7th Annual Egg Industry Issues Forum
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# Salmonella and Eggs: What do we really know?

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# Salmonella and eggs

- SE and eggs
- Relationships: routes, egg formation, serotypes
- Interventions
  - FDA Egg Safety Rule
  - Vaccinations
- ISU Testing
- Final comments

# SE Emergence

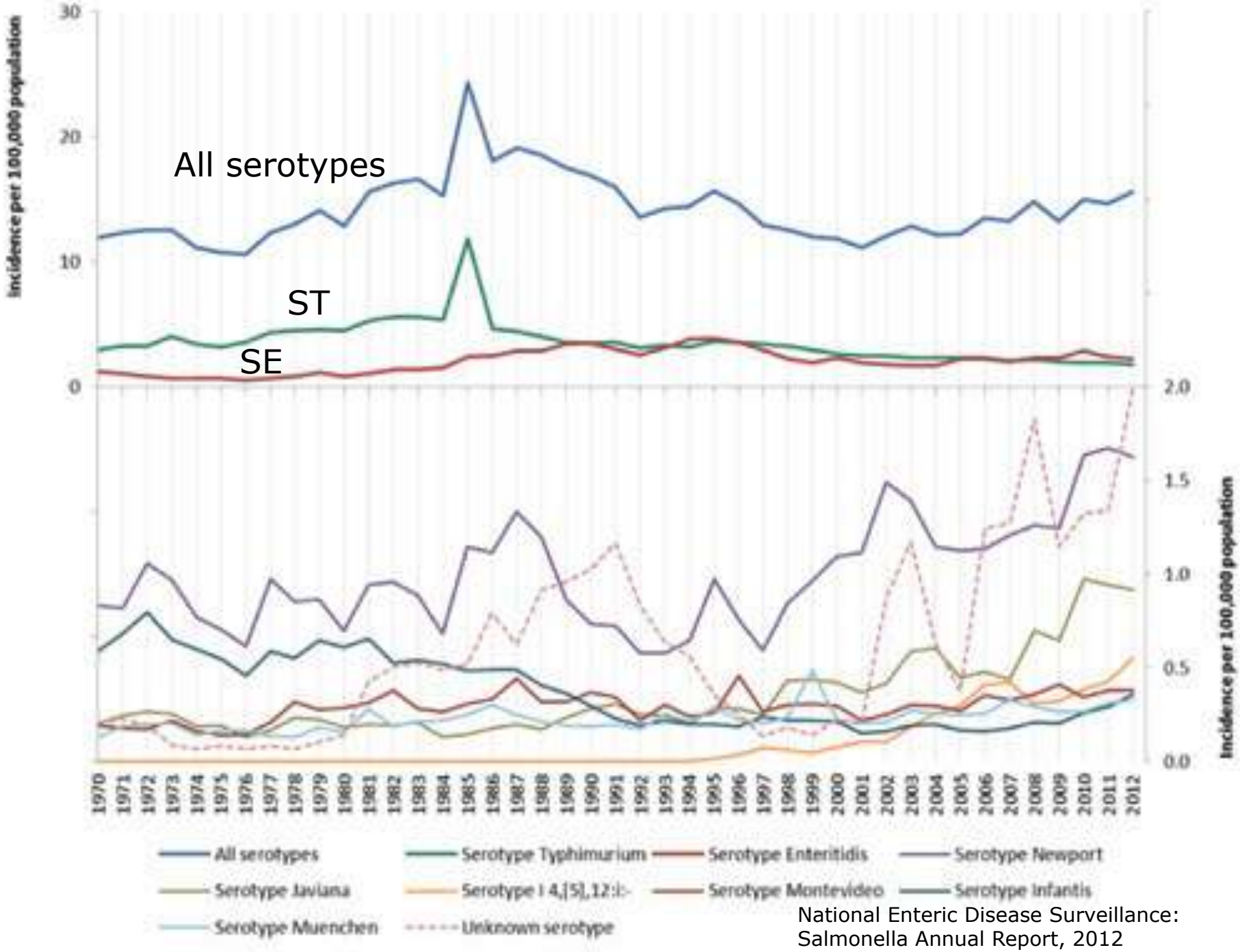
- Late 1980's became predominate Salmonella serotype found in human cases
- Linked to eggs and egg products
- Industry and government take measures to address
- Outbreaks decrease but SE remains common serotype

# SE Emergence

St Louis ME, Morse DL, Potter ME, DeMelfi TM, Guzewich JJ, Tauxe RV, et al.

**The emergence of grade A eggs as a major source of Salmonella enteritidis infections. New implications for the control of salmonellosis**.

