Salmonella in the Caribbean

A Classroom Case Study

INSTRUCTOR'S VERSION

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NOTE: This case study is based on real-life investigations undertaken in Trinidad and Tobago in 1998-1999 and published in Clinical Infectious Diseases and the West Indian Medical Journal. (See Appendix for abstracts.) Some aspects of these investigations (and the circumstances leading up to them) have been altered to assist in meeting the desired teaching objectives and some details have been fabricated to provide continuity to the storyline.

Target audience: public health practitioners with knowledge of basic epidemiologic concepts, especially non-epidemiologists (e.g., laboratorians, environmental health specialists, sanitarians, public health nurses, veterinarians, MPH students)

Level of case study: basic

Teaching materials required: graph paper, calculator

Time required: 3-4 hours

Language: English

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Public Health Service
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INSTRUCTOR'S VERSION
***Salmonella* in the Caribbean**

Learning objectives:

After completing this case study, the student should be able to:
1) describe the signs and symptoms, means of diagnosis, and control of salmonellosis
2) describe how *Salmonella* serotyping can be used in public health practice
3) given a disease, describe the desired characteristics of a surveillance system for that disease
4) discuss how the inclusion of the laboratory in the surveillance of a disease impacts the characteristics of the surveillance system and the usefulness of the data
5) calculate the incidence of a disease if given the number of cases and population size
6) characterize a health problem by time, place, and person (e.g., perform the descriptive epidemiology)
7) create and interpret a graph
8) interpret the measure of association for a case-control study

Part I – Background on *Salmonella*

Salmonellosis is a gastrointestinal illness caused by bacteria from the genus *Salmonella*. The illness is characterized by the sudden onset of headache, abdominal pain, diarrhea (which may be bloody), nausea, and sometimes vomiting. Fever is almost always present. The illness typically lasts for 5-7 days and usually does not require treatment unless the patient becomes severely dehydrated or the infection spreads from the intestines. In the immunocompromised host or an overwhelming infection in a normal host, *Salmonella* may spread to the blood stream and other body sites, and can cause death unless treated promptly with antibiotics.

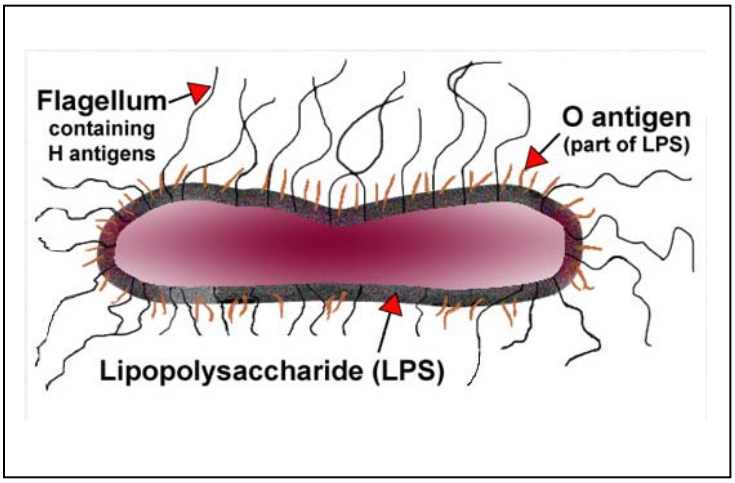
Salmonella live in the intestinal tracts of humans and other animals, including mammals, birds, and reptiles. *Salmonella* are usually transmitted to humans by eating foods contaminated with animal feces. Implicated foods are typically those of animal origin, such as beef, poultry, milk, or eggs, but all foods, including vegetables, may become contaminated. The incubation period for salmonellosis is usually 12-36 hours, but can be as long as a week.

Question 1: How is salmonellosis diagnosed? How does the method of diagnosis impact our understanding of the occurrence of salmonellosis in the community (e.g., burden of disease, trends over time, high-risk populations)?

