

# **A Multi-County and Multi-State Outbreak of Salmonella enteritidis (group D) Among Oriental Restaurant and Market Patrons**

**Bill L. Toth, MPH, Donna Walsh, RN, BSN, Zuber Mulla, PhD, MSPH,  
Dean Bodager, RS, MPA, Roberta Hammond, PhD**

## **Introduction**

In the reporting week of May 7, 2001, Infection Control Practitioners from two large Orlando, Florida area hospitals reported unusual numbers of *Salmonella sp.* Group D cases to the Orange County Health Department. Cases were diagnosed with diarrheal illness, treated and released or admitted. Reported cases totaled approximately twice the number seen in background surveillance. Exposure questioning revealed that fourteen of the first 15 reported cases had history of eating at Asian restaurants or purchasing foods from oriental markets within three days of onset. A rapid assessment of food items consumed indicated that mung bean sprouts was the single common food item among fourteen of the initial fifteen (93.3%) reported ill. Hospital laboratories that isolated cultures were contacted to request all *Salmonella* group D subcultures be forwarded to the Florida Department of Health Laboratories in Jacksonville for Pulsed Field Gel Electrophoresis (PFGE) analysis.

Laboratory confirmed cases have been reported from Orange (29) and Seminole (4) counties in Florida with two (2) cases reported from Minnesota. Twenty-four of Orange County were laboratory confirmed and five were epi-linked. One of the Orange County cases was diagnosed with *Salmonella* septicemia. These restaurant facilities (13), primarily of Vietnamese or Thai management and cuisine, were located in a relatively small geographic area of Orlando. Of the two markets, one was in Orange County located in the same area as were the restaurants, the second was located in neighboring Seminole County. Since there the number of facilities was suggestive of a product supply issue, Florida Department of Business and Professional Regulation, Hotels and Restaurants (BPR), and Florida Department of Agriculture and Consumer Services (DACCS) were notified to ask their assistance in determining supply sources.

## **Materials and Methods**

A case-control study was conducted to identify risk factors for illness. The following case definition was used: An individual with a laboratory-confirmed *Salmonella* serogroup D infection, or an ill individual who is epidemiologically linked to a confirmed case, who ate a meal(s) at or from an Oriental restaurant located in Orange County or Seminole County during their incubation period, and had onset of illness during April or May of 2001. Two ill individuals who were included in the general epidemiologic investigation were excluded from the case-control study because they did not report recent patronage of an Oriental restaurant. One of these cases had purchased food at an Oriental grocery store.

Controls were selected from healthy meal partners of the cases. A total of 35 cases and 18 controls were included in the study. Four of the 31 cases were not laboratory-confirmed cases but were linked in an epidemiologic fashion to a confirmed case. Demographic information and food histories were obtained using a questionnaire.

Data were entered into the Epi Info Version 6.04b statistical package (CDC, Atlanta, GA). Epi Info was used to perform univariate analyses. For each of the most popular food items, the maximum likelihood estimate of the odds ratio was calculated along with its exact 95% mid-P confidence interval. The SAS statistical package, Release 8.00 for Windows, (SAS Institute, Cary, NC) was used to perform multiple logistic regression [1]. Due to a small sample size, not all of the variables that were examined in the univariate analysis could be included in the multivariate analysis. The four strongest risk factors were included in the multivariate analysis. A backward elimination procedure was used to choose the strongest predictors of illness from this group of select variables [2]. In this approach the null hypothesis was  $\beta=0$  for each variable. The significance level chosen for a variable to stay in the model was 0.10. Crude and adjusted odds ratios were calculated along with 95% confidence intervals.

## Results

Reported cases ate at Asian restaurants or consumed food from Asian markets from April 22 through May 9, 2001. All of the cases experienced diarrhea while 74% had fever (23/31), 71% had abdominal cramps (22/31), 48% had chills (15/31), 45% reported vomiting (14/31), 42% had a headache (13/31), 32% had blood in their stools (10/31), 32% had nausea (10/31), 32% had myalgia, and 23% had mucous in their stool (7/30). A 75 year-old man diagnosed with *Salmonella* bacteremia was reported with an onset date of June 11, 2001, however his exposure was consistent with others in regard to time and place of contact. The epidemic curve is shown below. Seven cases had onset of illness on May 4, 2001.

