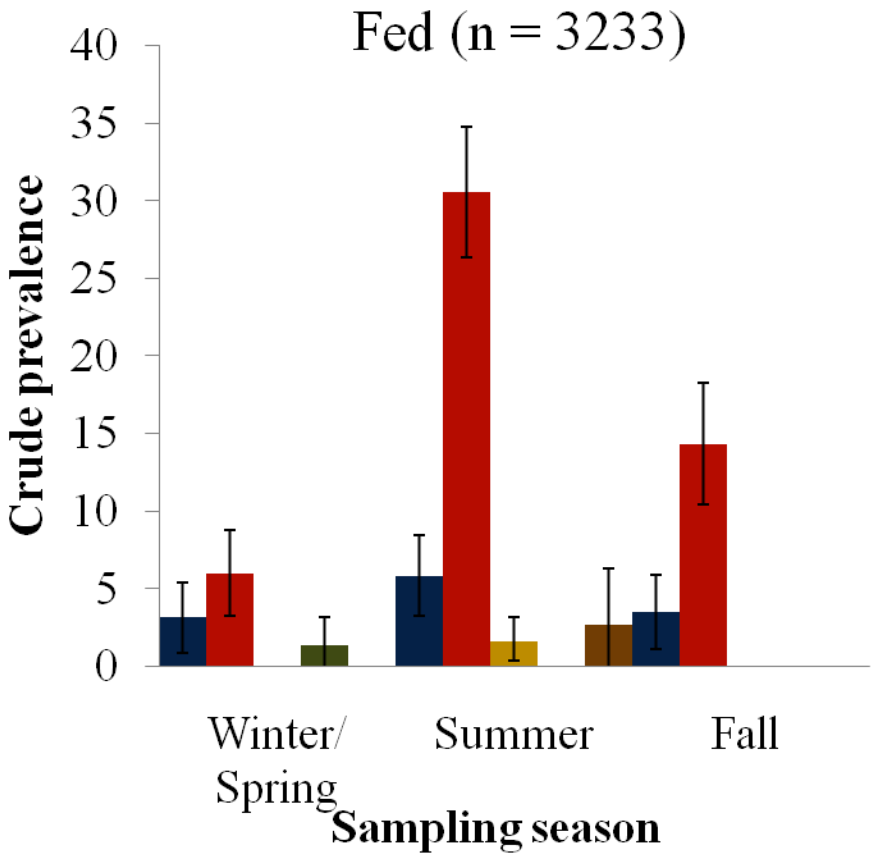
2012 Surveillance

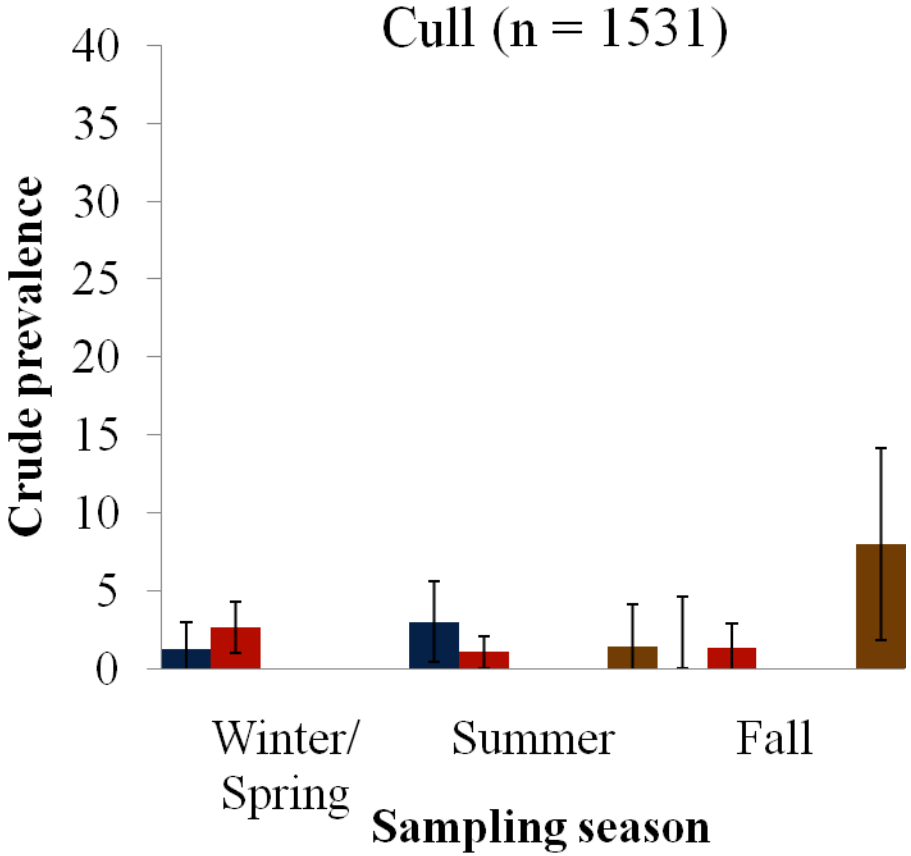


- 12 Plants by BIFSCO regions
  - 5 in region 3
  - 3 in region 2
  - 2 in region 4
  - 1 each in regions 8 & 5
- 4,764 lymph nodes assayed
  - 6.0% positive

