

# Chasing the Dragon: Investigating an Outbreak of Rare *Salmonella* Serotypes Linked to Pet Bearded Dragons

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**July 27, 2016**

Presenting on behalf of the outbreak investigation team:

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# Overview

- ❑ **Salmonellosis and reptiles**
- ❑ **U.S. reptile and bearded dragon industries**
- ❑ ***Salmonella* Cotham and *Salmonella* Kisarawe outbreak investigation**
- ❑ **Recommendations for public health officials, the reptile industry and reptile owners**

# Salmonellosis

- ❑ **Most common bacterial enteric illness in United States**
  - 1.2 million illnesses per year
  - Multiple sources
    - Food and water
    - Animal contact
- ❑ **11% due to animal contact, or zoonotic**
  - Direct or indirect
  - Highest morbidity and mortality among enteric zoonoses
  - Children disproportionately affected

# Reptile-Associated Salmonellosis

- ❑ ***Salmonella* normal gut flora for reptiles**
- ❑ **Human infections**
  - Contact with fecal matter from reptile
  - Direct or indirect animal contact
  - Serious illnesses can result
- ❑ **Young children often at higher risk**
  - Certain reptiles marketed toward young children
  - Environmental contamination

# Reptile Industry

- ❑ **\$1.0 to \$1.4 billion in earned revenues for reptile products**
  
- ❑ **U.S. households (2013–2014 APPA Survey)**
  - 5.6 million own a reptile
  - 11.5 million reptiles total
  - Increasingly popular pets – fastest growing sector
  
- ❑ **Reptile owners**
  - First-time and novice owners
  - Enthusiasts
  - Hobbyists

# Reptile Industry

## ❑ Reptile Producers

- Large-scale (mass) breeders
- Small-scale breeders and specialty morphs
- Hobbyists and part-time breeders

## ❑ Import / Export

## ❑ Distribution and sales

- Wholesalers and distributors
- Pet superstores
- Pet stores
- Reptile shows
- Online



- The Modern Reptile Industry, Georgetown Economic Services LLC (2011)
- American Pet Products Association: <http://www.americanpetproducts.org>
- Descriptive Analysis Report of Wild Reptile Imports to the United States (2004 – 2009); USDA /APHIS/VS/CEAH

# Reptile Industry

- ❑ **U.S. reptile exports and imports (Jan 2009–Apr 2014)**
  - ❑ **Shipments: ~80,000**
  - ❑ **Reptiles: >50 million**



## **Pet Bearded Dragon Industry**

# Inland Bearded Dragon

## *Pagona vitticeps*

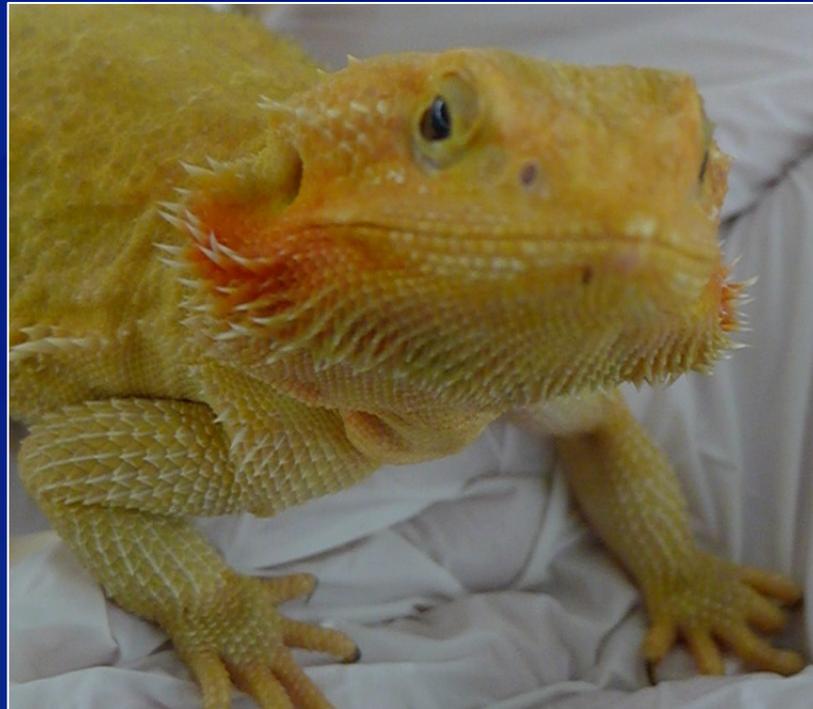
- ❑ **Native to Australia**
- ❑ **Lifespan**
  - 3–10 years (or more)
- ❑ **Size**
  - 12–24 inches
- ❑ **Pet industry breeding population**
  - Australia prohibits export of native wildlife
  - Original breeding stock smuggled out of country



Source: <http://beardeddragon.org>

# U.S. Bearded Dragon Industry

- ❑ **2009–2013 exports and imports:**
  - **Overall 153% increase in bearded dragon numbers**
  - **Net import of bearded dragons**



# Inland Bearded Dragon

## *Pagona vitticeps*

### ❑ Increasingly popular pet

- Docile
- Pleasing colors
- Easy to keep
- “Personality”