The only food item that was significantly associated with illness in the univariate analysis was mung bean sprouts (Table 1). A total of 89% of the cases (24/27) had eaten food items containing bean sprouts (see Appendix 1, Contingency Tables). Individuals questioned gave descriptions of the sprouts was consistent with that of mung bean sprouts.

Shrimp roll, basil, cilantro, and bean sprouts were subjected to stepwise logistic regression. The only significant predictors of illness at the 0.10-level were shrimp rolls and bean sprouts (Table 2). Each of the odds ratios shown in Table 2 is adjusted for any confounding by the other variable. Only bean sprouts is statistically significant at the 0.05-level (Adjusted odds ratio=9.33, 95% confidence interval: 1.58 – 55.03).

PFGE analyses performed on 17 *Salmonella enteritidis* subcultures from cases in Orange and Seminole counties matched each other utilizing primary enzyme PFGE-XbaI; two additional cases added in later analyses matched as well. Nine of nineteen randomly selected subcultures from the original set of samples matched in secondary PFGE analysis utilizing PFGE-BlnI enzyme. Tertiary analysis utilizing PFGE-SpeI enzyme on seven of 19 randomly selected samples matched (see Fig. 1).

Dice (Tol 2.0%-3.0%) (H>0.0% S>0.0%) [0.0%-100.0%]  
**PFGE E-Xba I**

**PFGE E-Bln I**

**PFGE E-Spe I**

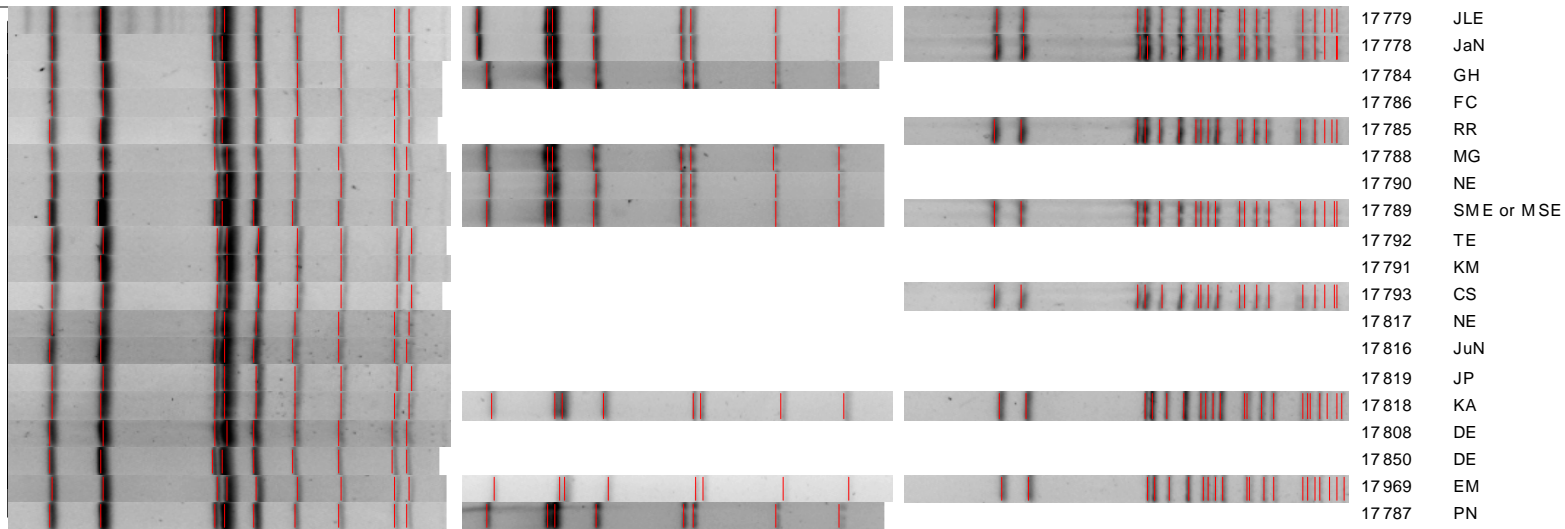


Figure 1. Primary, secondary and tertiary PFGE analyses of *S. enteritidis*, cluster 0025

PFGE analyses performed on two samples of *S. enteritidis* utilizing Enzyme-XbaI were compared to four other clusters or groups in Florida. Results indicate the Orlando and Seminole County cluster (cluster 0025) were different from the four other clusters and were perhaps unique from all other Florida samples (see Fig. 2).