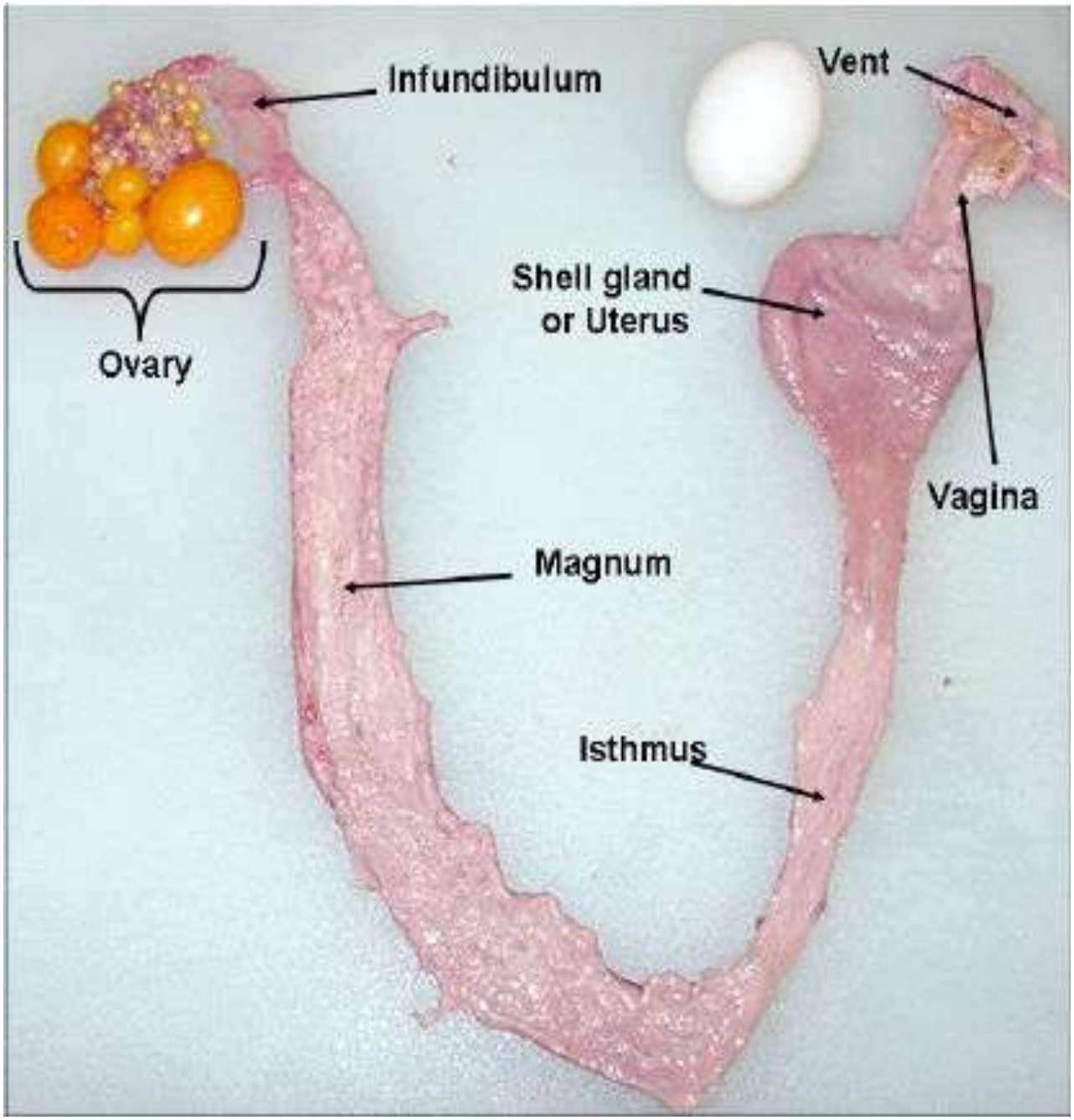
JAMA 1988 Apr;259(14):2103-7.




National Enteric Disease Surveillance: Salmonella Annual Report, 2012

# How does Salmonella get into an egg?

- Direct contamination
  - Colonization of reproductive organs -> contamination of yolk, membranes, albumen, shell before oviposition
- Egg shell penetration
  - Colonization of gut-> deposition of organisms on shell after oviposition and translocate through cracks, pores
- SE vs non-SE