### ❑ Retail

- Entry-level reptile
- Sold as juveniles

### ❑ Hollywood effect



Source: <http://pikdit.com/i/big-dreams/>



***Salmonella* Cotham and *Salmonella* Kisarawe outbreak investigation**

# One Health



## ❑ One Health approach

- Detection
- Control
- Prevention

## ❑ Integration of human, animal, and environmental health

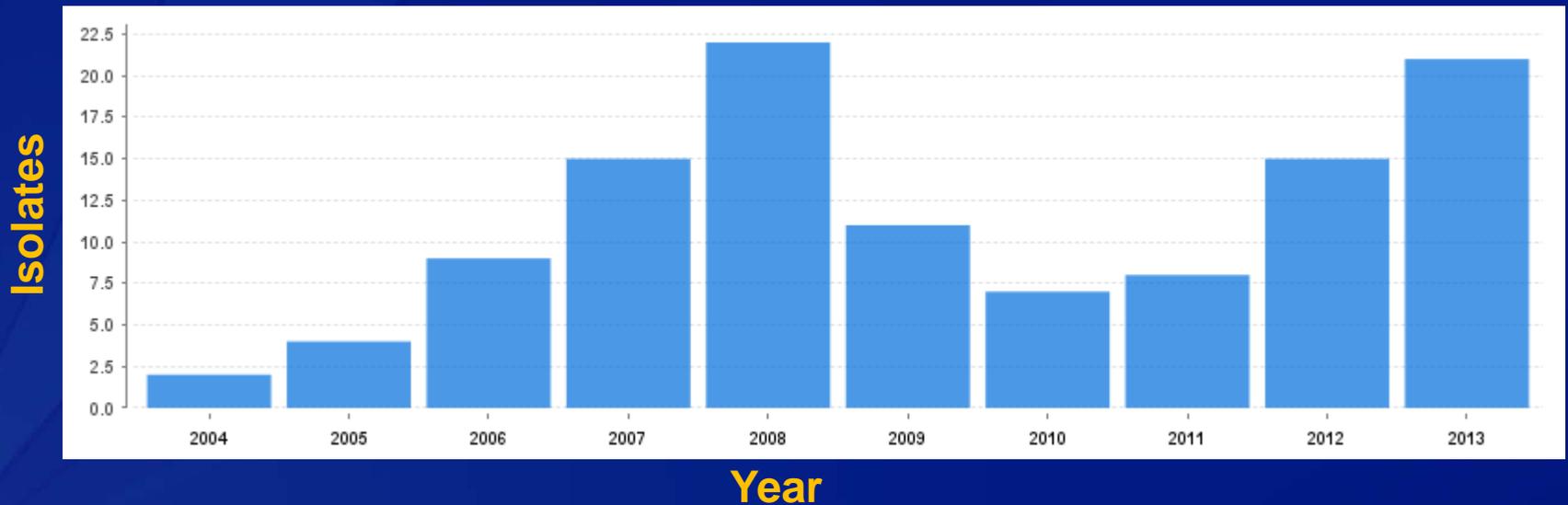
# Initial Notification

## January 22, 2014

- ❑ **Wisconsin Division of Public Health**
  - 11 cases of *Salmonella* Cotham since January 2012
  - 10/11 (91%) reported bearded dragon exposure
  - Median age of 4 years
- ❑ **Five households purchased at pet retail chains**
- ❑ **Two infants linked to same bearded dragons**
- ❑ **Isolated from bearded dragon in case household**

# Digging for Background

- ❑ SEDRIC data on *Salmonella* Cotham
  - 114 isolates uploaded to PulseNet since 2004
  - Median age 1 year old (<1 – 79)
  - Rare serotype
- ❑ Temporal increase in isolates



# The Chase Continues...

## ❑ Isolated from lizards

### Prevalence and antimicrobial susceptibility of salmonellae isolates from reptiles in Taiwan

Chun-Yu Chen, Wan-Ching Chen, Shih-Chien Chin, Yen-Hsueh Lai, Kwong-Chung Tung, Chien-Shun Chiou, Yuan-Man Hsu, Chao-Chin Chang<sup>1</sup>

## ❑ Isolated from bearded dragon and 8 month old infant

### Evidence for the transmission of *Salmonella* from reptiles to children in Germany, July 2010 to October 2011

M Pees (pees@vmf.uni-leipzig.de)<sup>1</sup>, W Rabsch<sup>2</sup>, B Plenz<sup>1</sup>, A Fruth<sup>2</sup>, R Prager<sup>2</sup>, S Simon<sup>2</sup>, V Schmidt<sup>1</sup>, S Münch<sup>2</sup>, P G Braun<sup>3</sup>

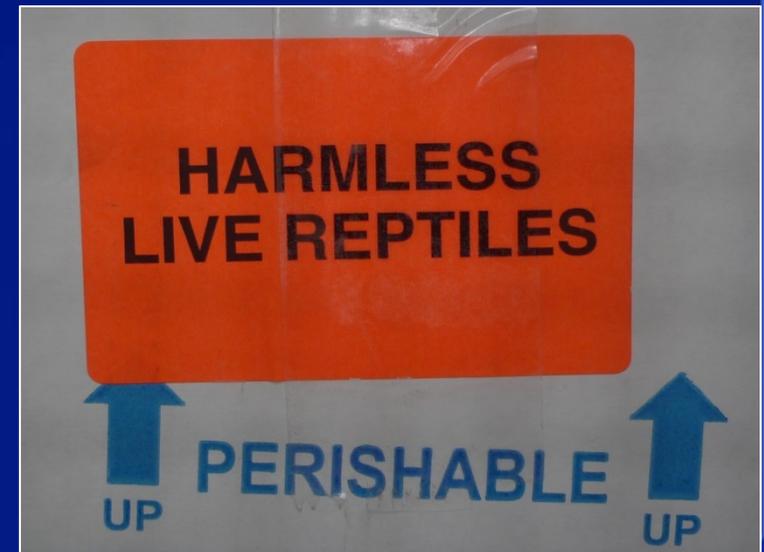
1. Clinic for Birds and Reptiles, University of Leipzig, An den Tierkliniken, Leipzig, Germany