Dice (Tol 2.0%-3.0%) (H>0.0% S>0.0%) [0.0%-100.0%]  
**PFGE-Xba I**

**PFGE-Xba I**

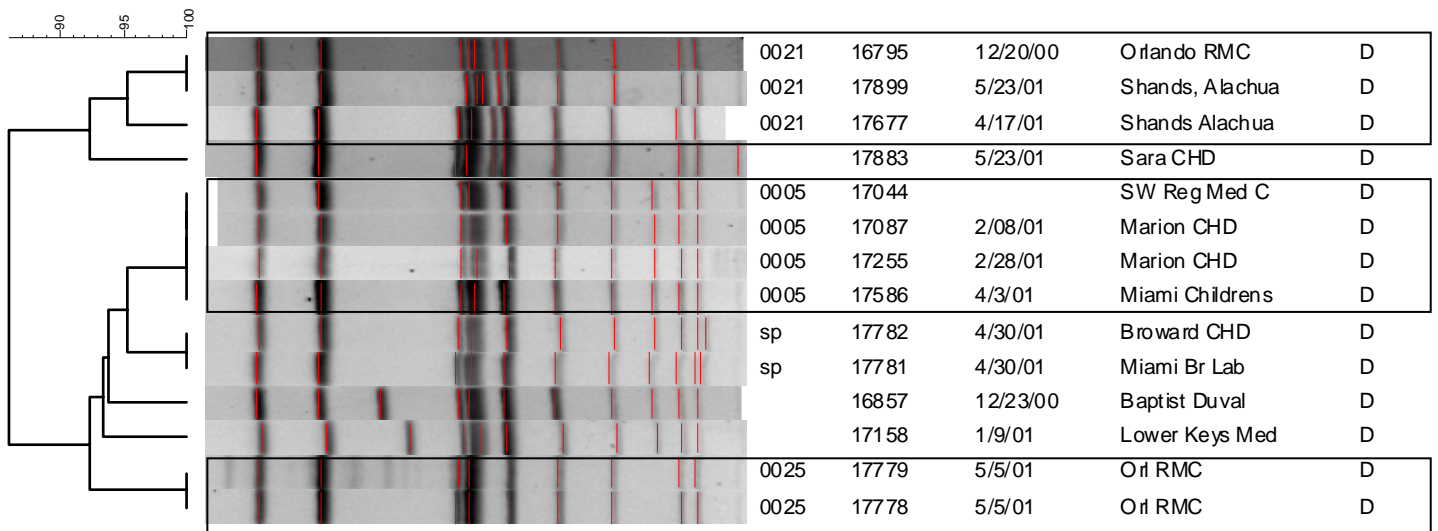
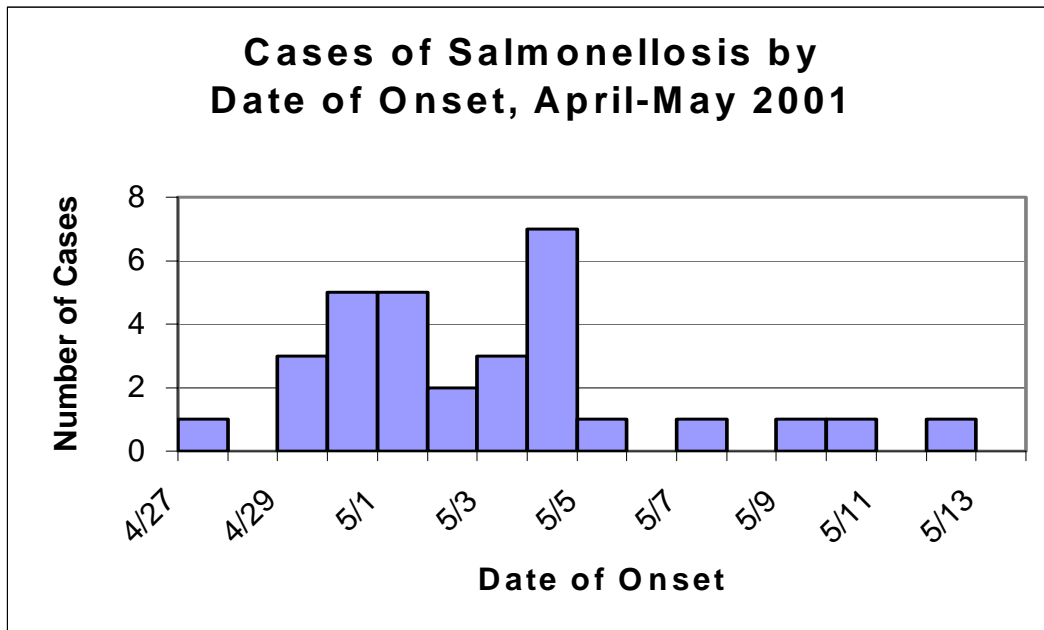