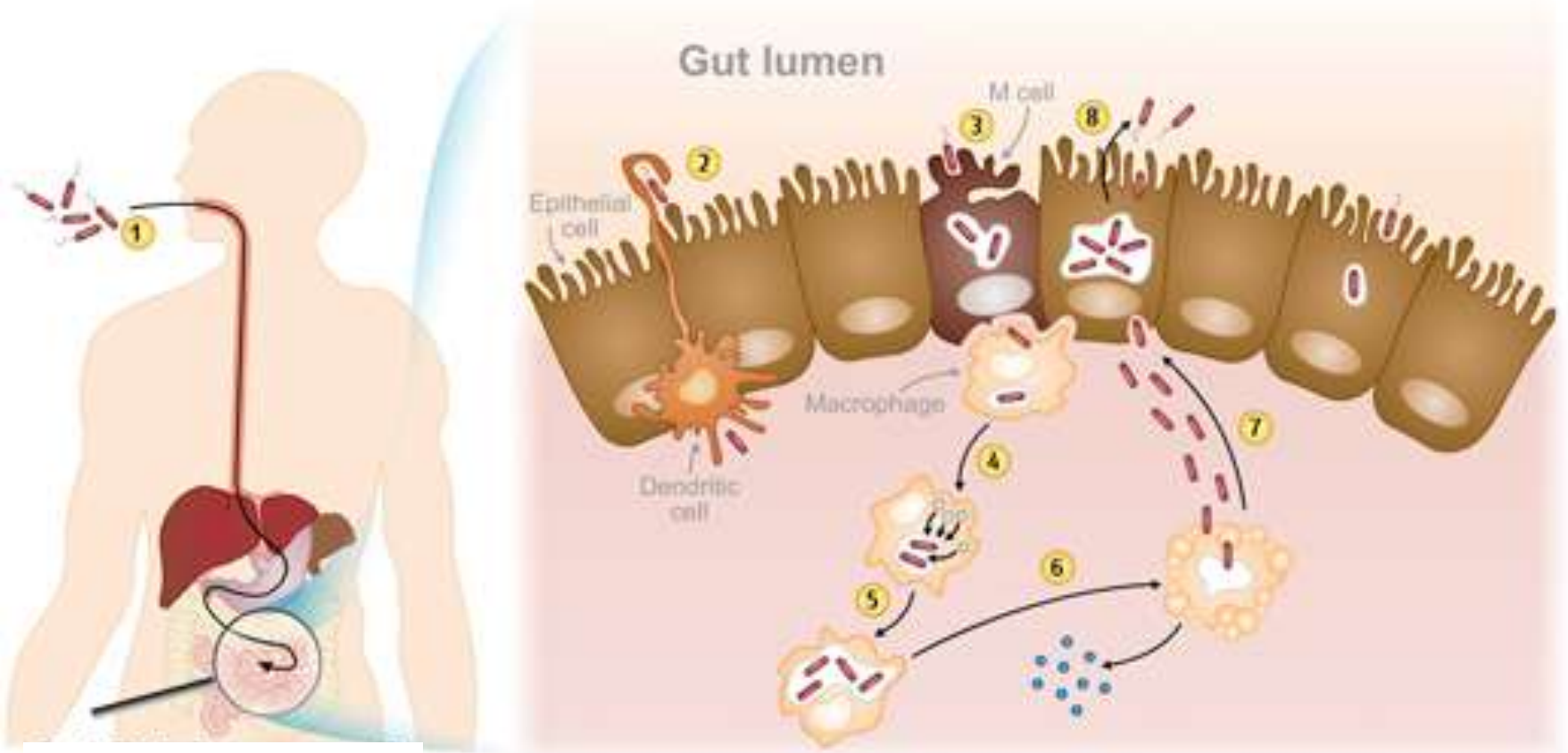



**Major Events in Egg Formation**

- Ovary: follicle to yolk formation.
  - Vitelline membrane
- Infundibulum: 15 minutes
  - Captures ovum/yolk
  - Fertilization
- Magnum: 3 hours
  - Largest section
  - Albumen
- Isthmus: 75 minutes
  - Shell membranes
- Uterus: 20+ hours
  - Longest stage
  - Egg shell
- Vagina: just a few minutes
  - Oviposition
  - Cuticle added to shell

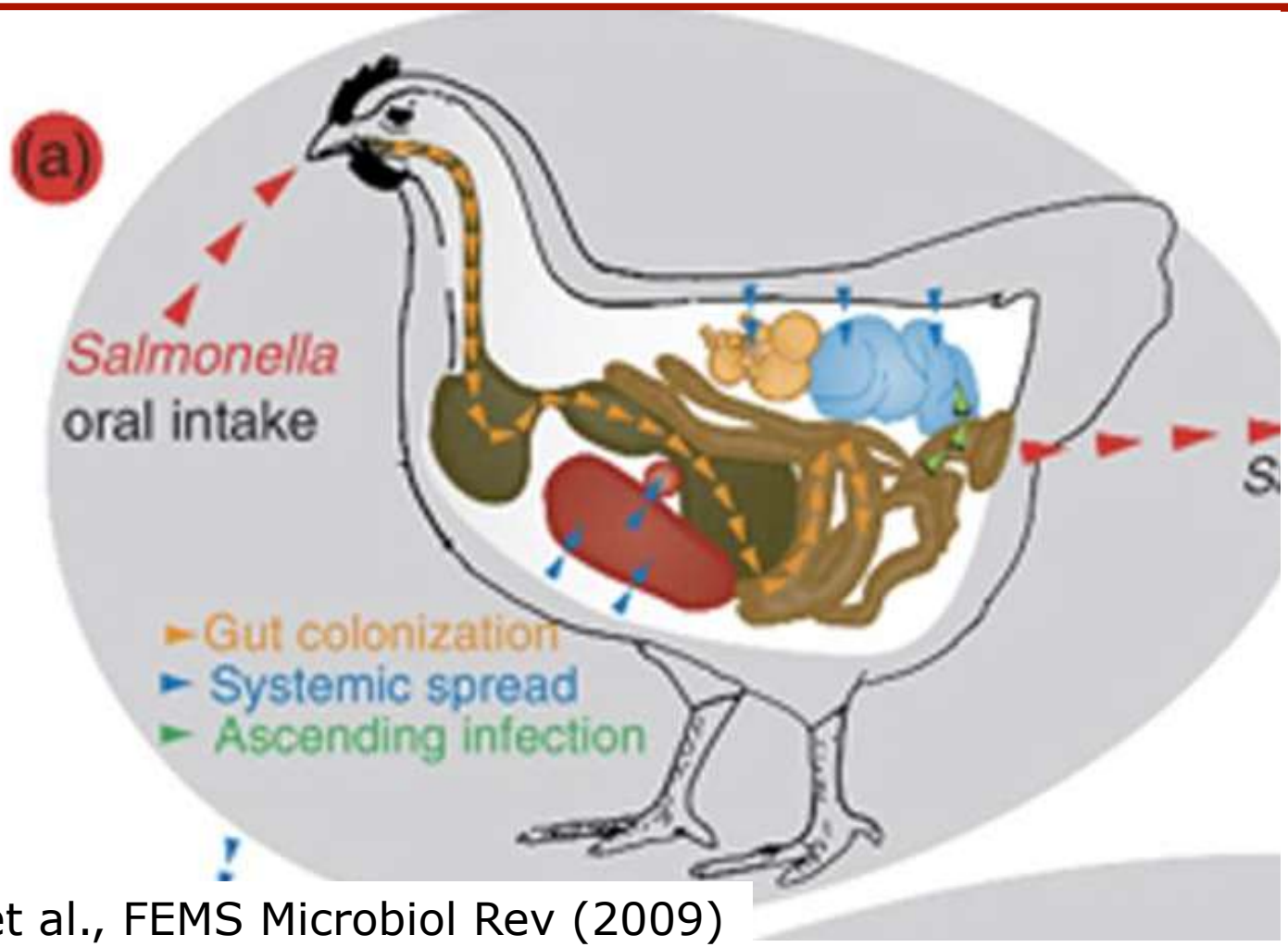
http://freefromharm.org/eggs-what-are-you-really-eating