2. National Reference Centre for Salmonella and other bacterial Enterics, Robert Koch Institute, Wernigerode, Germany

3. Institute of Food Hygiene, An den Tierkliniken, Leipzig, Germany

# Laboratory Based Enteric Disease Surveillance (LEDS)

- Human *Salmonella* isolates (1964–2014)
  - 1,720,978 total
  - 221 (0.01%) Cotham
  - First isolate in 1994
- Non-human *Salmonella* isolates (1968–2012)
  - 542,986 total
  - Five (0.001%) Cotham
  - First isolate in 2000
  - Two from reptiles



# An Outbreak is Born...

## □ Case definition

- Diarrheal illness in a person
- Clinical specimen yielding *Salmonella* Cotham isolate
- All matching cases from January 1, 2012



## International Scope

- ❑ **UK – at least one case with bearded dragon exposure**
- ❑ **Germany – nine cases with information**
  - 8/9 (89%) reptile exposure
  - 6/8 (75%) bearded dragons
- ❑ **Canada – nine cases, four with information**
  - 4/4 (100%) reptile exposure
  - 3/4 (75%) bearded dragons
- ❑ **Mostly children and infants**

# Early Outbreak Description

- ❑ **133 cases of *Salmonella* Cotham**
  - Onset dates ranged from February 20, 2012 until March 17, 2014
  - 32 states
  - Median age 2 years (<1–79)
  - 52% female
  - 48 PFGE patterns



# National Veterinary Services Laboratories (NVSL)

- ❑ One Health investigation
- ❑ Offer of assistance for specimen testing
  - *Salmonella* isolation
  - Serotyping
  - PFGE
  - Antimicrobial resistance testing



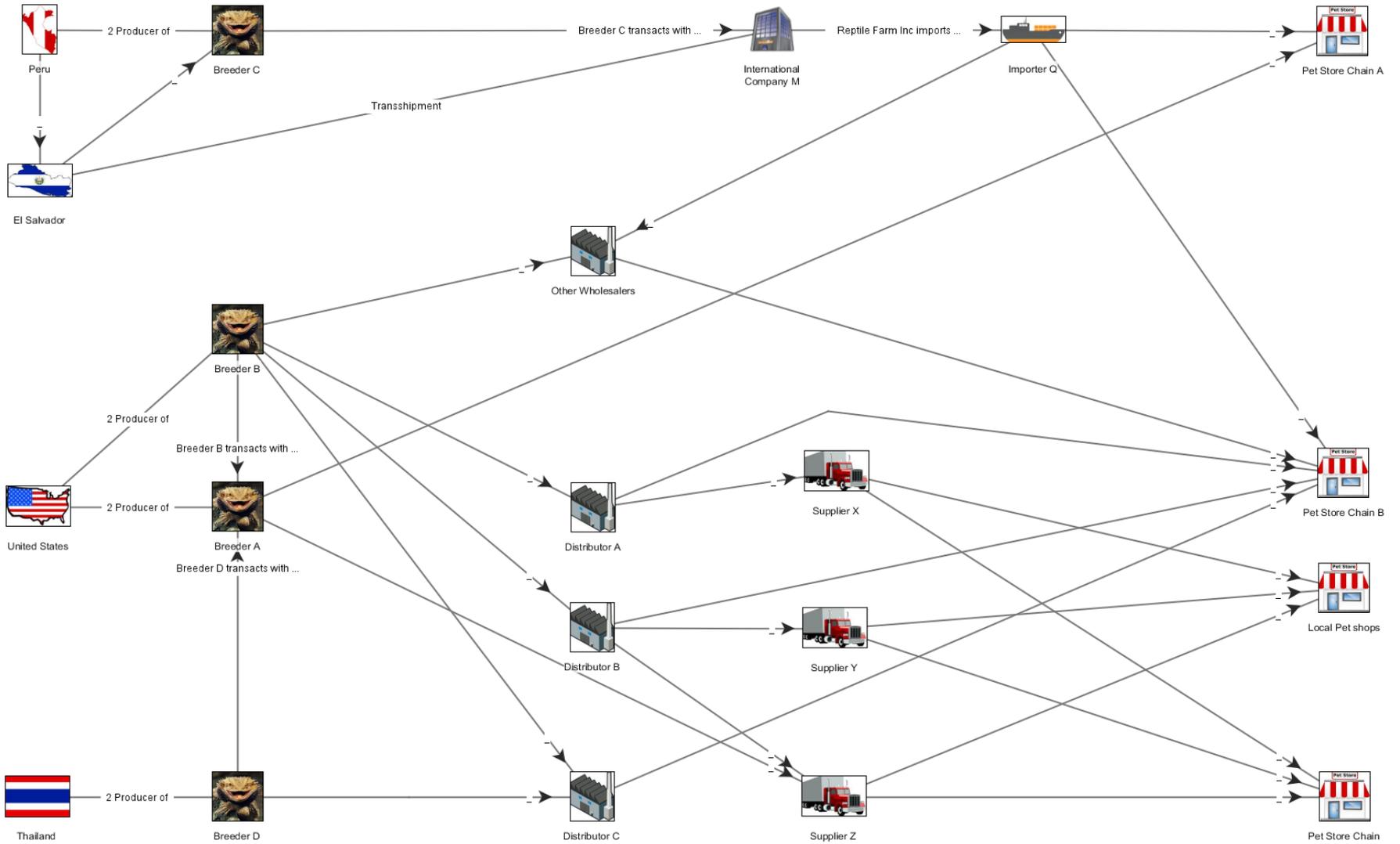
# Traceback

## ❑ Pet Industry Partnership

- Traceback
  - Primarily through Company A – halted sales in response
  - Limited from other retail chains
  - Purchase date ranges and store locations
- PIJAC – emergency alert issued



# SEDRIC Traceback



# Supplemental Questionnaire

## Bearded Dragon Colors

### Standard Colors

Standard colors of bearded dragons are generally a mix of green, tan, yellow, and red colors. Most bearded dragons come in these shades and you generally only find solid colored bearded dragons from selective breeders.