Many diseases can cause fever, diarrhea, and abdominal cramps. As a result, ***salmonellosis cannot be diagnosed based on symptoms alone****. Because Salmonella most often reside in the gastrointestinal tract, salmonellosis is usually diagnosed by isolating the organism from the stool of the patient, although it can sometimes be isolated from blood and other bodily fluids. Stools specimens should be collected during the period of active diarrhea (preferably as soon after onset of symptoms as possible).*

The need to confirm the diagnosis in the laboratory impacts our understanding of the occurrence of salmonellosis. To be laboratory confirmed: 1) the patient has to seek medical care, 2) a specimen has to be collected (while the patient is still shedding the organism), and 3) appropriate laboratory tests/cultures must be performed. Since only a fraction of patients with salmonellosis follow this course, laboratory-confirmed cases of Salmonella will ***underestimate*** *the number of Salmonella infections in the community. Furthermore, because patients from whom specimens are collected are likely to be sicker and have better access to health care (e.g., have higher incomes, be employed and have access to health insurance, be located in an urban setting) than patients from whom specimens are not collected, their characteristics* ***may not be representative*** *of all patients with the infection.*

Figure 1. *Salmonella* surface antigens



The genus *Salmonella* consists of only two species: *S. enterica* and *S. bongori*. The latter species, however, is very rare.

Members of the species *Salmonella enterica* can be divided into smaller groups (i.e., serotypes) based on two structures on the cell surface: the O antigen and the H antigen. The O antigen is a carbohydrate antigen in the lipopolysaccharide of the bacterium's outer cell membrane. The H antigen is a protein antigen in the bacterium's flagella. (Figure 1)

O antigens and H antigens are detected using antisera that react with a single antigen or group of related antigens. All *Salmonella* serotypes can be designated using a formula based on the O and H antigens they express. Many serotypes are also given a name (e.g., *Salmonella* Typhimurium, *Salmonella* Agona, *Salmonella* Muenchen). (NOTE: The serotype name is capitalized and not italicized.)

Although extensive serotyping of surface antigens can be used for identification of a *Salmonella* isolate, the reagents are costly, the process is time-consuming, and the results are not likely to affect treatment of the individual patient. As a result, in many countries clinical laboratories perform only a few O antigen reactions that allow them to group an isolate into broader, less specific categories called serogroups. The isolate is then forwarded to a state or national reference laboratory for complete serotyping.

There are over 2,500 recognized *Salmonella* serotypes. In 1995, *Salmonella* Enteritidis, Typhimurium, and Typhi accounted for over three-quarters of the isolates reported in a global survey.

Question 2: Describe how serotype results can be used in public health practice.

Because outbreaks of Salmonella are typically caused by contamination of food and water with a single serotype, routine serotyping of isolates can provide critical information to investigate and control outbreaks. Serotyping can help determine:

- *if **cases of the same disease are related** (i.e., are likely to represent an outbreak)*
- *if a **vehicle** (e.g., a food item) that is contaminated with bacteria is **related to a particular outbreak***