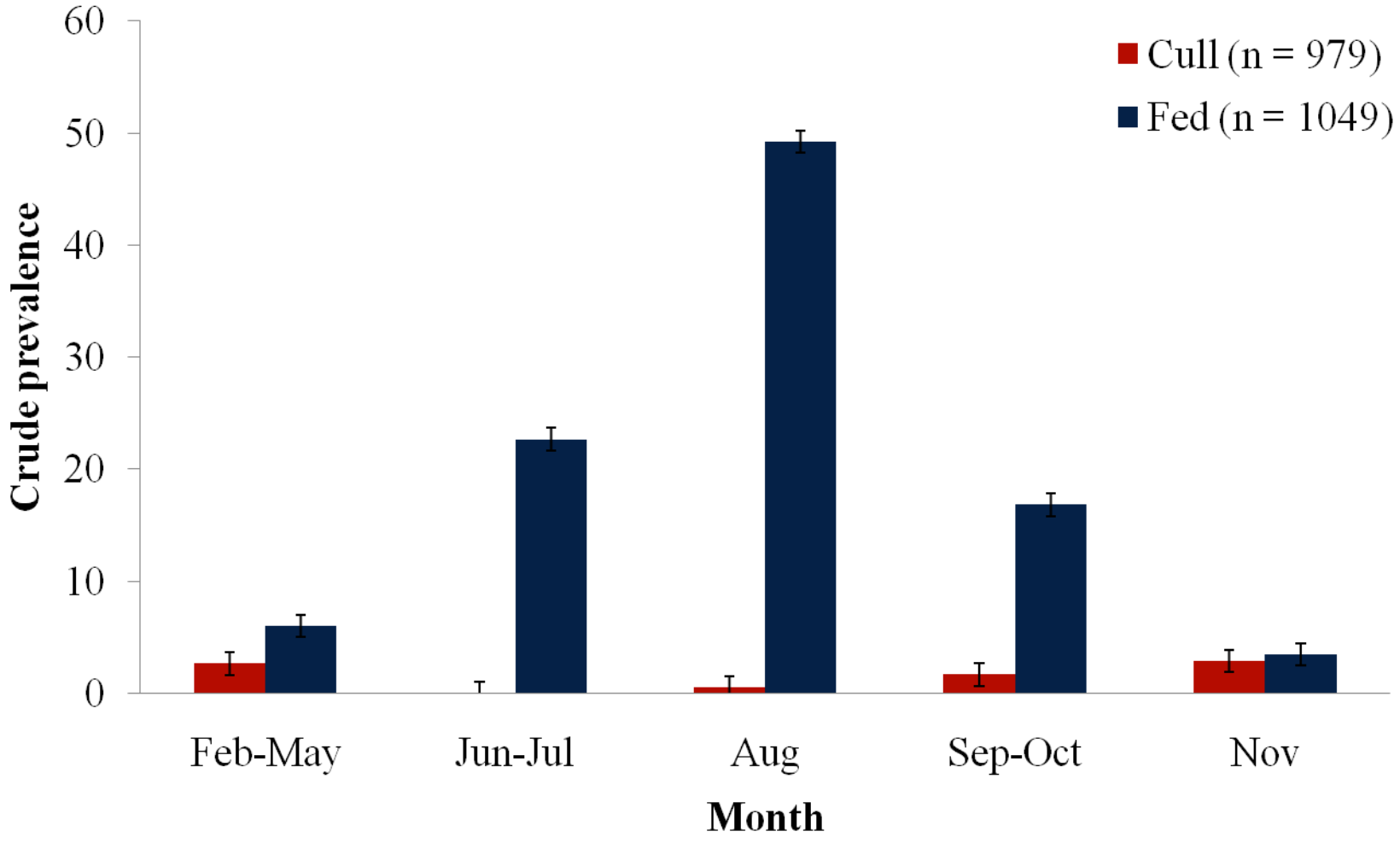
Prevalence by region and season

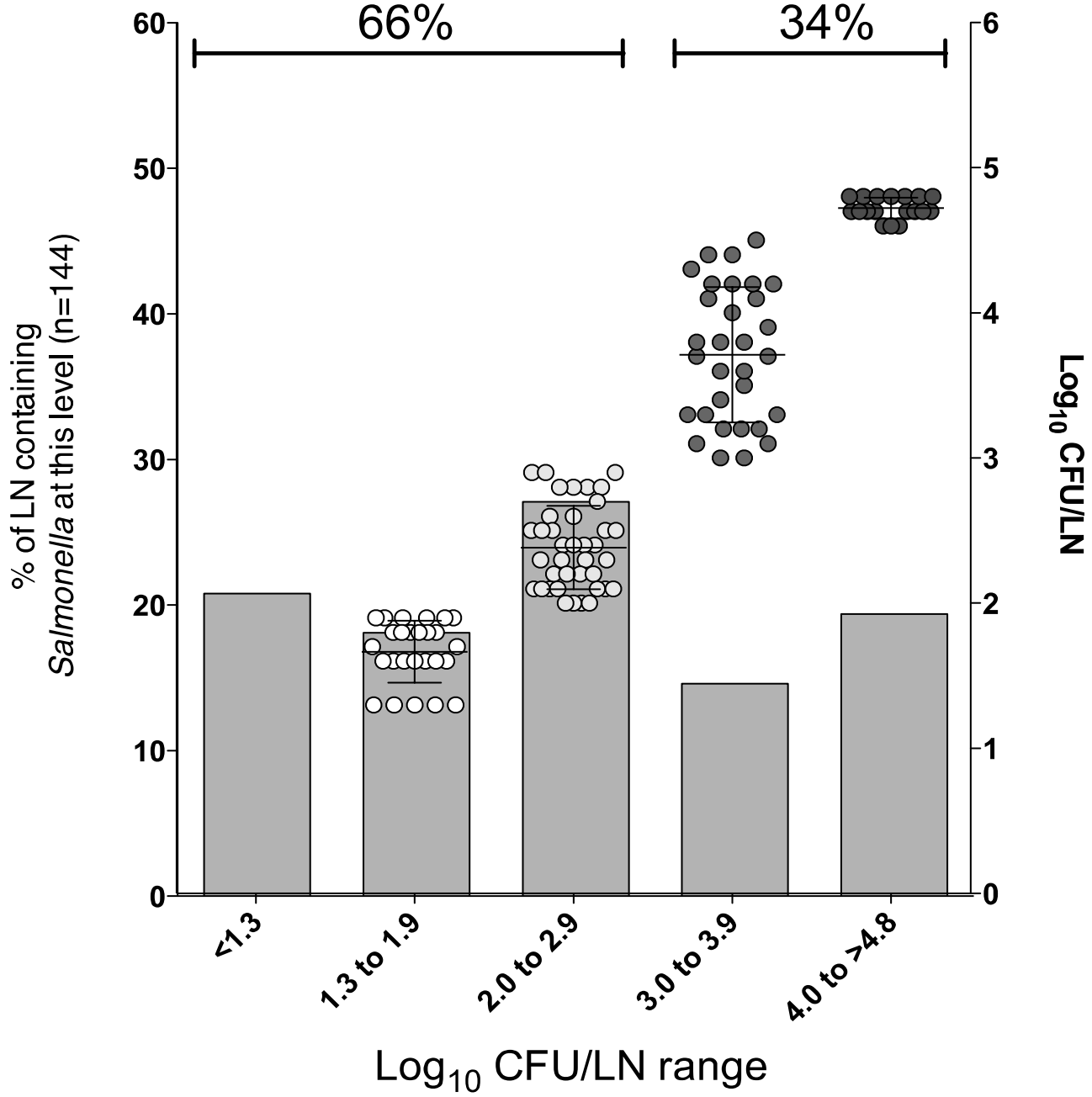




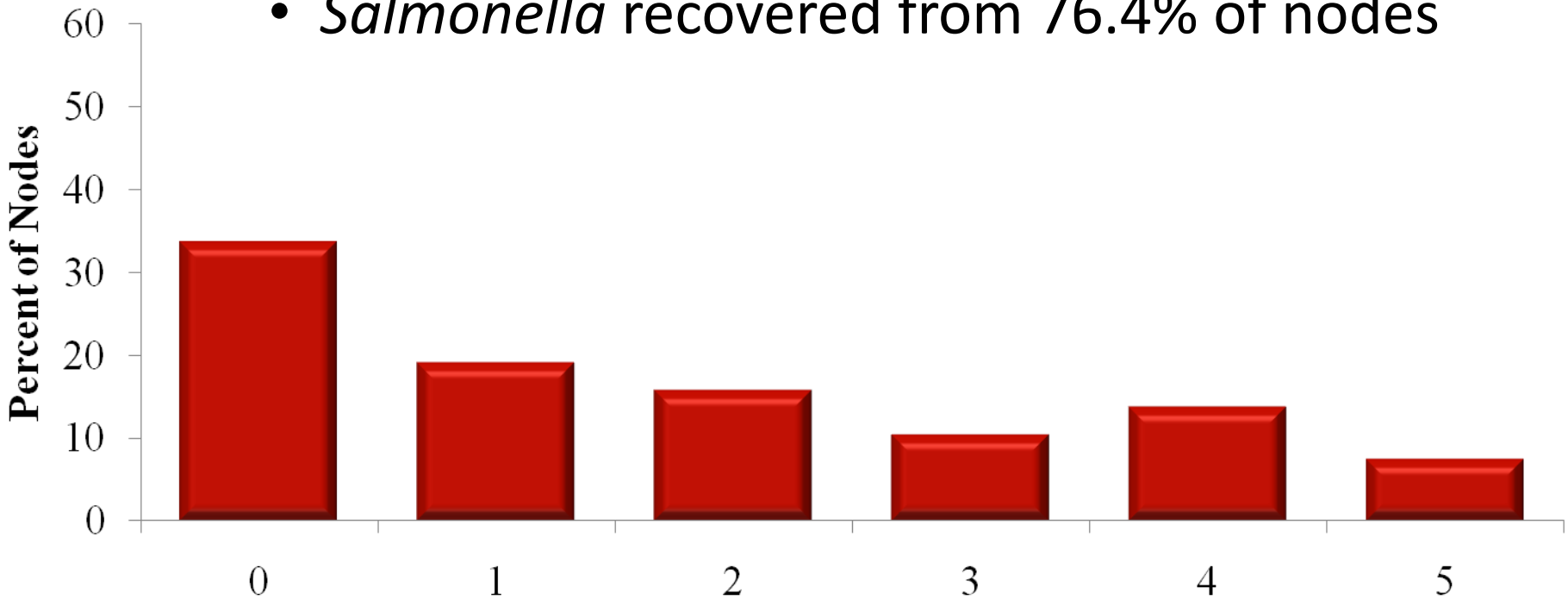


Prevalence in region 3 by month




66%
34%
60
50
40
30
20
10
0
6
5
4
3
2
1
0
% of LN containing
Salmonella at this level (n=144)
Log10 CFU/LN
<1.3
1.3 to 1.9
2.0 to 2.9
3.0 to 3.9
4.0 to >4.8
Log10 CFU/LN range

- A single subiliac lymph node collected from 300 carcasses across 12 pens of cattle
  - *Salmonella* recovered from 76.4% of nodes