# Salmonella infection



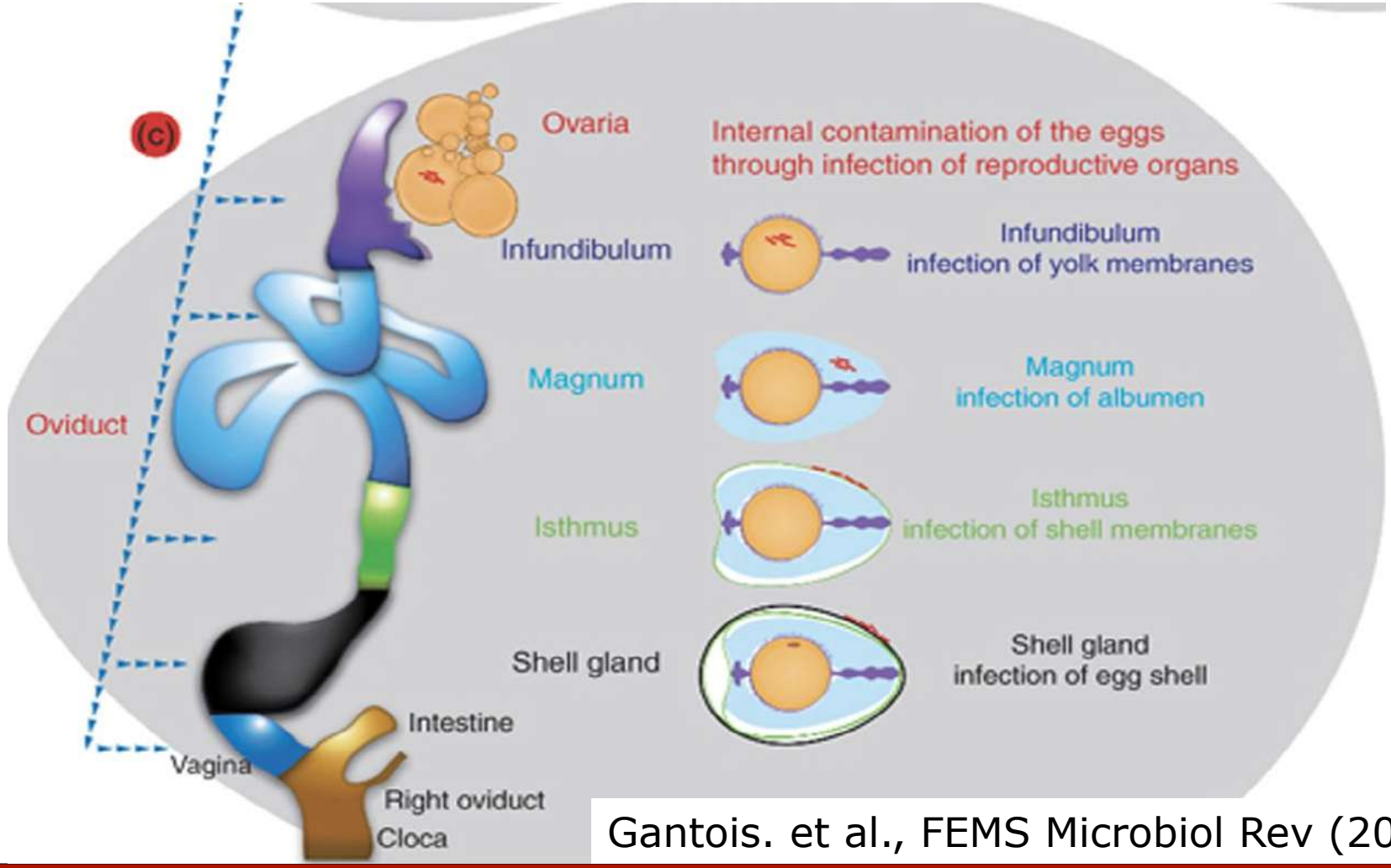

http://eng.sheba.co.il/Research_and_Development/Infectious_Diseases_Research_Lab/

# Salmonella infection



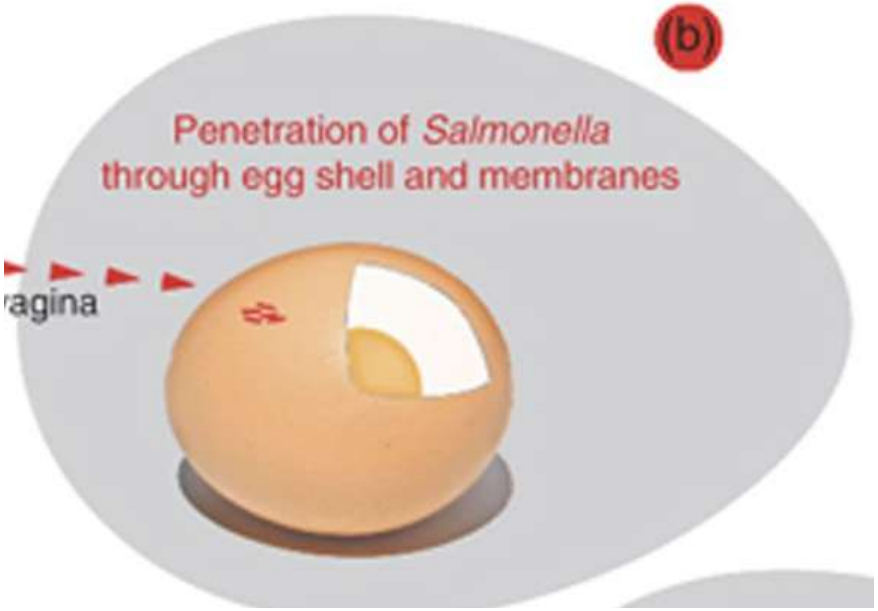

Gantois. et al., FEMS Microbiol Rev (2009)