### Red

The following is a list of bearded dragons that have a mostly red appearance:

- » Red bearded dragon
- » Blood-red bearded dragon
- » Ruby red bearded dragon

Red

Blood-Red



Interviewer: \_\_\_\_\_ Case ID: \_\_\_\_\_ Abstracted:  Yes  No  
 Interview date: \_\_\_/\_\_\_/\_\_\_ PulseNet ID: \_\_\_\_\_  
 Date First Contact Attempted: \_\_\_/\_\_\_/\_\_\_ Number of Contact Attempts: \_\_\_\_\_

### Salmonella Cotham (cluster 1203WICOT-1) Supplemental Questionnaire

Respondent:  self  parent  guardian  other (please specify) \_\_\_\_\_  
 Patient DOB: \_\_\_/\_\_\_/\_\_\_ Age: \_\_\_\_\_ Sex:  M  F

#### Section A. Background Information

1. Date of illness onset (MM/DD/YYYY): \_\_\_/\_\_\_/\_\_\_
2. Did you/your child have diarrhea (defined as 3 more loose stools in a 24 hr period) during this illness?  
 Yes  No  DK
  - a. If YES, when did the diarrhea start (MM/DD/YYYY): \_\_\_/\_\_\_/\_\_\_
  - b. If YES, did you/your child have bloody diarrhea?  Yes  No  DK
3. How many days did you/your child's illness last? \_\_\_\_\_ days  Illness Ongoing
4. Did you/your child take antibiotics for your illness?  Yes  No  DK
5. Were you/your child hospitalized for this illness?  Yes  No  DK  
 If YES, how many nights were you/your child hospitalized? \_\_\_\_\_ nights
6. Did you/your child develop severe illness resulting from this infection (e.g. blood stream infection, brain infection, etc)?  
 Yes  No  DK  
 If yes, please describe: \_\_\_\_\_

#### Section B. Reptile Exposure Information

1. Did your child attend daycare or school during the 7 days prior to illness onset?  Yes  No  DK  NA  
 If yes: Name: \_\_\_\_\_ Address: \_\_\_\_\_
2. Does your child's school or daycare keep pets in the classroom?  Yes  No  DK  NA  
 If yes, please list type(s) of pet (check all that apply):  
 Snake: (please specify) \_\_\_\_\_  
 Lizard: (please specify) \_\_\_\_\_  
 Turtle: (please specify) \_\_\_\_\_  
 Rodent: (please specify) \_\_\_\_\_  
 Bird: (please specify) \_\_\_\_\_  
 Fish: (please specify) \_\_\_\_\_  
 Other: (please specify) \_\_\_\_\_
3. In the week prior to your/your child's illness, did you have ANY contact with reptiles or their habitat (cage or enclosure), or were you in a location where reptiles were kept? (Reptile: snake, lizard, turtle, etc.)  
 Yes  No  DK  
 If yes, please specify which contact occurred:  
 Reptile  Reptile Habitat  Both Reptile and Reptile Habitat  
 If you/your child had contact with a reptile, please specify which contact occurred:  
 Pet Reptile  Wild Reptile  Both Pet and Wild Reptile  
 Please specify the type of contact: (mark all that apply)  
 Contact with the reptile habitat (e.g., spilled water, opening, closing, cleaning, changing the water)  
 Touched animal  
 Held animal  
 Fed animal  
 Kissed animal  
 Allowed animal to crawl or sit on lap  
 Allowed animal to crawl or sit on shoulders or head  
 Reptiles are kept in a home, classroom or other place where I/my child spent time  
 Other (specify) \_\_\_\_\_

This section is for interviewees who had any REPTILE exposure. If the interviewee did not have any reptile exposure, please skip to 'Section E. Rodent Exposure Information'. Otherwise, please continue to question 3a.