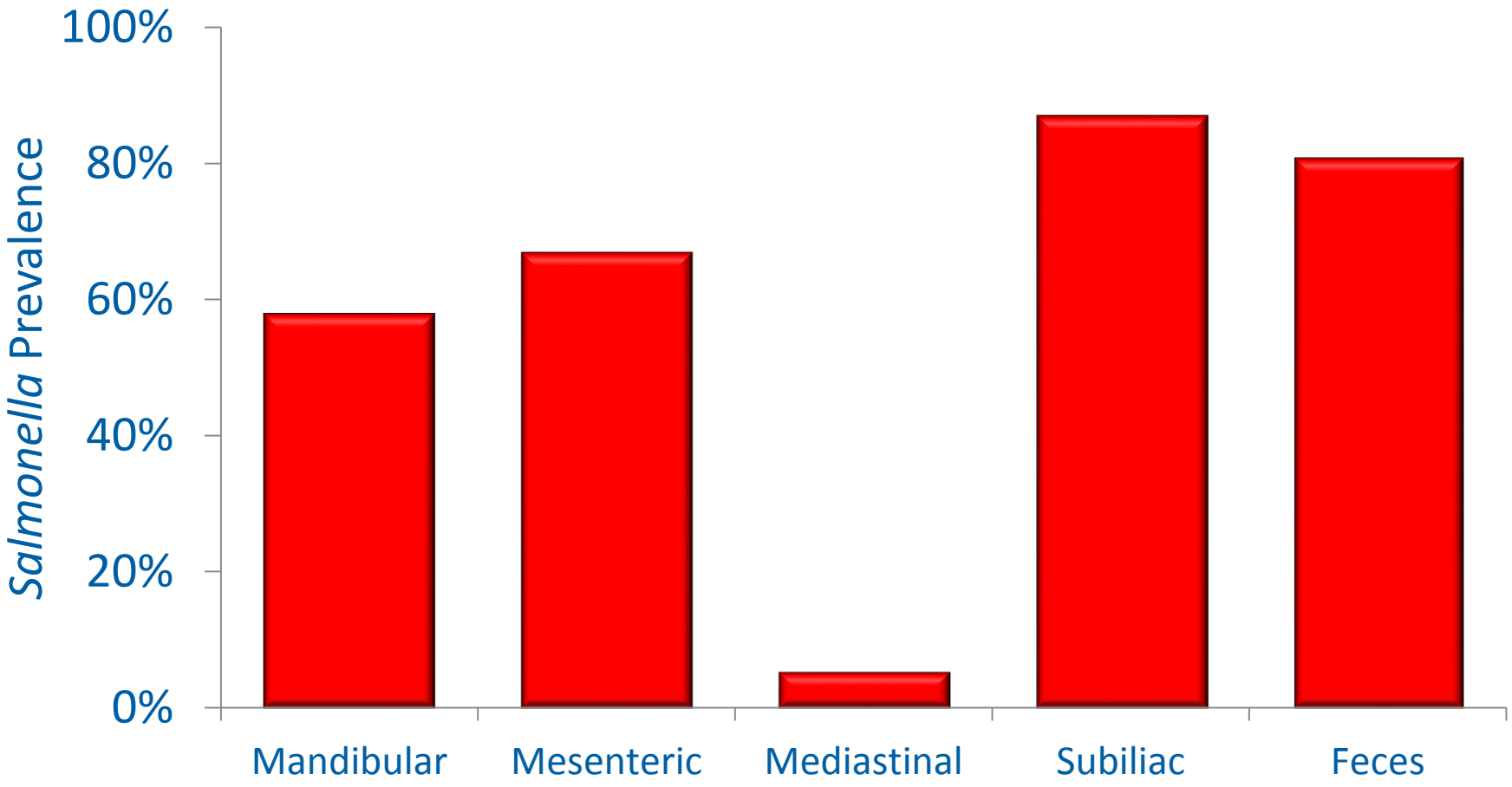


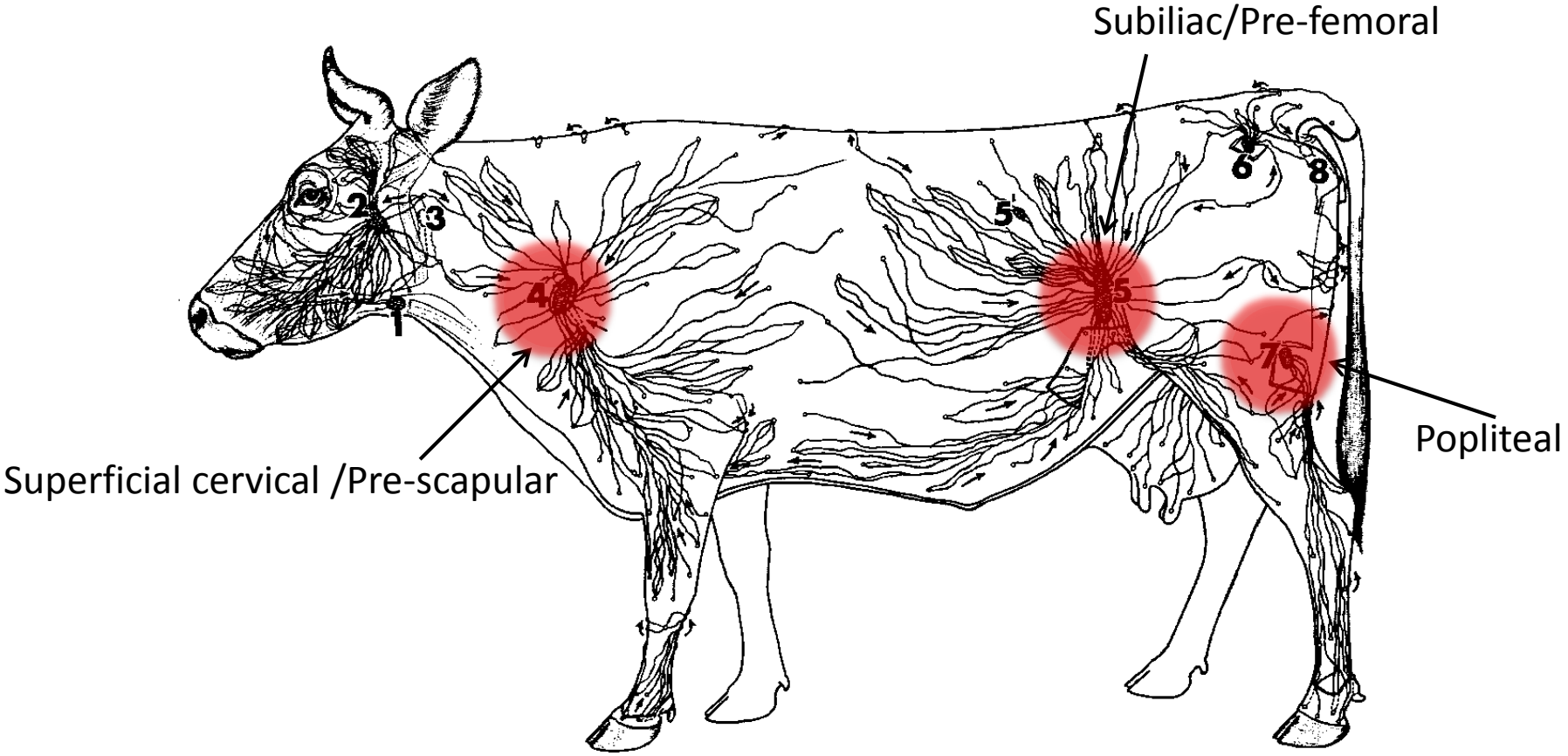
0: positive & le 1 log10 cfu/g
1: > 1 log10 cfu/g & le 2 log10 cfu/g
2: > 2 log10 cfu/g & le 3 log10 cfu/g
3: > 3 log10 cfu/g & le 4 log10 cfu/g
4: > 4 log10 cfu/g & le 5 log10 cfu/g
5: > 5 log10 cfu/g

Mexico Lymph Nodes

- An integrated Feedlot-Packing plant in Mexico
  - From a selection of animals and carcasses, multiple samples were collected
    - Feces & hides swabs
    - Subiliac, mandibular, mesenteric, and mediastinal
- Brought back to the U.S. (under APHIS permit) and cultured for *Salmonella*
  - PFGE, gene interrogation, serotyping

Mexico Lymph Nodes




Subiliac/Pre-femoral
Superficial cervical /Pre-scapular
Popliteal
1
2
3
4
5
5
6
7
8