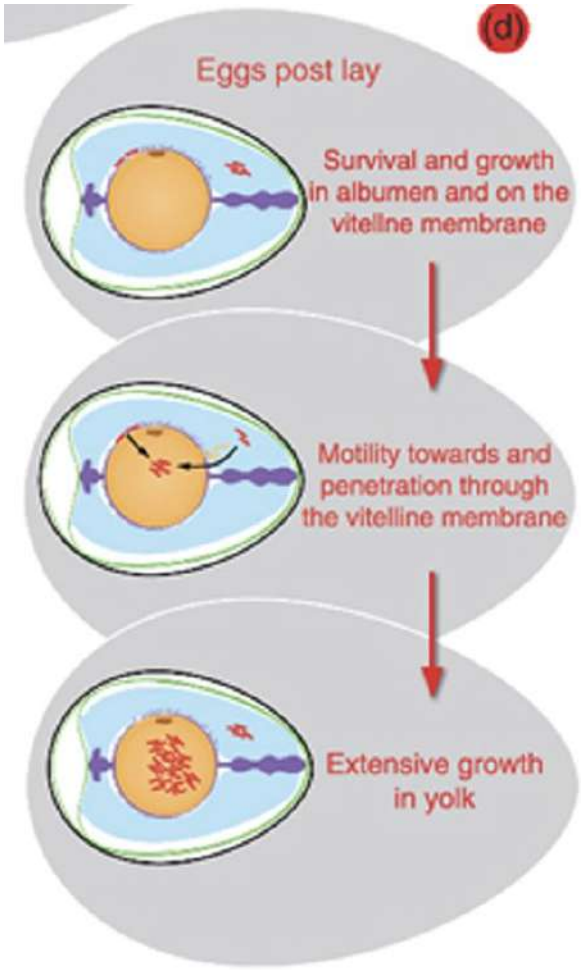
# Salmonella infection



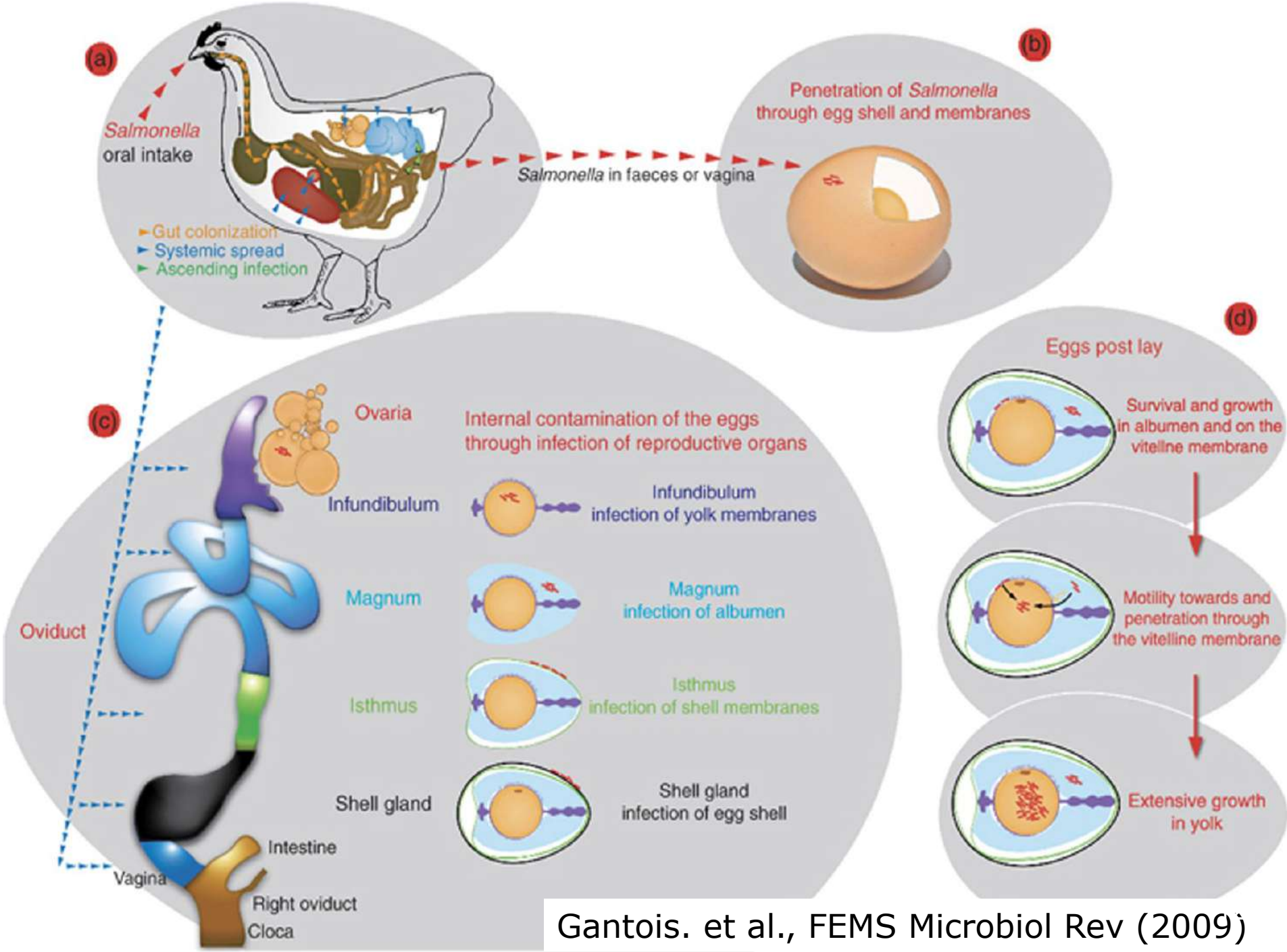

Gantois. et al., FEMS Microbiol Rev (2009)

# Salmonella infection




Gantois. et al., FEMS Microbiol Rev (2009)




Gantois. et al., FEMS Microbiol Rev (2009)

# SE advantages - Direct

- Relationship to S. Gallinarum
- Colonization of reproductive tissues
  - Lipopolysaccharide, fimbria, flagella,
- Other virulence factors
  - Survival in hostile environment
    - Antimicrobial peptide, inhibitory proteins
    - Stress tolerance
  - Secretion systems
- Strain variation within SE and other serotypes

# SE advantages - Egg Shell

- Survival on egg shell?
- Penetration of egg shell?
  - Motility?
- Survival in albumen?
  - Motility? Resistance?
- Growth in yolk?

