The Isolation of Salmonella from Fresh Cucumbers: A Laboratory Investigation

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NOTHING TO DISCLOSE
Lecture Objectives

1. Describe the distribution of Salmonella in nature

1. Explain the current nomenclature of Salmonella spp.

1. List the diseases caused by Salmonella

1. Describe the isolation of Salmonella in clinical samples

1. Explain the procedures involved in the isolation of Salmonella in foods
Distribution of Salmonella in nature worldwide in cold-blooded and warm-blooded animals.

http://www.cdc.gov/salmonella/poona-09-15/index.html
SALMONELLA FACTS

Gram negative bacilli

Ox(-), motile, non-lactose fermenter

Member of the Enterobacteriaceae family

# 1 cause of bacterial foodborne infection in U.S.

Most common infection is gastroenteritis

http://www.cdc.gov/salmonella/poona-09-15/index.html
Foodborne Diseases Active Surveillance Network (FoodNet)

a collaborative program of the CDC, 10 state health departments, the USDA-Food Safety and Inspection Service (FSIS), and FDA. It is a population-based active surveillance for lab-confirmed infections caused by:

7 bacterial pathogens:

1. Campylobacter
2. *Listeria monocytogenes*
3. *Salmonella*
4. Shiga toxin-producing *Escherichia coli* [STEC]
5. *Shigella*
6. *Vibrio*
7. *Yersinia*

http://www.cdc.gov/salmonella/poona-09-15/index.html
Scientific Classification

* Kingdom: 
  Bacteria

* Phylum: 
  Proteobacteria

* Class: 
  Gammaproteobacteria

* Order: 
  Enterobacteriales

* Family: 
  Enterobacteriaceae

* Genus: 
  Salmonella

* Species 
  S. enterica

S. bongori
Current Nomenclature for Salmonella species

Two recognized species:

1. *S. enterica* (6 subspecies) >2000 serovars
   - *S. enterica* (serotype I)
   - *S. salamae* (serotype II)
   - *S. arizonae* (IIIa)
   - *S. Diarizonae* (IIIb)
   - *S. Houtenae*
   - *S. indica* (VI)

2. *Salmonella bongori*

Serotypes are usually put into subspecies groups after the genus and species, with the serovars/serotypes capitalized, but not italicized. Ex: *Salmonella enterica* serovar Typhimurium or simply

**Salmonella Typhimurium**

**Salmonella Poona**
Sources of Infection

1. Contaminated food or water
   beef, poultry, milk, fish, or eggs, fruit & vegetables, or processed foods.

2. Contact with infected animal.
   poultry and other birds, amphibians, and reptiles (turtles, lizards, and snake)

Beware!!

Contamination with small amount of feces may look or smell fine.

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Diseases caused by Salmonella

Typhoidal infection
- **Typhoid fever and paratyphoid fever** (Salmonella Typhi, Paratyphi A, Paratyphi B & Paratyphi C) in humans & primates
- Salmonella pass through lymphatic of intestines into blood (liver, spleen, kidneys) to form secondary foci causing hypovolemic or septic shock

Nontyphoidal infection:
- common self-limiting *gastroenteritis* disease aka “food poisoning” passed between humans and animals.
- can be invasive (iNTS) causing *septicemia*, (S. Typhimurium & S. Enteritidis seen in sub Saharan Africa due to the burden of HIV, malaria, and malnutrition

http://www.cdc.gov/salmonella/poona-09-15/index.html
The recognition of the outbreak.
Isolation of Salmonella from Fresh Cucumbers, Negado, L. 2016
Increase in Salmonella G submissions to PHL...

More than usual number for serogroup @ any given time

???
• Epidemiology staff notifies lab of increase in *Salmonella G* reports in web CMR

• Epi investigation points to a common source

• DEH to check out a local produce distributor

• May send samples to State Lab for testing
DAY 1
AUGUST 31, 2015, 10AM

Epidemiology notified lab for possible cucumber testing. Department of Environmental Health was asked to investigate the produce distributor a facility and collect samples.
DEH delivers samples about 3PM
DEH turns over Chain of Custody to the lab

Isolation of Salmonella from Fresh Cucumbers, Negado, L. 2016
The Samples

9 lots received
2 cucumbers per lot

Isolation of Salmonella from Fresh Cucumbers, Negado, L. 2016
Testing Begins
Salmonella Enrichment: Cucumbers + 250 ml Lactose broth, Incubated 24±2 hr @ 35°C air
DAY 2
SEPTEMBER 1, 2015

Subculture Lactose broth washings to:

Tetrathionate broth and Rappaport-Vasiliadis Broth
Day 3
September 2, 2015
Subculture: TT & RV to SS, XLD & HE

Isolation of Salmonella from Fresh Cucumbers, Negado, L. 2016
DAY 4
SEPTEMBER 3, 2015

Suspicious colonies...
Set up: screening bios (TSI, LIA, MIONPG)
DAY 5
SEPTEMBER 4, 2015
screening tests

Isolation of Salmonella from Fresh Cucumbers, Negado, L. 2016
DAY 5
SEPTEMBER 4, 2015

Read biochemicals: Bios typical Salmonella Agglutinate with salmonella antisera
Set up commercial ID system

Salmonella serogroup G
Lot: 03128528
Lot: 03128549

Report to Epidemiology, DEH, State MDL
Isolates Shipped to State FDA Lab in Richmond by 5PM via Golden State for PFGE & serotyping.
Testing continues...
Two more lots tested positive for Salmonella G

Report to:
Epi, DEH, State MDL

Isolation of Salmonella from Fresh Cucumbers, Negado, L. 2016
California State MDL Identified
Salmonella Poona (cucumber isolate)
Isolate forwarded to Foodborne and Waterborne Disease Section of State lab for PFGE
Testing done at the State MDL

**Patient isolates** (S. Poona)

**Serotyping**: Immunodiagnostics Unit

**PFGE**: Foodborne and Waterborne Disease Section

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**Environmental samples** (cucumbers) S. Poona

**Serotyping and PFGE**: 2 isolates done at MDL

**PFGE**: 2 isolates done at CDPH Food & Drug Laboratory Branch
AS OF JANUARY 21, 2016 (CDC POSTING)

- **Case Count:** 888
- **States:** 39
- **Deaths:** 6
- **Hospitalizations:** 191
- **Recall:** Yes

http://www.cdc.gov/salmonella/poona-09-15/index.html
Significance of Salmonella

California Code of Regulations, Title 17, Section 2505 requires laboratories to report laboratory testing results suggestive of the following diseases of public health importance to the local health department:

SALMONELLA (Section 2612)
California Code of Regulations, Title 17, Section 2612 requires that a culture of the organisms on which a diagnosis of salmonellosis is established must be submitted to the local public health laboratory and then to the State’s Microbial Diseases Laboratory for definitive identification.

Update: (January 21, 2016)

• **Whole genome sequencing** (WGS) of Salmonella Poona from patients and cucumbers distributed by Andrew & Williamson Fresh Produce are closely related genetically.

• The source of contamination for Andrew & Williamson Fresh Produce has not been identified.

• Number of reported illnesses declined but has not returned to the expected number (about 1 every month this time of year).

• This investigation is ongoing. CDC will provide updates when more information is available.

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Salmonella-free???
Thank You!