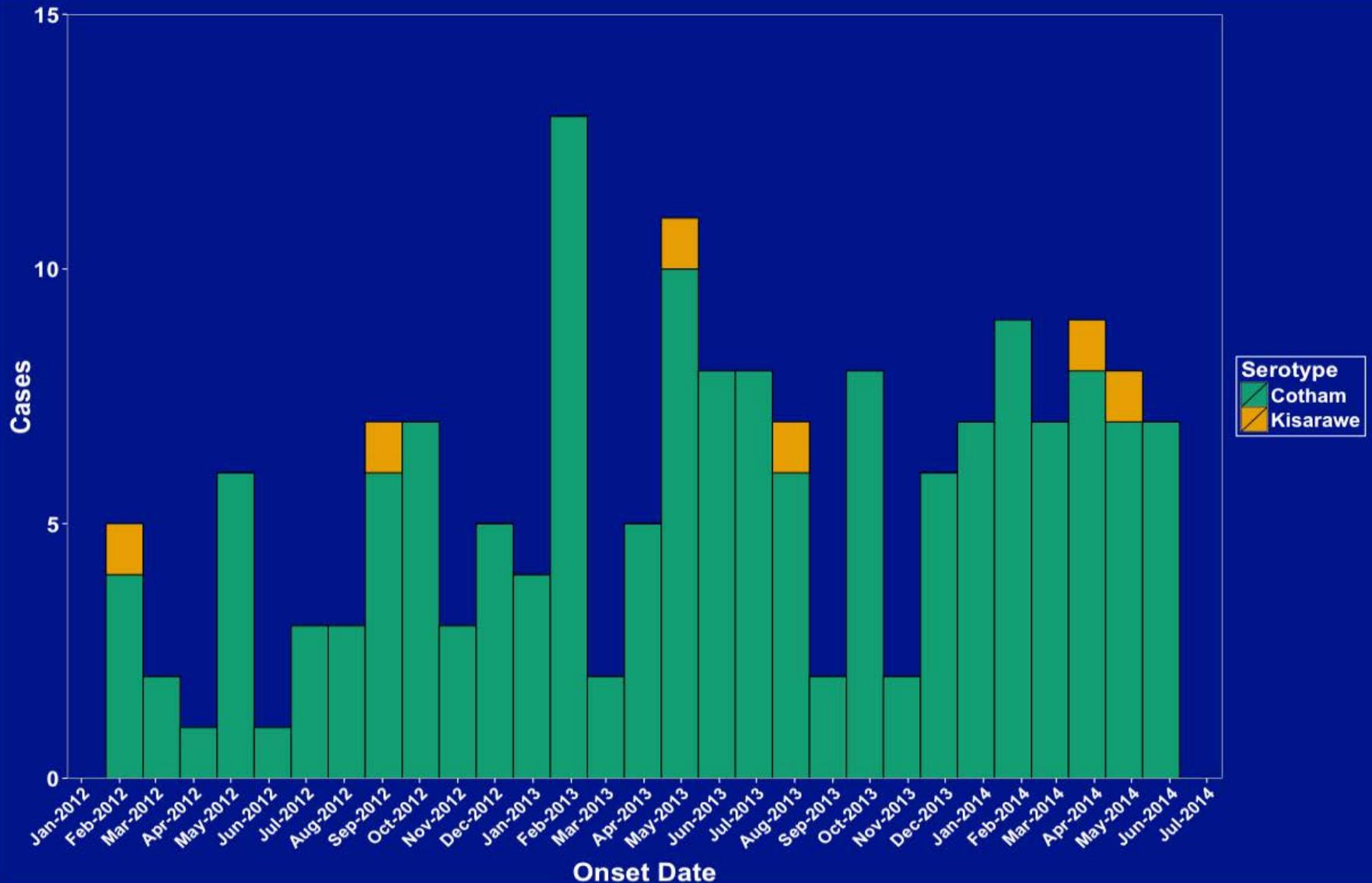
# *Salmonella* Kisarawe

- ❑ Bearded dragon serotype in separate investigation
- ❑ NVSL reported five from January 2012
  - All in bearded dragons
- ❑ WI identifies past isolates
  - Human case (2010) – household had three bearded dragons
- ❑ TX case patient bearded dragon
  - Isolated both *Salmonella* Cotham and *Salmonella* Kisarawe



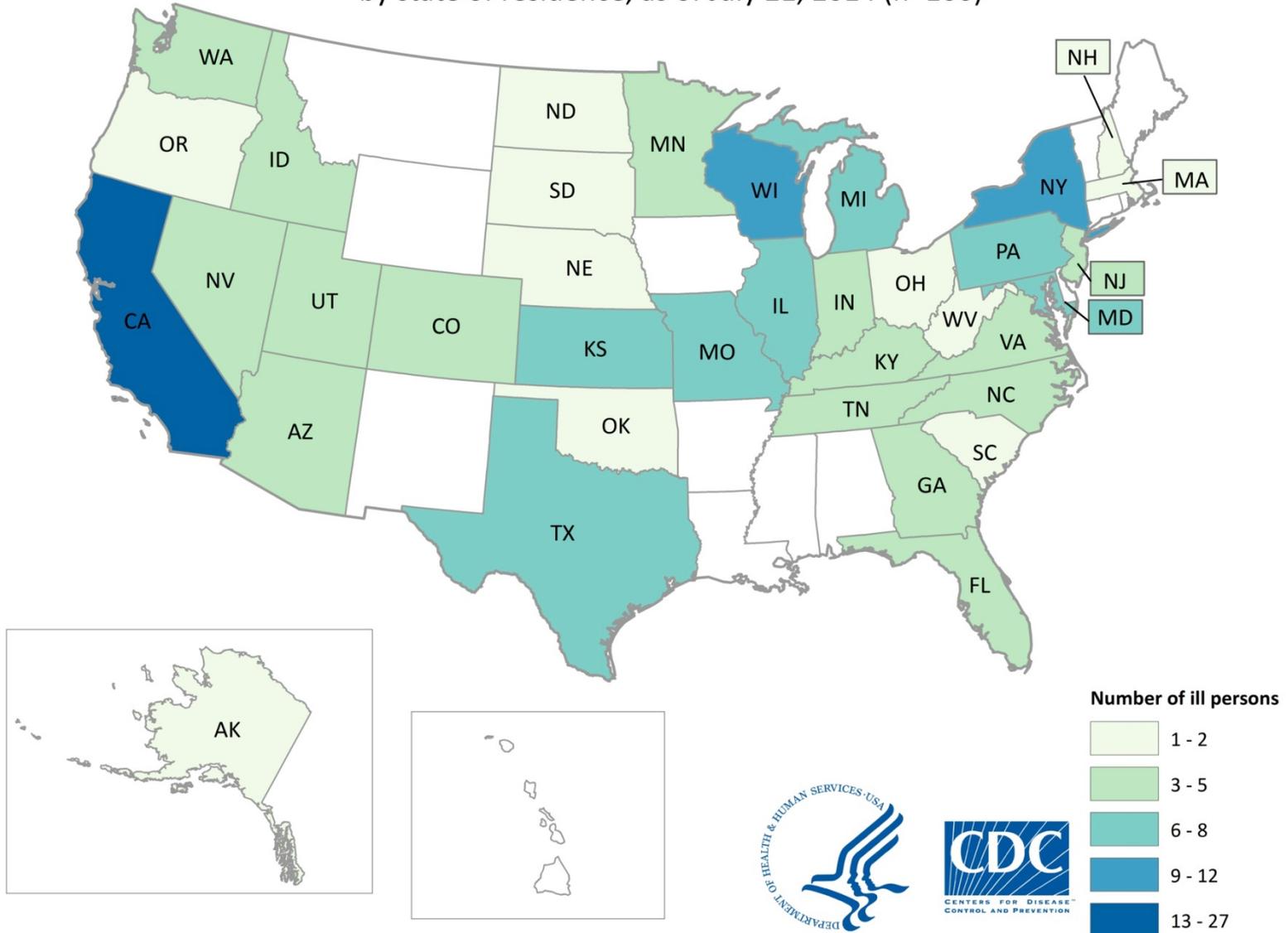
Source: [www.beardeddragon.org](http://www.beardeddragon.org)