# Interventions

- FDA Egg Safety Rule
  - SE targeted
    - prevention plan
    - biosecurity measures
    - pest control programs
    - storage and refrigeration conditions
    - testing of environment and eggs
- Vaccination not required but generally done since implementation of ESR

# Appropriate for Non-SE?

- ✓ prevention plan
  - ? Expanded NPIP, serotypes/serogroups, niches
- ✓ biosecurity measures
  - ? Feed
- ✓ pest control programs
- ✓ storage and refrigeration conditions
- ✓ testing of environment and eggs
  - Methods?
- ? Vaccination??

# Vaccination??

- Shedding versus systemic protection?
- Immune response?
- Route of administration?
- Type of vaccine?
- Cross protection?
- In ovo protection?
- Other Interventions?
  - Probiotics, prebiotics, supplements, exclusion

# ISU VDL Testing

# FDA testing requirements

- Testing to detect SE in environmental samples must be conducted by the method entitled “Environmental Sampling and Detection of Salmonella in Poultry Houses” or an equivalent method
- Testing to detect SE in egg samples must be conducted according to Chapter 5 of FDA’s Bacteriological Analytical Manual (BAM) or an equivalent method

# FDA Egg Safety Rule

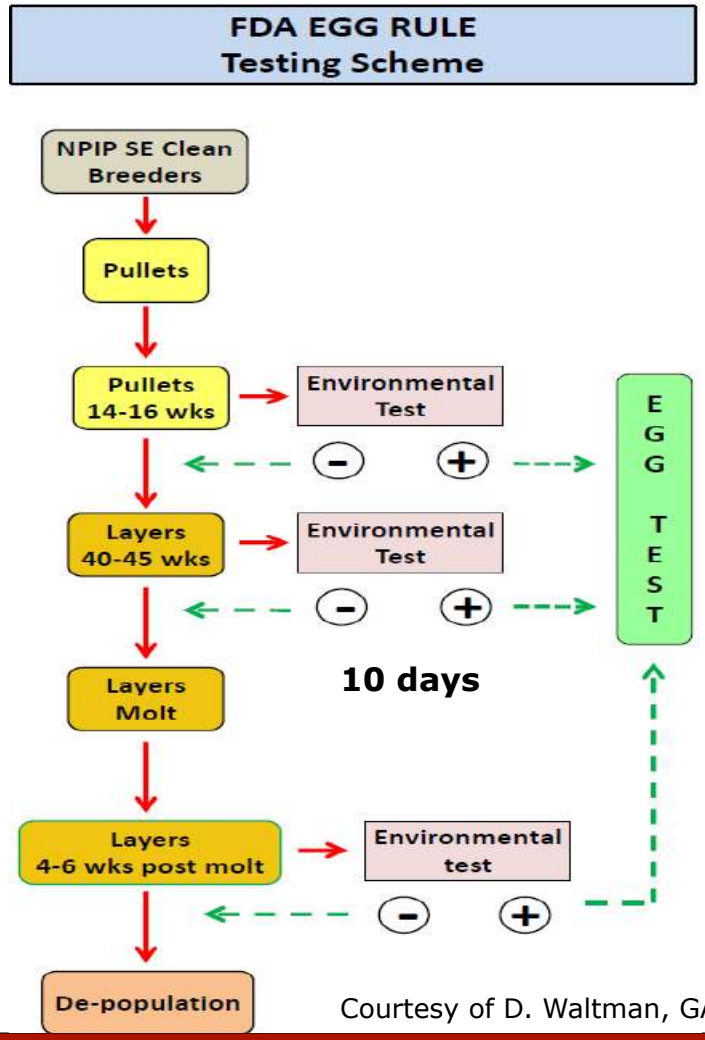
## Environmental testing

- 14-16 weeks
- 40-45 weeks
- 4-6 weeks after molt

## Egg testing

- 1,000 eggs @ 2 week intervals X 4
- 4,000 eggs/flock

## Effective 7/11/2010 and 2012




Courtesy of D. Waltman, GA Poultry Lab

**Guidance & Regulation**

Guidance Documents & Regulatory Information by Topic

Eggs

# Testing methodology for *Salmonella* Enteritidis (SE)

<< Egg Safety Final Rule Main Page

FDA has determined that the following methods are equivalent to "Environmental Sampling and Detection of *Salmonella* in Poultry Houses" (April 2008) in accuracy, precision, and sensitivity in detecting *Salmonella* Enteritidis:

1. "Procedures for collection, isolation and identification of *Salmonella* from environmental samples, cloacal swabs, chick box papers, and meconium samples," 9 CFR 147.12.(September 2010)
2. SDIX RapidChek SELECT™ *Salmonella* Enteritidis Test System
3. Neogen Reveal *Salmonella* Enteritidis (SE) Test System
4. Applied Biosystems TaqMan® Salmonella Enteritidis Detection Kit from Life Technologies.

FDA has determined that the following methods are equivalent to Chapter 5 (*Salmonella*) of FDA's Bacteriological Analytical Manual (BAM, December 2007 Edition) in accuracy, precision, and sensitivity in detecting *Salmonella* Enteritidis:

1. Applied Biosystems TaqMan® *Salmonella* Enteritidis Detection Kit from Life Technologies, both with and without the 96-hour hold time recommended by the BAM.
2. SDIX RapidChek SELECT™ *Salmonella* Enteritidis Test System, without the 96-hour hold time recommended by the BAM.
3. Neogen Reveal *Salmonella* Enteritidis (SE) Test System, but ***only*** with the 96-hour hold time recommended by the BAM. It is ***not*** considered equivalent without the 96-hour hold time.
4. The BAX® System PCR Assay for *Salmonella* and the BAX® System PCR Assay for *Salmonella* 2, without the 96-hour hold time recommended by the BAM.
5. Chapter 5 (*Salmonella*) of FDA's Bacteriological Analytical Manual (BAM, February 2014 Edition).