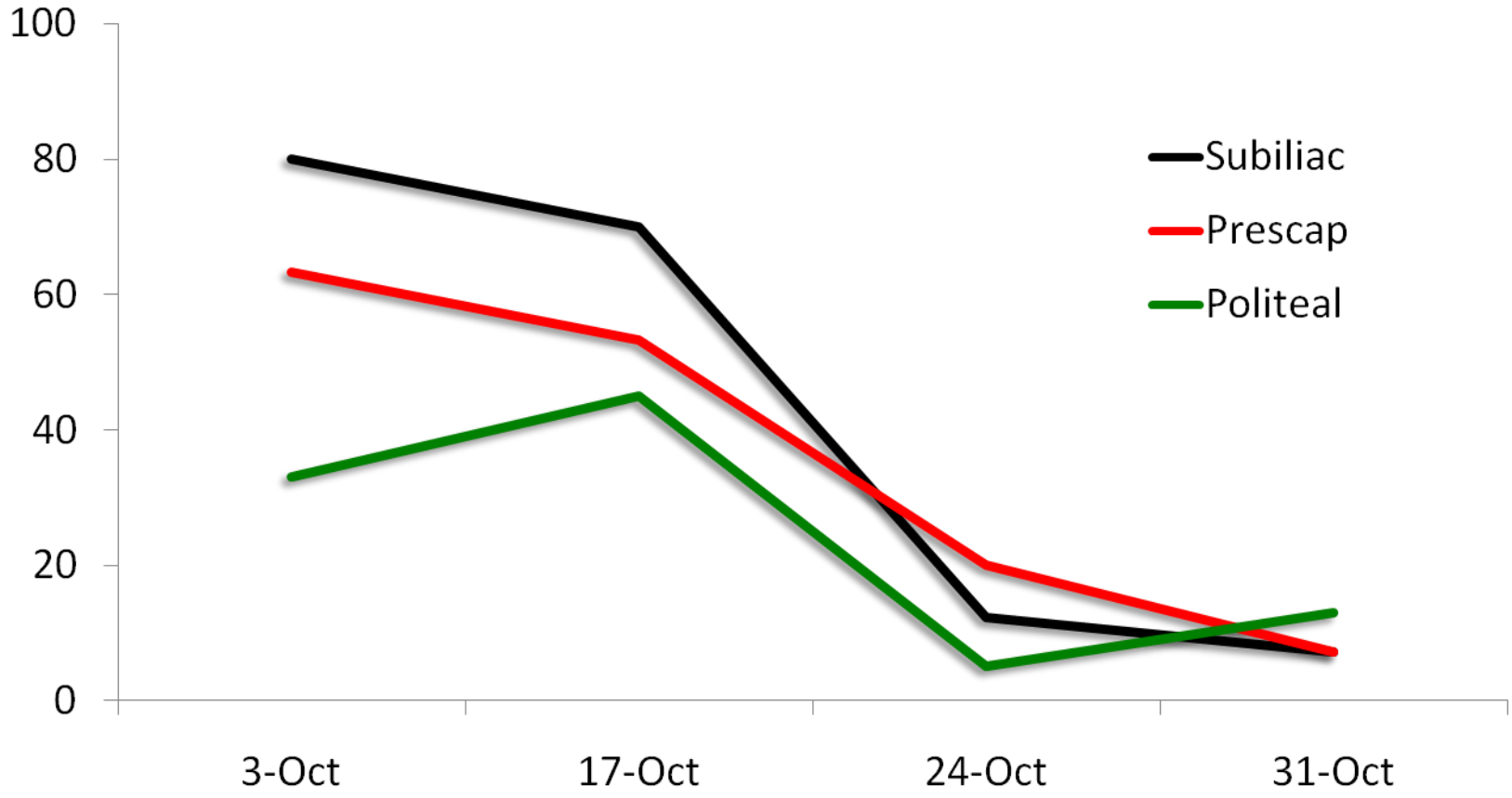
Multiple lymph nodes per carcass

Visit	Carcasses Sampled	Fecal prevalence	Node prevalence	Positive in 1 or more	Positive in all 6
03OCT	15	80%	58.9%		
17OCT	30	100% (n=6)	56.1%		
24OCT	20	95%	15.8%		
31OCT	35	47.8% (23)	9.1%		

Multiple lymph nodes per carcass

Visit	Carcasses Sampled	Fecal prevalence	Node prevalence	Positive in 1 or more	Positive in all 6
03OCT	15	80%	58.9%	100%	20%
17OCT	30	100% (n=6)	56.1%	96.7%	23.3%
24OCT	20	95%	15.8%	50%	5%
31OCT	35	47.8% (23)	9.1%	37.1%	0%

Prevalence by Node Overtime



BEEF CHORIZO CON CARNE DE RES

ARTIFICIALLY COLORED WITH FD & C RED NO. 40

REMOVE CASING BEFORE COOKING

KEEP REFRIGERATED

P8000105

Nutrition Facts

Serv. Size 1 Link (45g)
Servings 5
Calories 150
Fat Cal. 120

*Percent Daily Values (DV) are based on a 2,000 calorie diet.

Amount/serving	%DV*	Amount/serving	%DV*
Total Fat 13g	20%	**Total Carb.** 3g	1%
Sat. Fat 6g	30%	Fiber 0g	0%
Trans Fat 0g		Sugars 1g	
Cholest. 60mg	20%	**Protein** 5g	
Sodium 550mg	23%		

Vitamin A 10% • Vitamin C 0% • Calcium 0% • Iron 4%

INGREDIENTS: BEEF SALIVARY GLANDS, LYMPH NODES AND FAT (CHEEK/TONGUE), BEEF, BEEF TRIPE, FLAVORING, CEREAL FLOURS (CORN, WHEAT, OAT, RYE, RICE), SALT, DEXTROSE, CONTAINS 2% OR LESS OF WATER, SODIUM NITRITE, FD&C RED NO. 40. CONTAINS WHEAT

MAY052012

SELL BY

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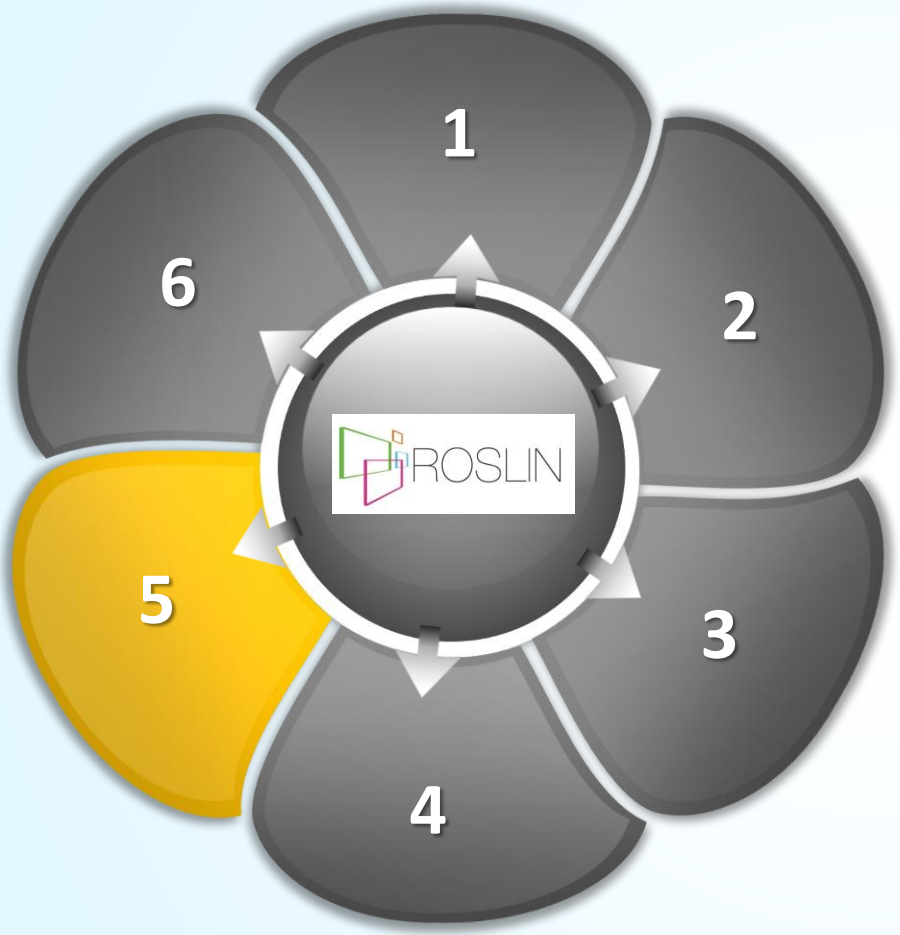
U.S. INSPECTED

University of Edinburgh



Mark Stevens

- 140 principal investigators
- $42 million annually
- 500 articles annually
- 19 research institutes