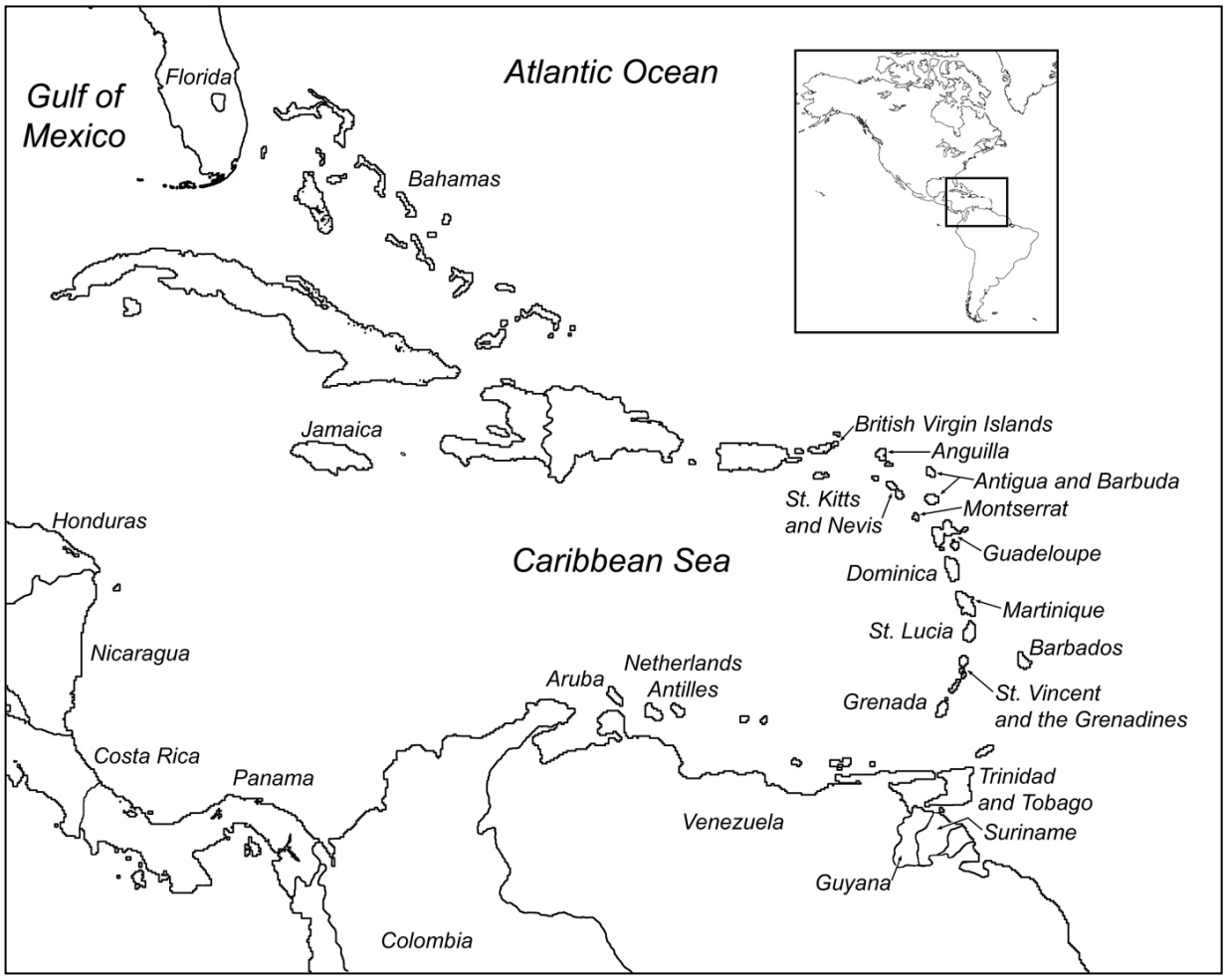
Serotype information can also be used to compare human isolates with animal and food isolates identified through other surveillance systems. The resulting information is useful in assessing the scope of a problem, investigating its source, and planning and evaluating interventions.

*Serotyping, however, is an **adjunct to epidemiologic investigation** and not a replacement for it. Similar serotypes should not be considered proof of a common exposure, merely that the isolates share a common ancestry. An epidemiologic investigation is necessary to demonstrate that there is a common source of infection.*

Part II – Surveillance of *Salmonella* in the Caribbean

As early as the mid-1980s, *Salmonella* became a pathogen of public health concern in the Caribbean (Figure 2) when it caused an increasing number of cases and outbreaks of diarrhea involving local and tourist populations. The communicable disease surveillance system in place at the time, however, did not support the timely detection of these outbreaks or the investigation of risk factors associated with infection. As a result, the incidence of *Salmonella* continued to grow.

Figure 2. Countries of the Caribbean and surrounding land masses.



Question 3: To detect outbreaks of infectious diseases (e.g., salmonellosis) and investigate risk factors for infection, what characteristics should a communicable disease surveillance system have?