Figure 2. PFGE comparative analyses, utilizing Enzyme X-baI, with five Florida Salmonella types.

PFGE patterns submitted to Pulsnet were examined by Canadian authorities, and compared to a mung bean sprout related outbreak in Canada during February 2001. Matches with two of three enzyme patterns in Florida suggest a similarity to the Canadian cluster. Since the Canadian group performed phage-typing analysis, subcultures from the Florida cluster were forwarded to The Centers for Disease Control in Atlanta for similar typing. **Phage-typing results for Florida cases matched Canadian cases with phage-type 913.**



**Table 1. Crude/Unadjusted Odds Ratios (OR) for Illness**

Exposure	Crude OR (95% Confidence Interval)	Available Sample Size	Significant?
Chicken entrée	0.91 (0.24 – 3.63)	31 cases, 18 controls	No
Pork entrée	0.84 (0.20 – 3.91)	31 cases, 18 controls	No
Beef entree	1.80 (0.18 – 50.61)	31 cases, 18 controls	No
Noodles entrée	2.73 (0.54 – 20.98)	31 cases, 18 controls	No
Spring roll	1.45 (0.32 – 7.83)	31 cases, 18 controls	No
Roll with peanut sauce	0.84 (0.20 – 3.91)	31 cases, 18 controls	No
Summer roll	0.82 (0.23 – 3.03)	31 cases, 18 controls	No

Shrimp roll	3.72 (0.77 – 27.98)	31 cases, 18 controls	No
Lettuce, salad	2.15 (0.41 – 16.88)	31 cases, 17 controls	No
Cucumber garnish	0.33 (0.05 – 1.83)	31 cases, 16 controls	No
Carrot garnish	1.66 (0.30 – 13.36)	31 cases, 16 controls	No
Green onion garnish	1.59 (0.16 – 44.98)	31 cases, 16 controls	No
Basil garnish	5.07 (0.71 – 123.93)	31 cases, 16 controls	No
Cilantro garnish	5.07 (0.71 – 123.93)	31 cases, 16 controls	No
Mint garnish	1.04 (0.16 – 8.94)	31 cases, 16 controls	No
Beverage	0.65 (0.15 – 2.50)	31 cases, 17 controls	No
Water with lemon	1.35 (0.30 – 7.35)	31 cases, 17 controls	No
Ice	0.45 (0.12 – 1.59)	31 cases, 17 controls	No
Bean sprouts	6.62 (1.40 – 38.31)	27 cases, 15 controls	<b>Yes</b>

NOTE: The sample size fluctuates with the exposure due to missing values.

**Table 2. Results of Stepwise Logistic Regression:  
Adjusted\* Odds Ratios (OR) for Illness,  
27 Cases and 15 Controls**

Exposure	Adjusted OR (95% Confidence Interval)	p-value
Bean sprouts	9.33 (1.58 – 55.03)	0.01
Shrimp roll	5.61 (0.79 – 39.77)	0.08

\* Each odds ratio is adjusted for the remaining exposure.

Information received from the Florida Department of Business and Professional Regulation, Division of Hotels and Restaurants, and Florida Department of Agriculture and Consumer Services in initial bean sprout trace-back data suggest thirteen restaurants and two markets were supplied through sources in Orlando and Tampa, Florida. A grower in the Orlando area supplied 74 stores in the general area with restaurants named as point of contact for several confirmed or epi-linked cases. Per invoice data, this grower received mung bean seeds from a large warehouse in Kentucky. Canada reported their trace-back procedures linked mung bean seeds to the same Kentucky warehouse company. Four other distributors were listed as sources in the Orlando area; their suppliers are linked to distributors in the Tampa area. Currently, the Florida trace-back procedure is continuing through a multi-agency task force. Data suggest distribution of mung bean seeds originated in China, and was brokered or supplied through a large company in Kentucky to Florida growers.