The Easter Bush Campus

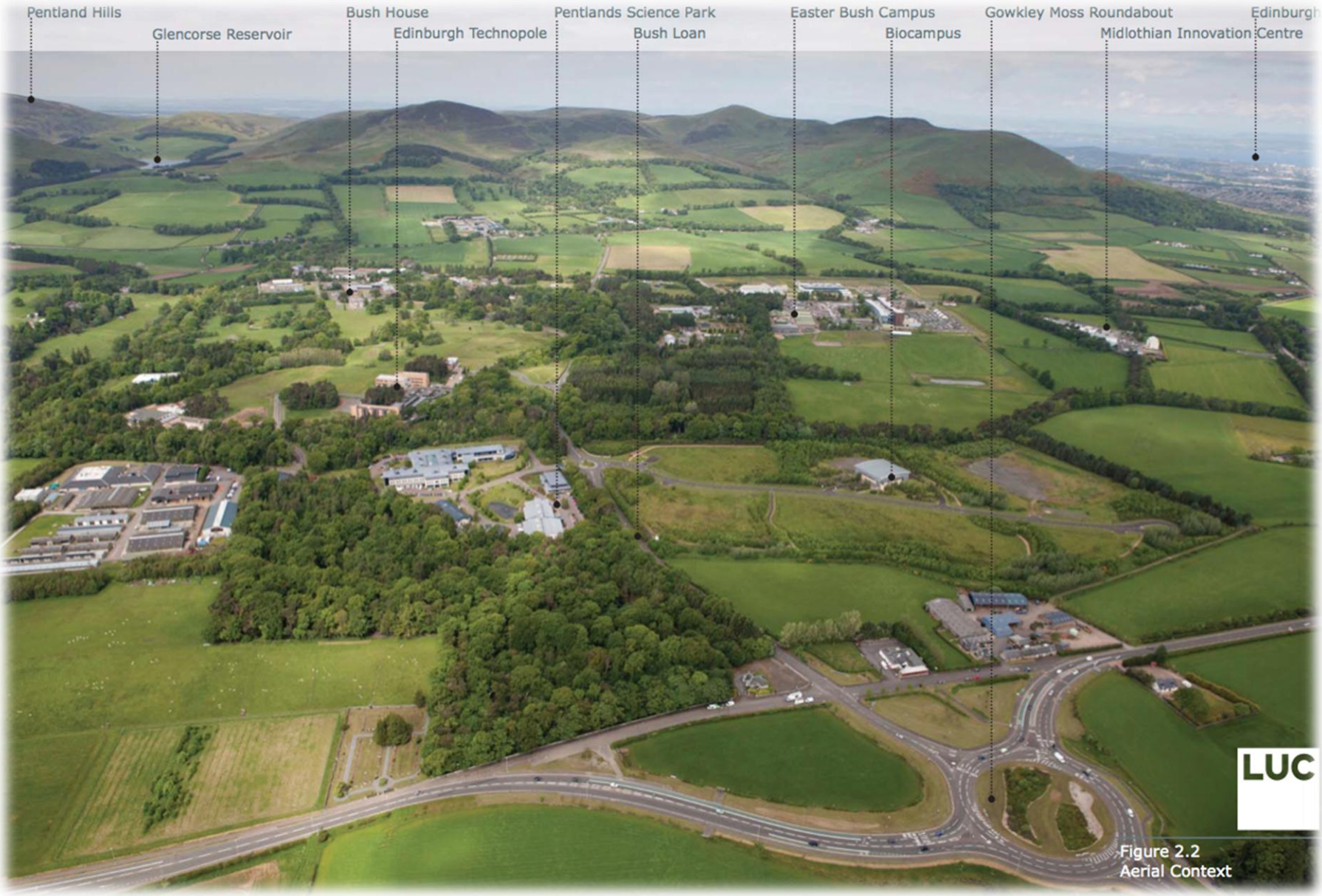
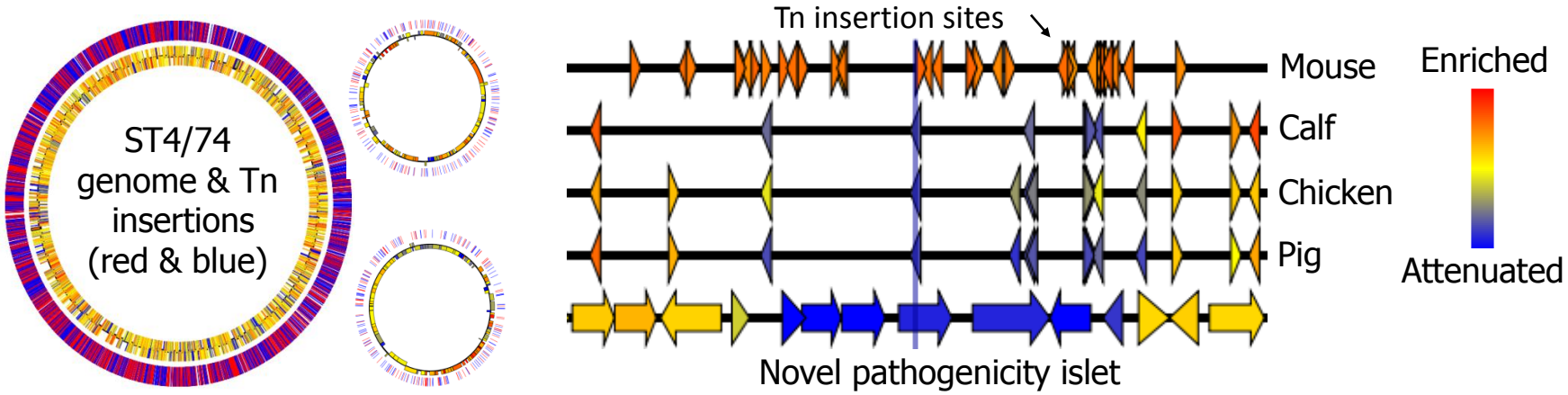


Figure 2.2
Aerial Context

Assignment of roles to *Salmonella* genes in intestinal colonisation



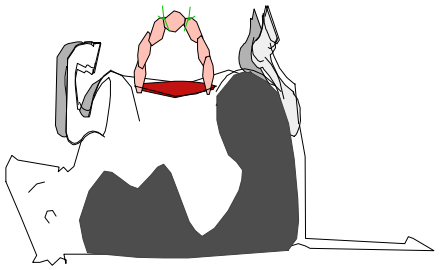
- Transposon-directed insertion-site sequencing (TraDIS) used to screen a library of 8550 *S.* Typhimurium mutants in cattle, pigs & chickens
- Simultaneously assigned the insertion site & fitness score for >90% of mutants screened
- Roles defined for 2721 different genes, including some that act in conserved- & host-specific ways
- Significant advance of 3R principles & provides valuable data for the design of control strategies

ROSLIN

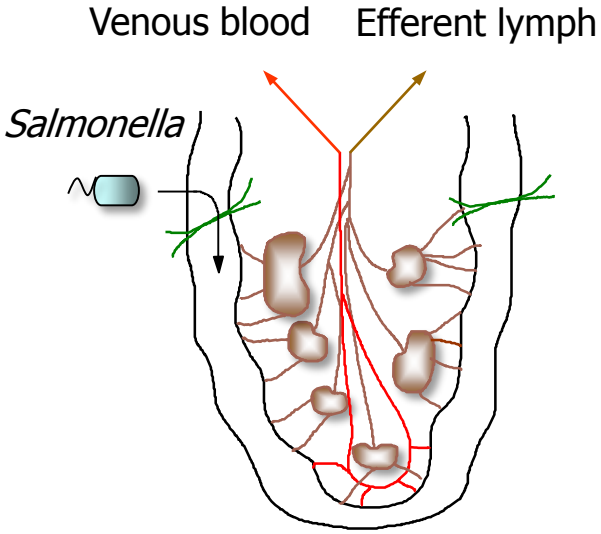
Chaudhuri et al. 2013. PLoS Genet 9:e1003456

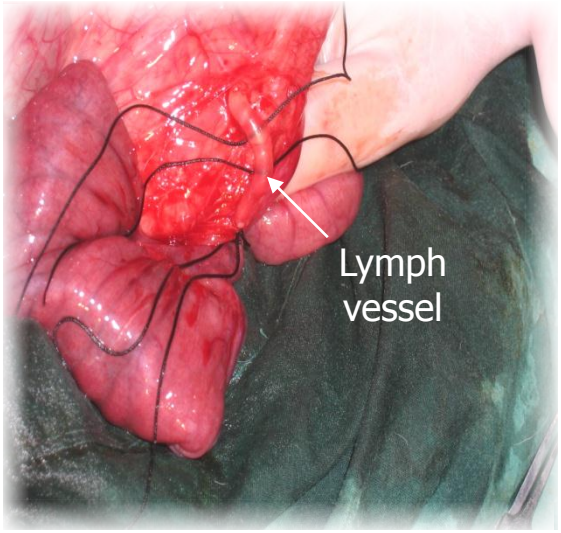
Cannulation of intestinal blood & lymph vessels to define the mode of systemic translocation of *S.* Dublin

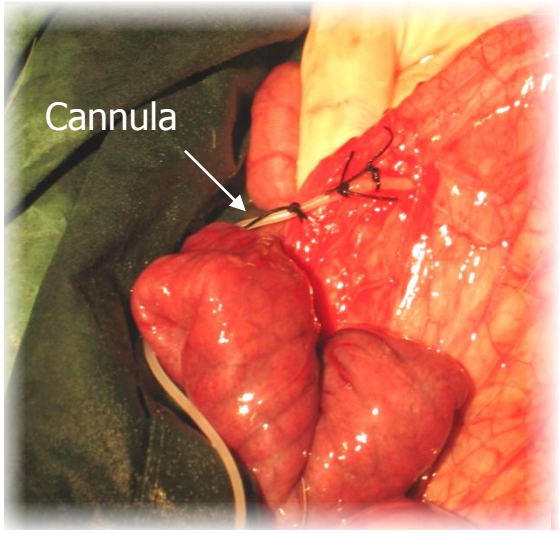
Laparotomy under terminal anaesthesia



Cannulation of venous and efferent lymph vessels serving distal ileal loop







How do *Salmonella* transit from the bovine intestines to draining MLN & distal lymph nodes?