A surveillance system should be developed to meet the intended purpose of the system. To detect outbreaks in the community and investigate risk factors for infection so that control measures can be implemented, a surveillance system needs to have the following characteristics:

- *It should be able to detect a large proportion of infections that occur in the community (i.e., have a **high sensitivity**).*
- *Reported cases, however, should have a high probability of being true cases (**high positive predictive value**) and should include serotype results to enhance the detection of potential linkages between cases.*
- *Finally, the system should be **timely**, with a minimal delay between onset of symptoms in the patient and receipt of the case report. This will allow public health officials to initiate investigations as quickly as possible and implement control measures to limit morbidity and mortality.*

NOTE TO INSTRUCTORS: Students should keep the above characteristics in mind as they learn about the Caribbean communicable disease surveillance system.

The communicable disease surveillance system in the Caribbean was based on notifiable disease reports from physicians and other health care providers in the community (i.e., clinician-based reporting). Surveillance of most communicable diseases included both laboratory-confirmed cases and cases diagnosed based on clinician suspicion. The laboratory did not report cases of communicable disease to the surveillance system or submit isolates for confirmation or further testing (e.g., serotyping).

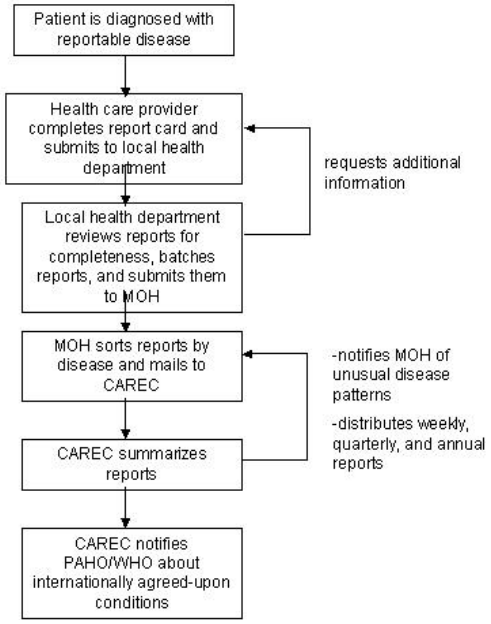
To report a communicable disease in the Caribbean, the health care provider completed a disease report card (Figure 3) and mailed it to the local health department within 7 days of diagnosis of the patient.

Figure 3. Communicable Disease Case Report Card

<table>
<tr><td colspan="2">CARIBBEAN EPIDEMIOLOGY CENTRE
Clinician-based Reporting
COMMUNICABLE DISEASE CASE REPORT CARD</td></tr>
<tr><td colspan="2">Case identification
Last name, First name, Middle initial:</td></tr>
<tr><td colspan="2">Address:</td></tr>
<tr><td colspan="2">City/Country:</td></tr>
<tr><td>Disease information
Diagnosis:
Lab-confirmed: ☐ Yes ☐ No
Date of onset:</td><td>Case information
Sex: ☐ Male ☐ Female
Age:
Current status: ☐ Alive ☐ Dead</td></tr>
<tr><td>Attending physician
Name:
Address:
Telephone number:</td><td>Person reporting case (if not attending physician)
Name:
Telephone number:</td></tr>
</table>

A clerk at the local health department reviewed the report cards for completeness (requesting additional information from the health care provider, where needed), batched the reports, and mailed them to the country's Ministry of Health where they were sorted by disease. The Ministry of Health forwarded the reports to the Caribbean Epidemiology Centre (CAREC).
An epidemiologist from CAREC reviewed and summarized the reports from the individual countries. If necessary, the epidemiologist contacted the Ministry of Health if an unusual disease pattern was noted. CAREC distributed weekly, quarterly, and annual communicable disease reports for the region and each country to all Ministries of Health. In addition, CAREC reported occurrences of selected diseases (e.g., cholera, plague, yellow fever) to the Pan American Health Organization/World Health Organization as required by International Health Regulations.

Question 4: Diagram the flow of information in the Caribbean communicable disease surveillance system.



In an evaluation of the Caribbean communicable disease surveillance system, it was determined that less than 40% of notifiable disease cases were actually reported by health care providers. The average reporting delay (i.e., from diagnosis to receipt of the report by CAREC) was 56 days.