## Conclusions

Exposure and statistical data are consistent in this cluster of *Salmonella enteritidis* to be linked to the ingestion of mung bean sprouts at several Asian restaurants and markets in Orange and Seminole Counties, Florida during the period, April 22 through May 9, 2001. The restaurants associated with this outbreak, primarily Vietnamese and Thai cuisine, serve entrées that include raw or undercooked mung bean sprouts.

Mung bean sprouts, and many other types of sprouts, have been implicated in multiple outbreaks, including *Salmonella*, in the United States and other countries (3). This outbreak included

exposures in several Asian style restaurants that were supplied by local growers in Florida. Since several growers may have been supplied through many sources, it is likely their primary source supplied seeds that were initially contaminated with *Salmonella* bacteria.

Decontamination activities at the grower's sites may have been inadequate to reduce the inoculum of growing beds. Ingestion of raw or undercooked sprouts was likely to have played a role in this outbreak; several Chinese style restaurants that were supplied mung bean sprouts in the Orange and Seminole County area of Florida were not named as establishments frequented by those ill. Chinese style cuisine is more likely to serve cooked sprouts and thus reduce or destroy *Salmonella* bacteria. Adequate supply, decontamination and cooking procedures could reduce exposure to bacterial agents.

*Acknowledgement: Pulsed Field Gel Electrophoresis (PFGE) laboratory analyses were expediently performed at the Florida Department of Health Laboratories by Paul Fiorella, PhD. We wish to thank Dr. Fiorella for his diligent work and his willingness to share information regarding the Canadian connection in their outbreak.*

## References

1. SAS Institute Inc. The LOGISTIC Procedure. In: *SAS/STAT User's Guide*, version 6. Fourth edition. V. 2. Cary, NC: SAS Institute Inc., 1989; 1071-1126.
2. Kleinbaum DG, Kupper LL, and Muller KE. Selecting the Best Regression Equation. In: *Applied Regression Analysis and Other Multivariable Methods*. Second edition. Boston: PWS-Kent, 1988; 324-325.
3. Taormina PJ, Beuchat LR, and Slutsker L. Infections Associated with Eating Seed Sprouts: An International Concern. *Emerging Infectious Diseases, CDC, Vol.5, No.5*



**9. Contributing Factors:** (See list on page 3, check all that apply)  
 Contributing factors unknown

**Contamination Factor:**  
C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12  
C13 C14 C15 (describe in Comments)  N/A

**Proliferation/Amplification Factor (bacterial outbreaks only):**  
P1 P2 P3 P4 P5 P6 P7 P8 P9 P10 P11 P12  
(describe in Comments)  N/A

**Survival Factor (microbial outbreaks only):**  
S1 S2 S3 S4 S5 (describe in Comments)  N/A

**Was food-worker implicated as the source of contamination?**  
If yes, please check **only one** of following:  
 laboratory and epidemiologic evidence  
 epidemiologic evidence (w/o lab confirmation)  
 lab evidence (w/o epidemiologic evidence)  
 prior experience makes this the likely source (please explain in Comments)

**10. Agency reporting this outbreak:**  
ORANGE CHD

**Contact Person:**  
NAME: BILL TOTH  
TITLE:  
PHONE NO: 407-623-1212  
FAX NO: 407-741-4689  
E-MAIL: BILL\_TOTH@DOH.STATE.FL.US

**Date of completion of this form:**  
Feb 25 2002 12:00AM

**Initial Report**  
 **Updated Report**  
 **Final Report**  
 **Additional data suggests this is not a foodborne outbreak**