- Define the mode of translocation of *S*. Dublin from the gut by cannulation of afferent & efferent lymph vessels
- Plan to study translocation from various sites, both early after injection into gut lumen and in orally dosed calves & steers
- Tissue & lymph to be collected for immunohistochemical & flow cytometric analysis

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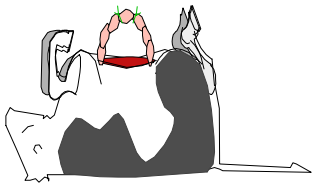
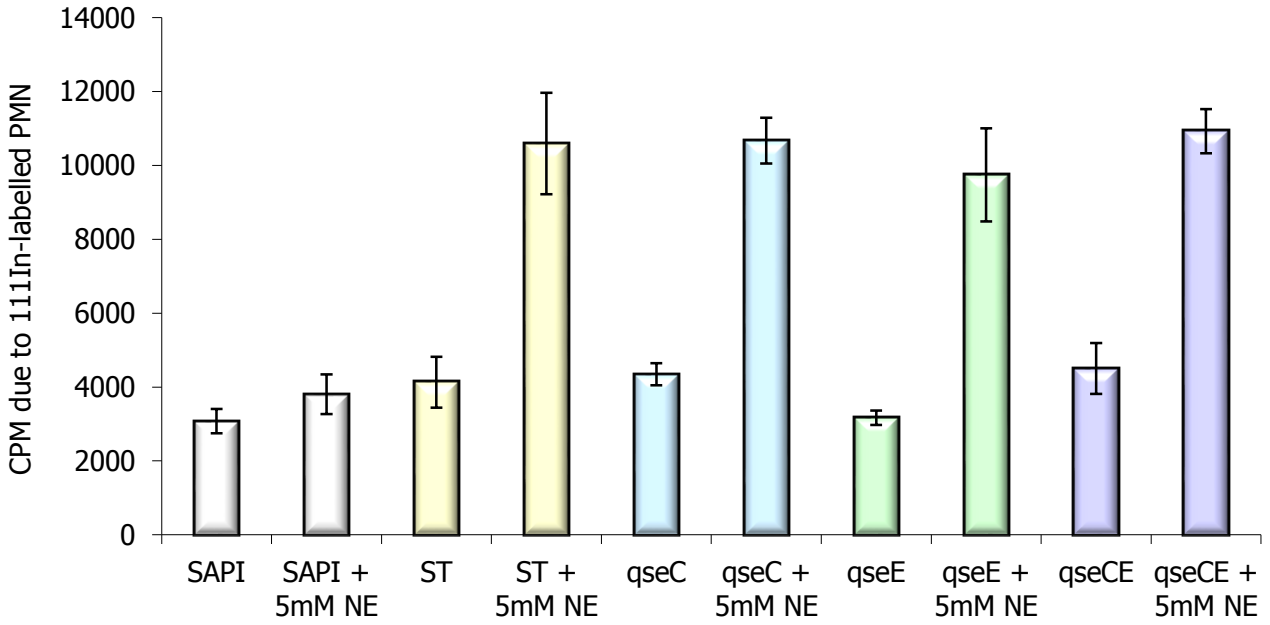
Analysis of the impact of stress-related catecholamines on *Salmonella* pathogenesis



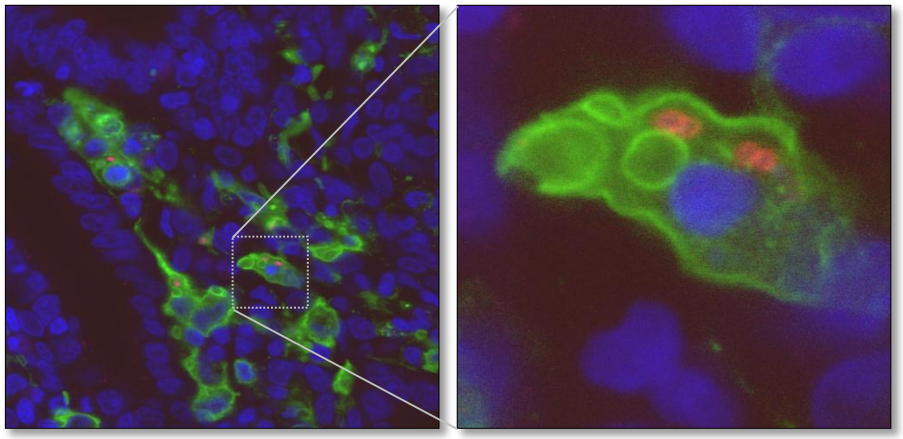


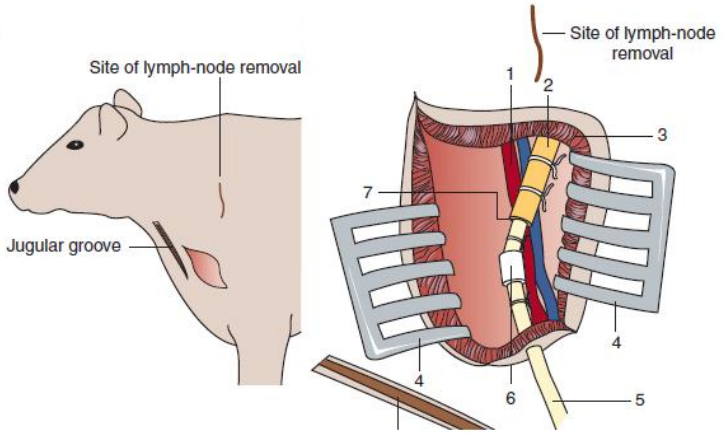
- Norepinephrine augments *Salmonella*-induced enteritis in bovine ligated intestinal loops
- Effect independent of putative bacterial adrenergic sensors
- Associated with increased net bacterial replication by analysis of plasmid-partitioning

ROSLIN

Pullinger et al. 2010. I&I 78:372-80

Which *Salmonella*-host cell interactions occur & what are their consequences?