Question 5: Evaluate the Caribbean communicable disease surveillance system with respect to the desired goals of outbreak detection and investigation of risk factors for infection. What changes would you make to the surveillance system? Why?

NOTE TO INSTRUCTORS: You may wish to use a white board or flip chart to create a list of strengths and weaknesses. To increase participation, have each student provide only one strength or weakness and move on to the next student.

Strengths:

- *Clinicians are a well-established and traditional source of reporting. As a result, the responsibility for reporting is generally accepted among most health care providers.*

- *Because clinicians are typically the first point of contact between the patient and the health care system, the system has the potential for increased sensitivity and timeliness.*
- *Because clinicians have more information on the patient (e.g., patient characteristics, risk factors), the system can provide a more accurate description of the population at risk. Clinicians also have better access to patients if additional information is needed or special investigations are undertaken.*
- *The Communicable Disease Case Report Card is short but includes the necessary information (e.g., patient identifying information, demographic information, clinical information, name of the health care provider). This brevity enhances the acceptability of the system and increases the likelihood that health care providers will report.*

Weaknesses:

- *Reporting of communicable diseases to the system is incomplete (40%) and lacks sensitivity.*
- *Lack of laboratory confirmation means that reported cases have a low positive predictive value.*
- *Lack of subtyping inhibits the system's ability to detect outbreaks.*
- *Clinician-based reporting involves a large number of individuals. This makes it more difficult to change reporting procedures (e.g., add new diseases, collect additional information).*
- *Mailing of reports from health care providers, the multiple parties involved with processing of reports (i.e., local health department, Ministries of Health, CAREC), and the batching and holding of reports at various points along the way decreases the timeliness of reporting.*

Desirable changes:

- ***Require laboratory confirmation** of diseases for which laboratory tests/cultures are necessary for a definitive diagnosis (e.g., shigellosis, salmonellosis, hepatitis A). Laboratory confirmation will increase the positive predictive value of the system.*
- ***Require clinical laboratories to report** the detection of notifiable diseases directly to the reporting authority. Because most laboratories are computerized, labs may be able to submit reports through automated computer-based systems that will likely increase the completeness and timeliness of reporting.*
- ***Require clinical laboratories to submit isolates** for selected diseases (e.g., Salmonella) to the national laboratory **for subtyping**. Through subtyping, the public health officials may be able to identify potential linkages between cases (and, therefore, possible outbreaks) and compare human, animal, and food subtype results.*
- ***Streamline the flow of information** and speed the transmission of reports to the final recipient (e.g., send reports on a daily basis where possible instead of batching or holding them).*
- ***Improve the communication network** between health care providers, clinical laboratories, and public health officials with respect to reporting and use of surveillance information.*

After extensive consultation with the Ministries of Health from the individual Caribbean countries, health care providers, professional medical organizations, and clinical laboratories, CAREC proposed a modification of communicable disease reporting in the region.

In addition to health care providers (i.e., clinician-based surveillance), clinical laboratories were enlisted to report the detection of notifiable diseases that were laboratory confirmed (i.e., laboratory-based surveillance). Clinical laboratories were also asked to forward all isolates of *Salmonella* to the national reference laboratory in Trinidad (i.e., the Public Health Laboratory). Staff at the Public Health Laboratory, who had specialized training in *Salmonella* serotyping and access to specialized reagents, were to perform serotyping and antimicrobial susceptibility testing on forwarded *Salmonella* isolates.

To further improve the timeliness of reporting, health care providers and clinical laboratories were to submit reports directly to newly designated surveillance officers in each country's Ministry of Health. Diseases that potentially could be spread through food or water or readily from person-to-person were to be reported within 24 hours of diagnosis. The remainder were to be reported within 3 days of diagnosis. Health care providers and clinical laboratories were encouraged to submit reports by telephone or FAX.