# Persons Infected with *Salmonella* Cotham or *Salmonella* Kisarawe, by Date of Illness Onset\*



\*n=166 includes reported and estimated dates as of July 21, 2014

Persons infected with the outbreak strains of *Salmonella* Cotham or Kisaware, by state of residence, as of July 21, 2014 (n=166)



## Characteristics of Persons Infected with *Salmonella* Cotham and Kisarawe

<b>Demographics</b>	<b>#</b>	<b>n (range)</b>	<b>%</b>
Age, median	3	(<1 - 79)	n/a
Age, ≤5 years	93	165	56
Female	92	165	56

<b>Outcomes</b>	<b>#</b>	<b>n</b>	<b>%</b>
Hospitalizations	44	118	37
Hospitalizations, ≤5 years	26	44	59

## Characteristics of Persons Infected with *Salmonella* Cotham and Kisarawe

<b>Exposures</b>	<b>#</b>	<b>n</b>	<b>%</b>
<b>Any Reptile</b>	<b>86</b>	<b>104</b>	<b>83</b>
<b>Lizard</b>	<b>87</b>	<b>112</b>	<b>78</b>
<b>Bearded Dragon</b>	<b>71</b>	<b>94</b>	<b>76</b>
<b>Lizard and Bearded Dragon</b>	<b>71</b>	<b>74</b>	<b>96</b>

***P* < 0.001**



## Onsite Breeder Sampling

# Sampling Protocol

## □ Sample Types

- Bedding
- Cloacal swab
- Egg
- Environmental swab
- Droppings
- Feed
- Tissue
- Water



# Sampling Protocol





# Sampling Results

## *Salmonella* spp.

<b>Sample Location</b>	<b># Pos</b>	<b>n</b>	<b>%</b>
<b>Breeder A</b>	<b>77</b>	<b>89</b>	<b>87</b>
<b>Breeder B</b>	<b>154</b>	<b>186</b>	<b>83</b>
<b>Breeder C</b>	<b>30</b>	<b>108</b>	<b>28</b>
<b>Retail A</b>	<b>59</b>	<b>71</b>	<b>83</b>
<b>Total</b>	<b>320</b>	<b>454</b>	<b>71</b>

# Sampling Results

## *Salmonella*

<b>Sample Location</b>	<b>Cotham</b>		<b>Kisarawe</b>	
	<b># Pos</b>	<b>%</b>	<b># Pos</b>	<b>%</b>
<b>Breeder A (n=89)</b>	<b>14</b>	<b>16</b>	<b>8</b>	<b>9</b>
<b>Breeder B (n=141)</b>	<b>13</b>	<b>9</b>	<b>7</b>	<b>5</b>
<b>Breeder C (n=108)</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>
<b>Retail A (n=71)</b>	<b>17</b>	<b>24</b>	<b>6</b>	<b>9</b>
<b>Total (n=409)</b>	<b>46</b>	<b>11</b>	<b>23</b>	<b>6</b>

## Results by Sample Type

Sample Type	<i>Salmonella</i> spp.		
	# Pos	n	%
Bedding	19	23	83
Cloacal swab	12	42	29
Egg	34	40	85
Environmental swab	128	167	77
Droppings	59	71	83
Feed	5	19	26
Tissue	59	71	83
Water	4	21	19
Total	320	454	71



**Recommendations for Stakeholders**

# One Health Approach to Zoonotic Salmonellosis Prevention and Control

- ❑ Industry
- ❑ Consumers
- ❑ Health professionals



# Recommendations for Industry: Breeding Facilities

- ❑ Retain a veterinary consultant
- ❑ Biosecurity
  - Cleaning and disinfection
  - Personal protective equipment
  - Improved pest control
  - Improved husbandry
  - Employee workflow controls
- ❑ Critical control points
  - Egg incubation
  - Juvenile handling
  - Environmental controls
- ❑ Routine monitoring and evaluation



# Recommendations for Industry

- ❑ **Strengthen the National Reptile Improvement Plan**
  - Focus on Best Management Practices for breeders and retail
  - Include monitoring requirements
  - Increase industry education



- ❑ **Increased consumer education**
  - Point of sale
  - Specialty workshops
  - Retail educational materials
  - One Health - support public / private partnerships

# Recommendations for Consumers

- ❑ Do not let reptiles roam in household
- ❑ Wash your hands right after
  - Touching reptiles
  - Contacting anything in the area where they live and roam
  - Cross-contamination
- ❑ Certain groups should not handle reptiles
  - Children younger than 5 years of age
  - Older adults
  - People with weak immune systems



# Recommendations for Consumers

- ❑ **Clean reptile equipment or materials outside the house**
- ❑ **Do not**
  - Snuggle or kiss reptiles
  - Touch your mouth, eat, or drink around reptiles
- ❑ **Avoid household environmental contamination**
  - Do not let your reptile roam in the household



Source: [www.beardeddragon.org](http://www.beardeddragon.org)

# Recommendations for Health Professionals - Increasing Awareness

## Love the Pets, Not the Germs: CDC Update on Enteric Zoonoses

[Recommend](#) [Tweet](#) [Share](#)

[CE](#) = [Free Continuing Education](#)

**Date:** Thursday, July 17, 2014

**Time:** 2:00 – 3:00 PM (Eastern Time)

### Presenter(s)

**Kara Jacobs Slifka, MD, MPH**

Epidemic Intelligence Service Officer

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National Center for Emerging & Zoonotic Infectious Diseases

Centers for Disease Control and Prevention

## COCA



THE OFFICIAL NEWSMAGAZINE OF THE AMERICAN ACADEMY OF PEDIATRICS

# AAP News

Volume 34 • Number 7  
July 2013  
[www.aapnews.org](http://www.aapnews.org)

## Focus On Subspecialties

### How to protect patients, families from enteric zoonoses

by **Karen P. Neil, M.D., M.S.P.H., FAAP**

Pediatricians can alert children and their families to emerging issues they may not be aware of that can help prevent illness. One such issue is an increased number of enteric illness outbreaks detected by public health surveillance that are linked to pet ownership and animal contact.