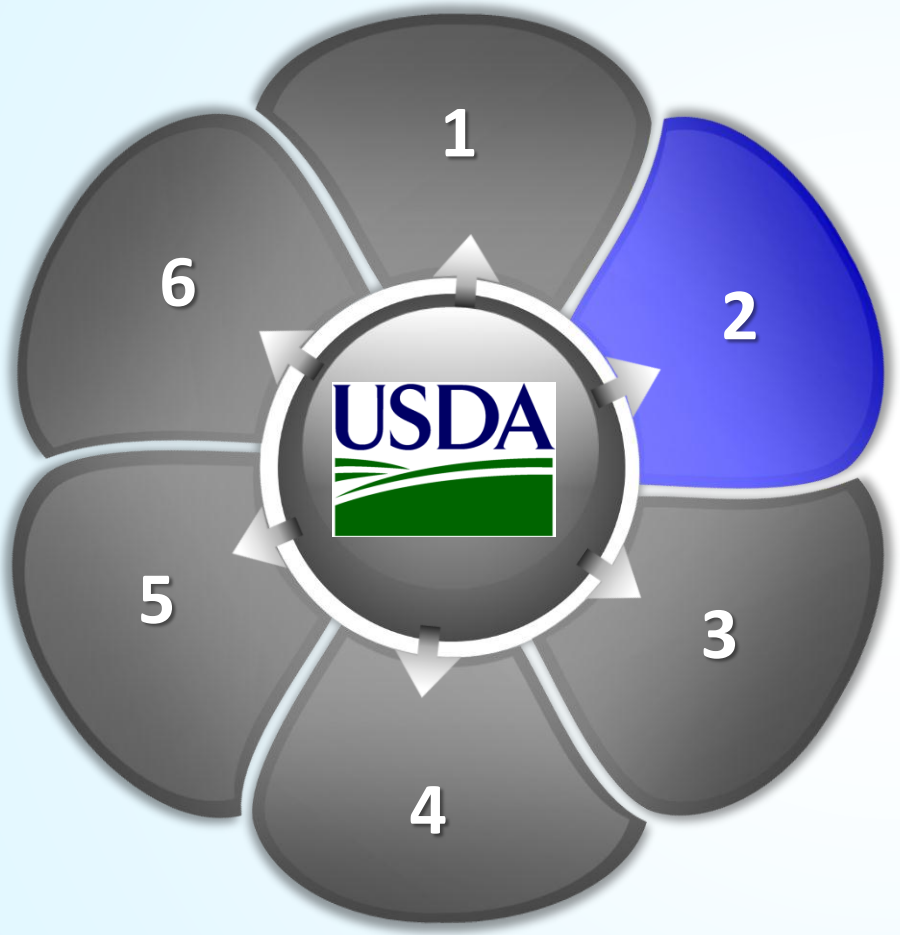




- The nature & consequences of *Salmonella*-APC interactions and basis of evasion or activation of immune responses are unknown
- Cell suspensions from mucosa, MLN & lymph to be analysed by multi-parametric flow cytometry for surface markers
- Live infected cells to be isolated & phenotyped by sorting for GFP-*S.* Dublin, esp. for expression of MHC, co-stimulatory molecules etc.
- Net bacterial replication to be analysed
- Cell tropism to be confirmed using afferent lymph cells sorted from skin

ROSLIN

USDA, College Station



Tom Edrington

- Preharvest Methods That Control Foodborne Pathogens
- Outstanding Relationships with TAMU Veterinary School
- Working relationship with USDA Livestock Insect Research Lab located in Kerrville, TX

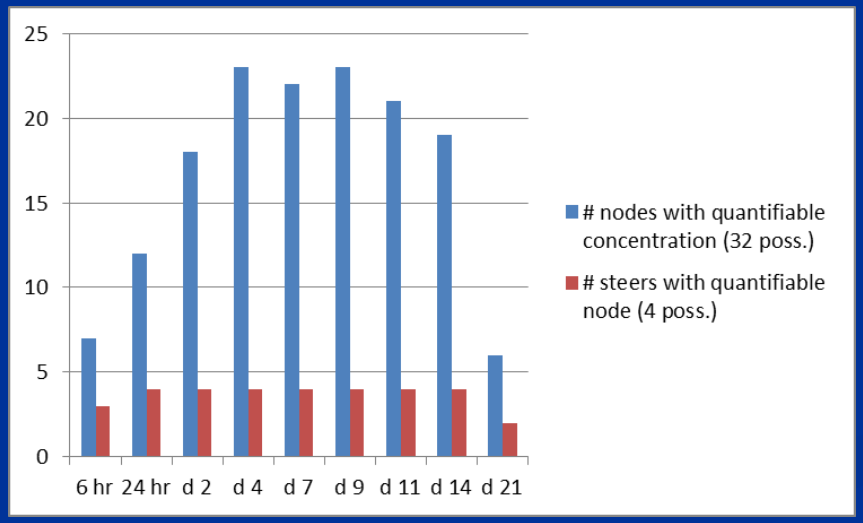
Transdermal Infection Model

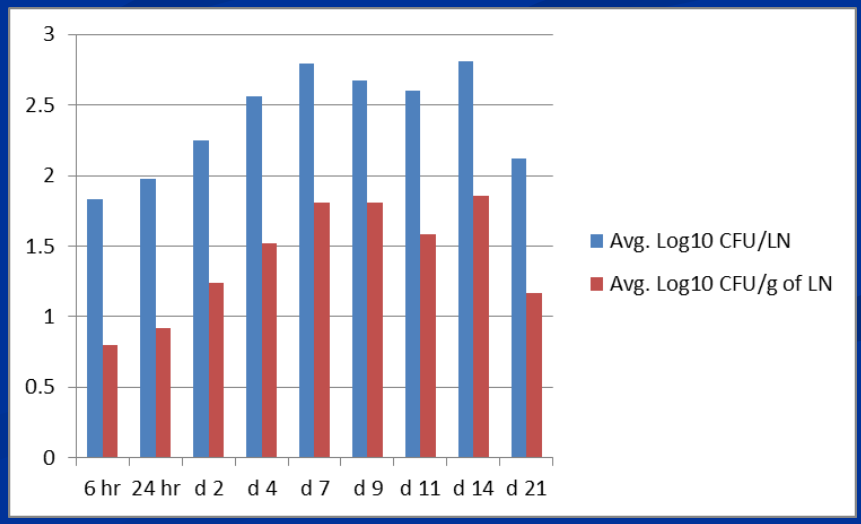
- Reliably produces *Salmonella* positive PLN
- To Date:
  - 3 pilot studies and 6 experiments (vaccine and other)
  - Strains used: MV, NP, Typ, Anat, Senft
  - Dose - 10^8 singly, 10^6 multiple
  - Time from last inoculation to necropsy: 2 – 28 d
  - Inoculation sites: lower legs, shoulders/back, paunch

T.S. Edrington, USDA-ARS-FFSRU,
College Station, TX

Duration of Infection

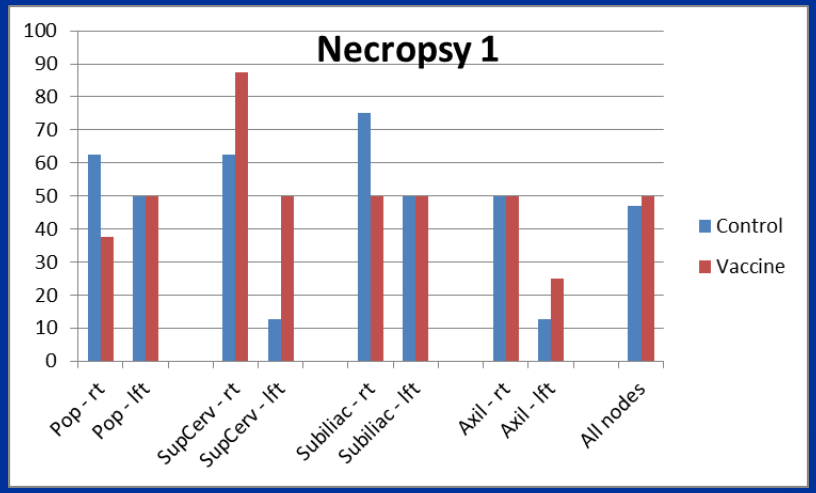
- Recently completed – data analysis in progress
- Inoculate day 0
  - *Salmonella* Montevideo, 1 x 10^9, 40 "bites" per leg, each side of belly and back
- Necropsy – 6 h, d 1, 2, 4, 7, 9, 11, 14 and 21

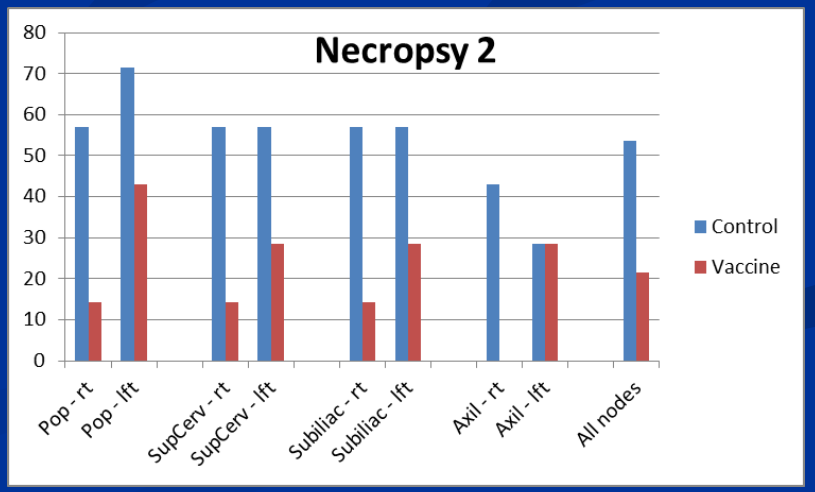




Transdermal Inoculation & SRP Vaccine

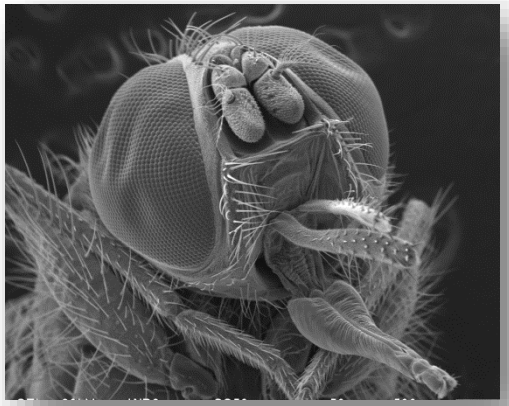
- Initial experiments indicate limited vaccine efficacy
- Modification of the experimental protocol allowed for better inoculation of peripheral lymph nodes and additional time for vaccine effect





What is the role of biting flies in producing *Salmonella* positive PLN?