# SE Testing Process

- Screen samples with immunoassay
- Culture samples that are positive
- Isolate and identify Group D1 organisms
  - If none found → reported as negative (rare)
  - If isolates found → forward to NVSL
- If SE confirmed from NVSL then reported as SE positive

Add Enrichment

Environmental Sample


Sample Submission

Inspect & Clean Eggs

Disinfect Eggs

Incubate Samples

Screening Test

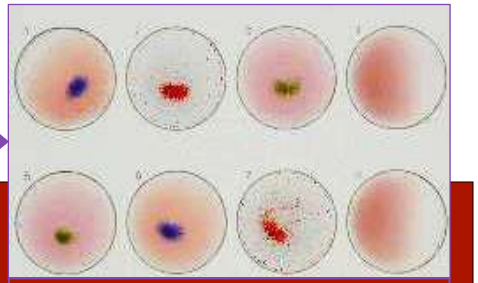
Incubate Samples

Pool Samples

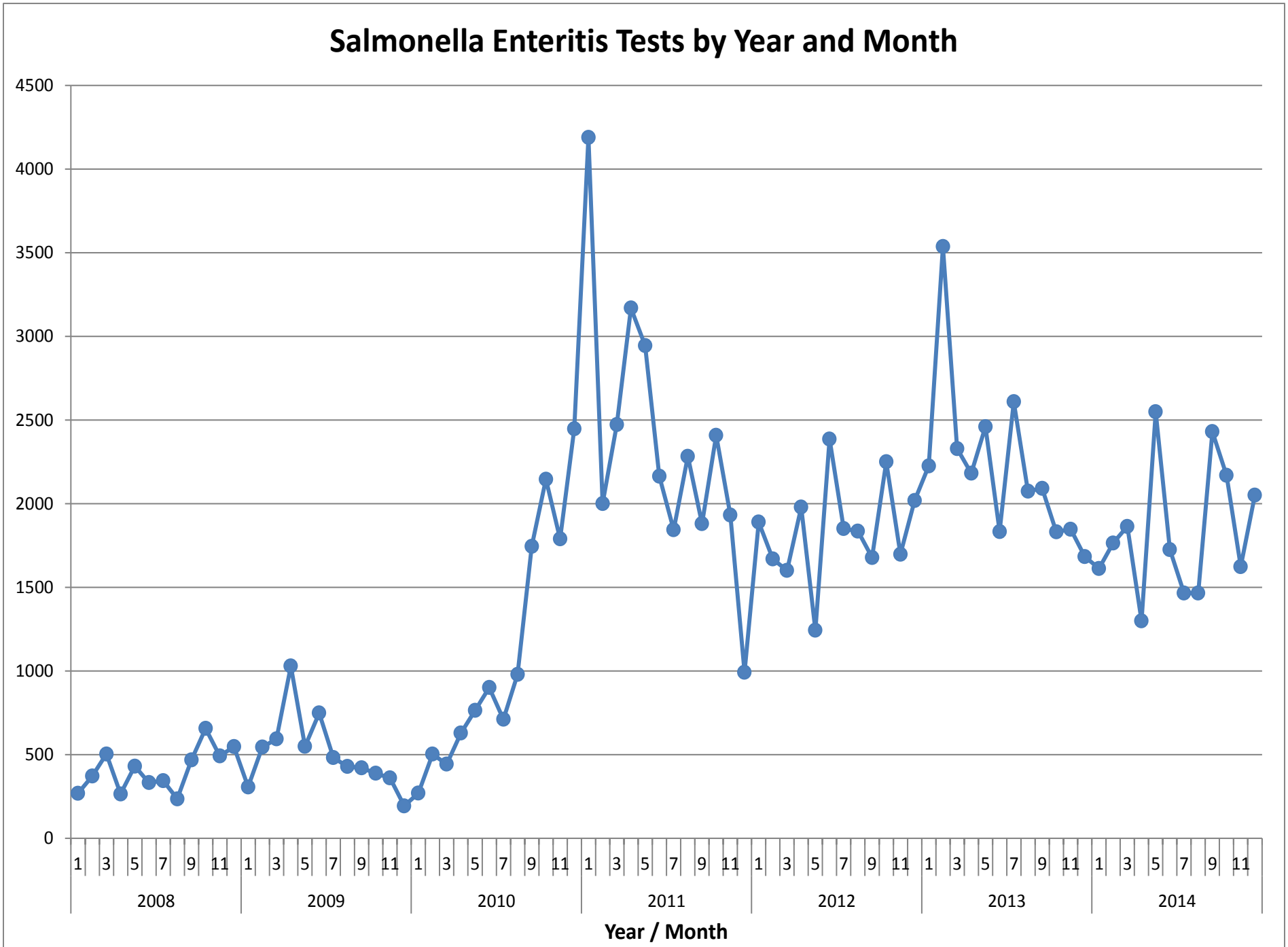
if positive

Culture

Isolate & Identify Serogroup D1 Organisms



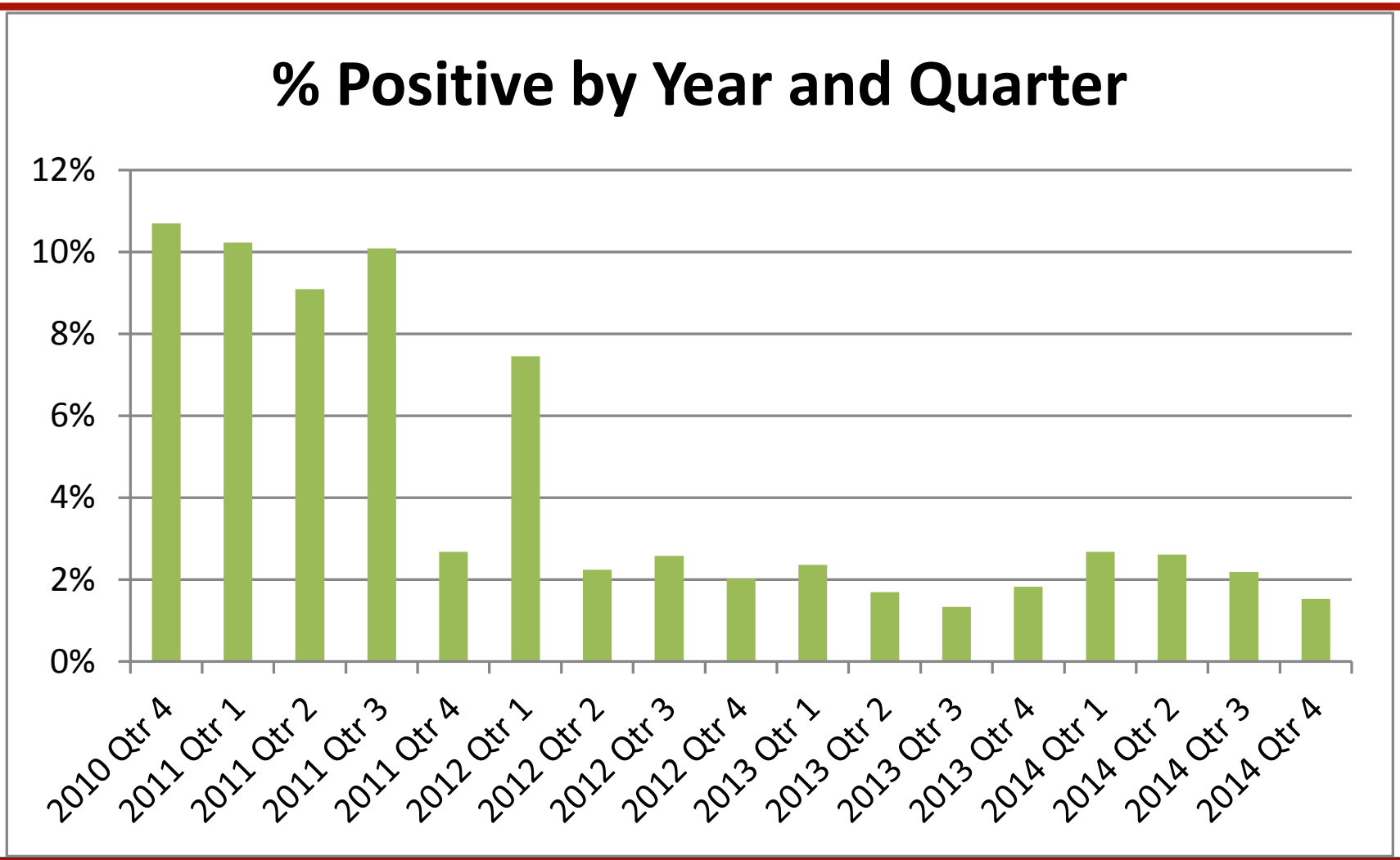
Serotyping at NVSL


Salmonella Enteritis Tests by Year and Month
4500
4000
3500
3000
2500
2000
1500
1000
500
0
1 3 5 7 9 11 1 3 5 7 9 11 1 3 5 7 9 11 1 3 5 7 9 11 1 3 5 7 9 11 1 3 5 7 9 11 1 3 5 7 9 11
2008
2009
2010
2011
2012
2013
2014
Year / Month

# Environmental (Jul 2010- Dec 2014)

- >58,200 Environmental Samples
  - 54,230 samples from 4917 submissions
  - ~12 samples/submission
- Results
  - 599(+)/54,230 samples
    - 1.10% samples positive SE positive
  - 278(+)/4917 submissions
    - 5.65% submissions SE positive

# SE + Environmental Results



# Eggs (Jul 2010- Dec 2014)

- >647,300 eggs
  - 647,340 eggs from 655 submissions
  - 32,367 pools tested (20 egg/pool)
  - ~1,000 eggs/submission
- Results
  - 1 SE (+) pool
    - 0.003% pools
    - 0.15% submissions