Roughly 74 million U.S. households have one or more pets, according to the American Veterinary Medical Association. Pet ownership and other types of animal contact provide many benefits to people. However, certain animals are not appropriate pets for high-risk groups, including children under 5 years of age, immunocompromised persons and adults over 65 years of age.

Enteric illnesses such as *Salmonella*, *Escherichia coli* O157:H7 and *Campylobacter* are most commonly transmitted through contaminated food. However, these bacteria are among the many zoonotic pathogens that pets and other animals can spread to people. Illnesses and outbreaks of enteric zoonotic diseases (i.e., zoonoses) have been linked to exposure to many different kinds of animals in public and private settings (*MMWR Recomm Rep.* 2011;60[RR-04]:1-24).

In addition to pet ownership, public venues such as animal exhibits, farms, stores, schools and child care facilities offer opportunities for children to contact animals. Petting zoos and backyard poultry flocks also are becoming increasingly popular.



While pets can provide many benefits, some animals can carry enteric zoonotic pathogens that can spread to people. Pediatricians have a role in educating patients and their families about the risks for contracting *Salmonella* infection associated with certain pets, including rodents such as mice, hamsters and guinea pigs.

cats and dogs, particularly puppies and kittens. Infected animals can appear clean and healthy while still shedding pathogens that can lead

AAP News 2013; Vol. 34, No. 7

# Gastrointestinal Diseases from Animals Website

## <http://www.cdc.gov/zoonotic/gi/>

### Zoonotic Diseases (Diseases from Animals)

#### Gastrointestinal (Enteric) Diseases from Animals

Outbreaks

Animals

Education

Places

#### Additional Resources

[Healthy Pets Healthy People](#)

[Reports of Selected Salmonella Outbreak Investigations](#)

[Multistate Foodborne Outbreak Investigations](#)

[Compendium of Measures to Prevent Disease Associated with Animals in Public Settings, 2011](#) [PDF - 28 pages]

[Clean Hands Save Lives](#)

[Feature: Wash Your Hands](#)

[CDC-TV: Put Your Hands Together](#)

 Recommend 1  Tweet 2  Share

## Gastrointestinal (Enteric) Diseases from Animals

Animals provide many benefits to people. However, some animals can carry diseases that can be shared with people. Zoonotic diseases or zoonoses are diseases caused by germs (pathogens) that can be spread between animals and humans. Many germs have been responsible for illnesses and outbreaks, including *Salmonella*, *E. coli* O157:H7, and *Cryptosporidium*. These germs can come from many types of animals, including pets, wild animals, and farm animals. Did you know that infected animals can make you sick, even if they appear healthy and clean?

Zoonotic diseases can cause many different illnesses in people. Gastrointestinal (enteric) zoonoses are one type of illness that can upset the digestive system (stomach and intestines) and can make people sick.



#### Outbreaks

Selected Multistate Outbreak Investigations Linked To Animals and Animal Products



#### Education

Educational Materials and Other Resources



#### Animals

Information for Selected Animals and Animal Products



#### Places

Information about Common Places Germs are Spread

 [Email page link](#)

 [Print page](#)

 [Subscribe to RSS](#)

#### Contact Us:

 Centers for Disease Control and Prevention  
1600 Clifton Rd  
Atlanta, GA 30333  
 800-CDC-INFO  
(800-232-4636)  
TTY: (888) 232-6348  
[Contact CDC-INFO](#)

# Conclusions

- ❑ **Human salmonellosis associated with pet reptiles is an important and preventable public health issue**
  - **Serious human illness**
  - **Young children, people with weakened immune systems, seniors at higher risk for serious illness**
- ❑ **Prevention and control depends on a One Health approach**
- ❑ **Interventions at breeding facilities and retail stores to reduce human illnesses**
- ❑ **Industry actions and public education critical for improvements**

# Acknowledgments

## CDC

Lyndsay Bottichio  
Casey Barton Behravesh

Craig Kiebler

LaToya Simmons

Colin Basler

Patrick Ayscue

Laura Burnworth

Kate Heiman

Matt Wise

Ian Williams

Rob Tauxe

Chris Braden

EDLB

PulseNet

NARMS

DFWED

PulseNet

## USDA-APHIS

Thomas Gomez

## NVSL

Matthew Erdman

Linda Schlater

Kristina Lantz

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Curtis Fritz

Annie Kao

Ernie Awa

Nikos Gurfield

Mark Lundberg

Linda Lewis

Kiyomi Bird

## Multiple state and local health departments

## Multiple state departments of agriculture

## Pet Industry

Tom Edling

Petco

Pet Industry Joint Advisory Council (PIJAC)

Multiple bearded dragon breeders

## European Centre for Disease Prevention and Control

## Public Health Agency of Canada

## U.S. Fish & Wildlife Service



# Thank You

**For more information please contact Centers for Disease Control and Prevention**

1600 Clifton Road NE, Atlanta, GA 30333

Telephone: 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348

E-mail: [cdcinfo@cdc.gov](mailto:cdcinfo@cdc.gov) Web: <http://www.cdc.gov>

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

National Center for Emerging and Zoonotic Infectious Diseases  
Division of Foodborne, Waterborne, and Environmental Diseases