**Part 2: Additional Information** (Please complete as much as possible)

11. Numbers of:			12. Incubation Period:	13. Duration of Acute Illness Among Those Who Recovered:																											
OUTCOME/SYMPTOM	Cases with Outcome / Symptom	Total cases for whom you have information available	Shortest: 17.00 Hours Longest: 30.00 Days Median: 32.00 Hours  <input type="checkbox"/> Unknown	Shortest: Longest: Median:  <input type="checkbox"/> Unknown																											
Healthcare Provider Visit	33		* Use the following terms, if appropriate, to describe other common characteristics of cases:  <table border="0"> <tr> <td>anaphylaxis</td> <td>diplopia</td> <td>myalgia</td> </tr> <tr> <td>arthralgia</td> <td>flushing</td> <td>paresthesia</td> </tr> <tr> <td>bradycardia</td> <td>headache</td> <td>septicemia</td> </tr> <tr> <td>bullous skin lesions</td> <td>hemolytic uremic syndrome (HUS)</td> <td>sore throat</td> </tr> <tr> <td>cough</td> <td>hypotension</td> <td>tachycardia</td> </tr> <tr> <td>coma</td> <td>itching</td> <td>thrombocytopenia</td> </tr> <tr> <td>descending paralysis</td> <td>jaundice</td> <td>temperature reversal</td> </tr> <tr> <td></td> <td>lethargy</td> <td>urticaria</td> </tr> <tr> <td></td> <td></td> <td>wheezing</td> </tr> </table>	anaphylaxis	diplopia	myalgia	arthralgia	flushing	paresthesia	bradycardia	headache	septicemia	bullous skin lesions	hemolytic uremic syndrome (HUS)	sore throat	cough	hypotension	tachycardia	coma	itching	thrombocytopenia	descending paralysis	jaundice	temperature reversal		lethargy	urticaria			wheezing	
anaphylaxis	diplopia	myalgia																													
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cough	hypotension	tachycardia																													
coma	itching	thrombocytopenia																													
descending paralysis	jaundice	temperature reversal																													
	lethargy	urticaria																													
		wheezing																													
Diarrhea	35																														
Bloody Stools	10																														
Fever	24																														
Abdominal Cramps	35																														
*mucus in stool	7																														
*headache	13																														
*other	1																														
*																															

**14. If Cohort Investigation Conducted:**   
Event-specific Attack Rate =  $\frac{34}{\# \text{ ill total}}$  /  $\frac{60}{\# \text{ of persons for whom you have illness info.}}$  x 100 = 57%

**15. Where was Food Prepared? (Check all that apply)**

<input type="checkbox"/> Restaurant or deli	<input type="checkbox"/> Nursing home
<input type="checkbox"/> Day care center	<input type="checkbox"/> Prison, jail
<input type="checkbox"/> School	<input type="checkbox"/> Private home
<input type="checkbox"/> Church, temple, etc	<input type="checkbox"/> Picnic
<input type="checkbox"/> Camp	<input type="checkbox"/> Fair, festival, other temporary/ mobile services
<input type="checkbox"/> Caterer	<input type="checkbox"/> Contaminated food imported into U.S.
<input type="checkbox"/> Grocery Store	<input type="checkbox"/> Commercial product, served without further preparation
<input type="checkbox"/> Hospital	<input type="checkbox"/> Other (please describe)
<input type="checkbox"/> Workplace cafeteria	

**16. Where was Food Eaten? (Check all that apply)**

<input type="checkbox"/> Restaurant or deli	<input type="checkbox"/> Nursing Home
<input type="checkbox"/> Day care center	<input type="checkbox"/> Prison, jail
<input type="checkbox"/> School	<input type="checkbox"/> Private home
<input type="checkbox"/> Church, temple, etc.	<input type="checkbox"/> Picnic
<input type="checkbox"/> Camp	<input type="checkbox"/> Fair, festival, temporary/ mobile service
<input type="checkbox"/> Grocery Store	<input type="checkbox"/> Other (please describe)
<input type="checkbox"/> Hospital	
<input type="checkbox"/> Workplace cafeteria	

**17. Other Available Info:**

Unpublished agency report (please attach)  
 Epi-Aid  
 Publication (please reference)  
 Not available

**18. Remarks:** Briefly describe important aspects of the outbreak not covered above (e.g., restaurant closure, product recall, immunoglobulin administration, economic impact, etc)

State Health Departments: If you have not entered this information into EFORS (Electronic Foodborne Outbreak Reporting System), please send this document to the Foodborne and Diarrheal Disease Branch, Centers for Disease Control and Prevention, 1600 Clifton Road Mailstop A-38, Atlanta, GA 30333, Phone: 404-639-2206, Fax: 404-639-2205