- Collaborators: Drs. Pia Olafson and Kim Lohmeyer
  - ARS-Kerrville, TX
- Questions:
  - If fly eggs are laid in *Salmonella* contaminated manure will *Salmonella*-positive adults emerge?
  - If an adult fly acquires *Salmonella* when feeding, does the *Salmonella* survive in the fly gut? Where? How long? Shed in feces?
  - What flies are important? Stable? Horn? House?
  - If a fly bites a *Salmonella*-contaminated hide, will that produce *Salmonella*-positive PLN?



Hypothesis:
Horn fly embryos deposited on *Salmonella*-contaminated manure will produce adult flies contaminated with *Salmonella*

Data from this study refutes the hypothesis;
however, pupal casings may serve as source of contamination

Conclusions

- The presence of *Salmonella* contamination in manure significantly reduced adult emergence

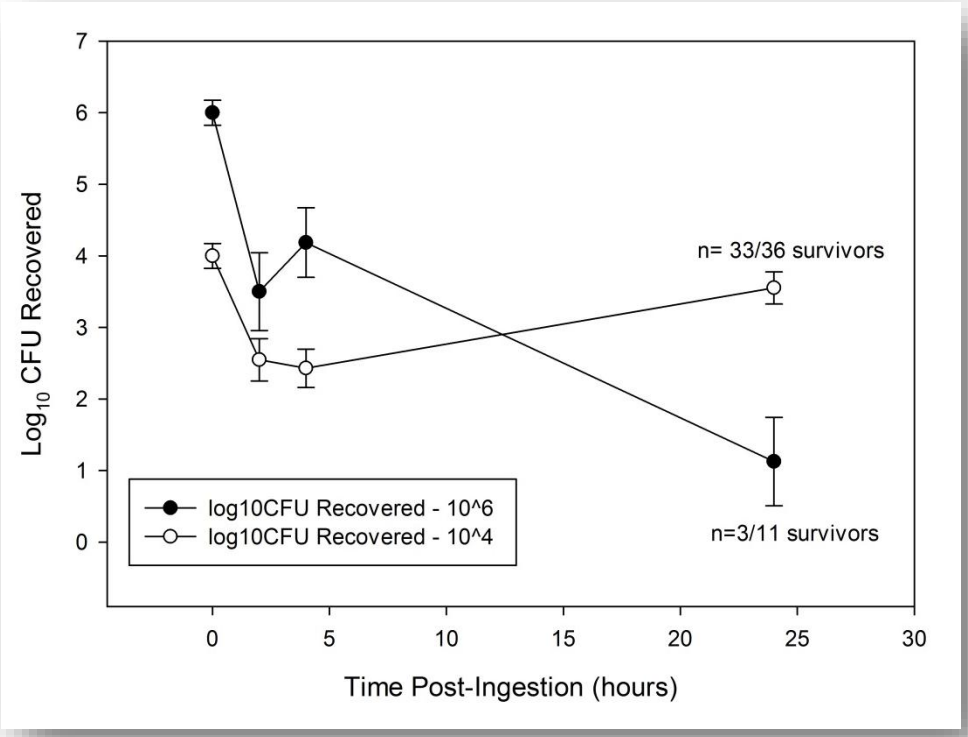
- A significant proportion (47%) of pupal casings from adults that emerge from *Salmonella*-contaminated manure are contaminated
  - Average recovery: **2.48 x 10^4 CFU Salm304/positive pupal case**

- **However, adults** that emerge from *Salmonella*-contaminated manure **are *rarely* surface-contaminated or infected with *Salmonella* (8%)**

Olafson and Lohmeyer, USDA-ARS,
Kerrville, TX

Recovery of *Salmonella* from Adult Horn Flies

Hypothesis:
The combination of the insect innate immune system and bacterial excretion will prevent or limit persistence of *Salmonella* ingested by adult horn flies



Results of bacteria recovery from starved flies at 24h PI support replication of *Salmonella* in the horn fly after ingesting a low dose**

Together with recovery of *Salmonella* at 24h post-ingestion in flies fed a high dose, **the hypothesis is rejected.**

Most importantly, the data suggest that horn fly adults are environmental reservoirs of *Salmonella*

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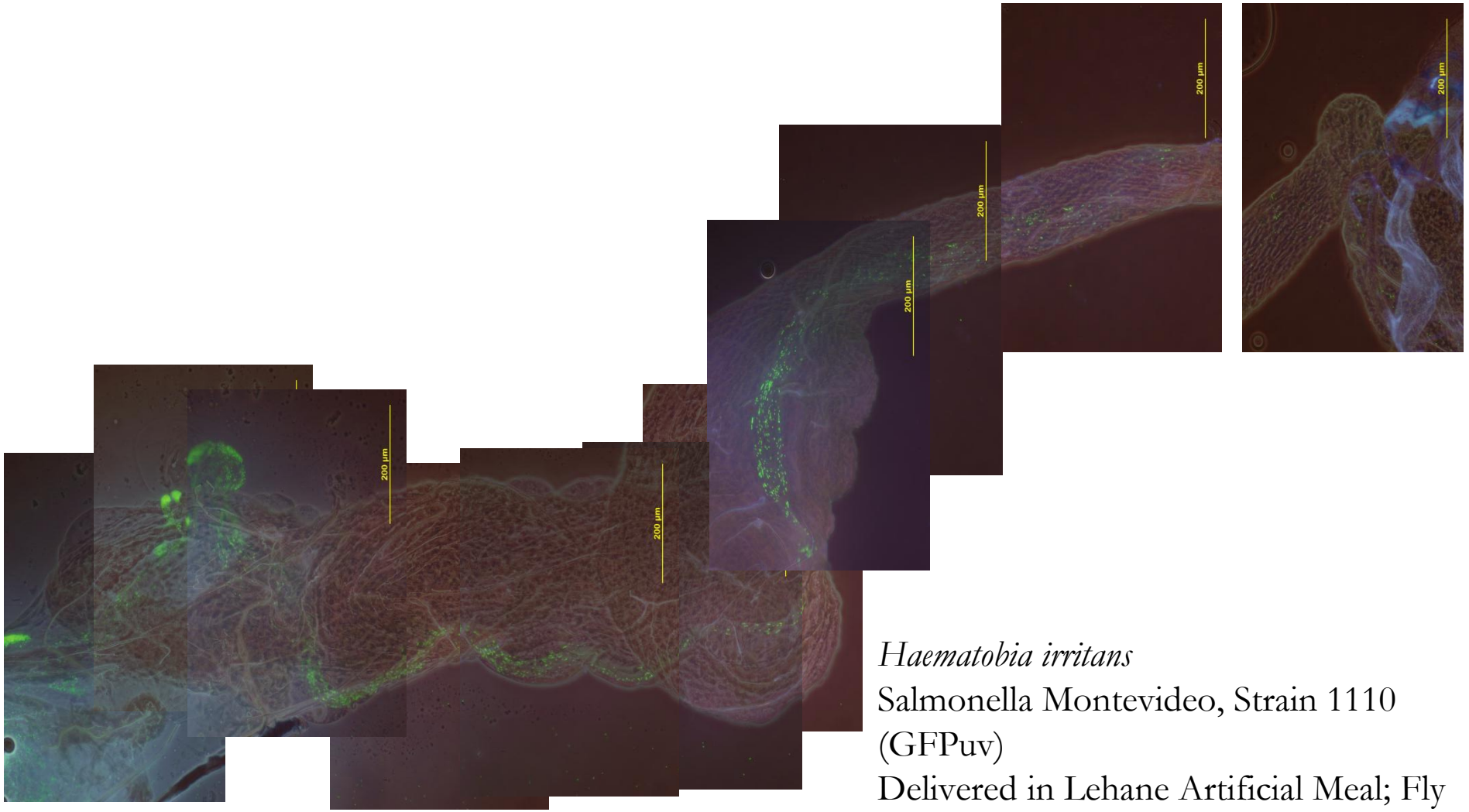
Kerrville, TX



Rectum

Haematobia irritans
Salmonella Montevideo, Strain 1110 (GFPuv)
Delivered in Lehane Artificial Meal; Fly observed feeding; Removed from receptacle upon completion
Estimated 0.5ul ingested = $8x10^5$ CFU
Alimentary canal dissected 2 hours post-ingestion

Olafson and Lohmeyer, USDA-ARS, Kerrville, TX

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