MEDICAL EXAMINER | HEALTH AND MEDICINE EXPLAINED. | MARCH 5 2014 11:44 PM

# Go Ahead, Lick That Spoon

I've consumed 360 raw eggs in my life and have never gotten salmonella poisoning. Here's why.

6.6k 302

*By L.V. Anderson*

Is cookie dough *really* dangerous?

Photo by Tyler Olson/iStock/Thinkstock

March 5, 2014. Slate.com

Since salmonella prevention practices have improved since then, the egg contamination rate is probably even lower now—indeed, according to Patterson, in Pennsylvania only **0.012 percent** of eggs *from salmonella-infected flocks* are contaminated.

Paul Patterson, Pennsylvania State University Professor.

# CDC SE Outbreaks

- **2014**
  - Bean Sprouts – Salmonella Enteritidis
- **2012**
  - Restaurant Chain A - Salmonella Enteritidis
  - Ground Beef - Salmonella Enteritidis
- **2011**
  - Alfalfa and Spicy Sprouts - Salmonella Enteritidis
  - Turkish Pine Nuts - Salmonella Enteritidis
- **2010**
  - Shell Eggs – Salmonella Enteritidis

http://www.cdc.gov/salmonella/outbreaks.html

# Final Comments

- Egg contamination w/ Salmonella not simple
  - Routes
  - Serotypes
  - Interventions
- Progress has been made
  - Few egg associated outbreaks
  - Testing reveals low egg contamination
- Salmonella in eggs is still a threat to public health and public perception

# Acknowledgements


- Egg Industry Center
- ISU VDL Bacteriology Section
- Melika Ibukic
- Gantois. et al., FEMS Microbiol Rev (2009)

# Questions?