## Supplemental Questionnaire results of Persons Infected with *Salmonella* Cotham and Kisarawe

<b>Reptile Ownership</b>	<b>#</b>	<b>n</b>	<b>%</b>
Owned reptile previously	15	22	68
Lizard ownership >1 year	13	14	93
Reptile and <i>Salmonella</i> awareness	23	32	72

<b>Husbandry</b>	<b>#</b>	<b>n</b>	<b>%</b>
Bearded dragon roams outside its enclosure	8	29	28
Bearded dragon ill w/in 2 weeks prior to patient illness	1	29	3

# Reptile Industry

- ❑ U.S. reptile exports and imports (Jan 2009 – Apr 2014)
  - ❑ Shipments: 82,142
  - ❑ Reptiles: 51,119,380



# U.S. Bearded Dragon Industry

- ❑ U.S. Bearded Dragon Exports and Imports (Jan 2009 – Apr 2014)
  - ❑ Shipments: 1,417
  - ❑ Dragons: 259,967



# Antimicrobial Susceptibility Testing (AST)

## ❑ Wisconsin Division of Public Health

- Single isolate resistant to 11 different antimicrobials
- Confirmed by CDC/NARMS
- Ceftriaxone resistant
- 5 month old patient

## ❑ Additional NARMS AST Results

- 10 human isolates from 7 states pan-susceptible
- 2 bearded dragon isolates pan-susceptible

# Sampling Scope

- ❑ Direct sampling at **Breeders A, B, C**
- ❑ **Breeder A**
  - Receives hatchlings for grow-out from **Breeder B**
  - Bearded dragons purchased from **Breeder D**
    - Isolated from rest of stock
    - Cannot rule out cross-contamination
  - Indirect sampling of **Breeder B** and **Breeder D**
- ❑ **Retail Store A**
  - Bearded dragons from stores in 33 states
- ❑ All testing conducted by NVSL using standard methodology
  - Some samples were not serotyped
  - No appreciable antimicrobial resistance

# Sampling Protocol

- ❑ CDC has no approved reptile sampling protocol
- ❑ Guidelines developed through CDC, NVSL, and state collaboration
- ❑ Naval Medical Research Unit (NAMRU-6)
  - ❑ Lima, Peru
  - ❑ All sampling at **Breeder B**

## RECOMMENDED GUIDELINES FOR COLLECTING SPECIMEN FOR CULTURE FROM REPTILES DURING SALMONELLOSIS INVESTIGATIONS April 2014

Contact your local public health laboratory, Environmental Health, and/or Veterinary Health section before collecting and submitting specimens so they can provide you with specific information on the services they offer as well as specimen collection, storage, and shipment guidelines that may pertain to your particular investigation.

Recommended specimens to collect (testing of the environment has the best yield):

- Swabs of reptile environment including (but not limited to) water dishes, food dishes, bedding material, gravel or other bedding at bottom of tank, inside surfaces of enclosure such as side walls and surfaces beneath bedding
- Swabs of reptile's body (cloacal area, skin, mouth)
- Stool specimens from each reptile or combined fecal samples from enclosure with multiple animals
- Swabs of any containers that held or transported reptile food (feeder mice/rats, crickets, worms, etc.)
- Reptile food specimens (pellets, feeder mice/rats, crickets, worms, etc.)
- Dry material (sand, soil, bedding material)

General Guidance for Collection of Reptile Specimens:

1. Collection Supplies (ideally do not use any materials present in the household)
  - a. Gloves and autoclave bag for disposing of contaminated waste (gloves and tongue depressors)
  - b. Ice chest and frozen ice packs
  - c. Swabs
    - i. Sterile water [or saline or pH 7.0 buffer or transport medium (Cary Blair) or sterile broth medium such as peptone, TSB, skim milk]
    - ii. Alternatives for swabbing
      1. Sterile cotton- or polyester-tipped swabs and sterile screw capped tubes or tubes of transport medium
      2. Gauze squares and Whirl-Pak bags or zipper-lock bags for larger surfaces
      3. Sterile sponges (Speci-Sponges for larger surface areas  
[http://www.fishersci.com/ecomm/servlet/fsproductdetail\\_10652\\_772518\\_1\\_0](http://www.fishersci.com/ecomm/servlet/fsproductdetail_10652_772518_1_0))
  - d. Fecal specimens or animal bedding or dry food – alternatives for collection
    - i. Tongue depressors for scooping up material (in a pinch, new, never-used plastic spoons can be used as long as these are not from household being sampled)
    - ii. Whirl-Pak or zipper-lock bags or new, never-used plastic food containers (Tupperware-type)
  - e. Water Collection Alternatives (at least 100 ml volume)
    - i. Sterile or new/never-used plastic wide mouth bottles (ideally)
    - ii. Or a new plastic container that has never been used because it is not likely to be contaminated with salmonella
    - iii. Whirl-Pak bags (double bag)
    - iv. Urine collection containers
    - v. Conical Centrifuge Tubes 50 ml
    - vi. Drinking water bottles (new and never opened) of purified drinking water (unchlorinated) such as Aquafina (pour out water first)
    - vii. New/never used (not contaminated with chlorine) water bottles from a pool supply store for testing water
2. Swabs of the reptile environment, especially in areas where fecal material may be concentrated, should be taken. Wet surfaces are best to culture. Thoroughly wet the swab by rolling it along the surface you are culturing. Limit excessive amounts of organic matter or debris on the swab as this can cause false negatives.
3. For large enclosures, such as reptile breeding facilities, swabs may be taken from the bottom of shoes worn at the site, or any object that had contact with the enclosure's ground, such as wagon