Initial acceptance and implementation of the new communicable disease reporting procedures were slow. Member countries had limited public health resources to initiate the changes and there was resistance among health care providers and clinical laboratories.
Question 6: What might be done to encourage acceptance of the surveillance system and improve reporting?

Efforts to improve the acceptance of the surveillance system are largely three-fold:

1) ***Make the reporting system as simple and straightforward as possible****. Minimize the burden of reporting by limiting the amount of information collected, using forms that are easy to complete, allowing for the submission of reports by phone/fax, and creating computer programs that can automatically generate reports when certain conditions are met. Where possible, provide support to health care providers and clinical laboratories in the form of finances, staff, and/or equipment that will facilitate reporting to the health department.*

2) ***Educate health care providers and clinical laboratories about reporting.*** *This includes not only education about the reporting process itself (e.g., what to report to whom and how), but also the rationale for the reporting. Health care providers and clinical laboratories need to understand why reporting is important and how the information will be used. They will become much more compliant with reporting if they understand the impact of the disease on the community (e.g., incidence, morbidity, mortality, socioeconomic impact) and the public health actions that will be taken based on the reports (e.g., contact investigations, treatment and/or prophylaxis of contacts, implementation of vaccination programs, investigations to determine the source, and implementation of control measures appropriate to that source).*

3) ***Provide feedback to health care providers and clinical laboratories that report cases.*** *Acknowledging the receipt of reports and providing routine information about cases back to health care providers and clinical laboratories (in the form of a weekly or monthly report that summarizes case counts with special articles about specific disease trends or investigations) is an ideal way to show them that the information is being used.*

Staff from CAREC visited member countries and, with the assistance of staff from the local Ministry of Health, provided training to both health care providers and staff from clinical laboratories. Training focused on the mechanics of reporting and how surveillance data would be used to monitor disease trends, detect outbreaks, and initiate controls measures. Many of the presentations were made at professional meetings, allowing for an open discussion of the reporting procedures and surveillance in general.

CAREC staff toured the larger clinical laboratories in the various countries and identified problems associated with testing, reporting, and the forwarding of *Salmonella* isolates to the national Public Health Laboratory in Trinidad. A resource person was identified at the Public Health Laboratory to provide ongoing support to all clinical laboratories.

A close working relationship developed between the Public Health Laboratory in Trinidad and CAREC. Laboratory staff forwarded laboratory results to epidemiologists at CAREC on a weekly basis and notified them by phone if an unusual case was noted or an increase in the isolation rate of a particular disease occurred.

CAREC staff summarized communicable disease surveillance results (including serotype and antimicrobial susceptibility test results) and distributed a weekly summary to the Ministries of Health and monthly updates to health care providers and clinical laboratories. They worked closely with staff from the respective Ministries of Health if an unusual disease pattern was noted or some reporting problem became evident.

Part III – Descriptive Epidemiology of *Salmonella* in Trinidad

Due to the close proximity of both CAREC and the national Public Health Laboratory, Trinidad and Tobago moved most quickly on the implementation of the new reporting procedures. As a result, several large outbreaks of salmonellosis were detected allowing local public health practitioners to initiate investigations and implement appropriate control measures. However, salmonellosis continued to occur at a high rate in the country.

In 1998, CAREC summarized the following data for laboratory-confirmed cases of salmonellosis reported in Trinidad and Tobago.

Table 1. Laboratory isolates of *Salmonella* by serotype and year of diagnosis, Trinidad and Tobago, 1988-1997.

	Year of Diagnosis									
Serotype	**88**	**89**	**90**	**91**	**92**	**93**	**94**	**95**	**96**	**97**
Enteritidis	0	0	0	0	1	0	18	47	107	73
Typhimurium	4	6	9	17	84	45	37	13	11	5
Other	27	18	27	48	21	37	44	49	57	31
TOTAL	31	24	36	65	106	82	99	109	175	109

Question 7A: Calculate the incidence of laboratory-confirmed salmonellosis (all serotypes combined) for Trinidad and Tobago in 1997. (Assume that only one isolate was received for each patient. The population of Trinidad and Tobago was estimated to be 1,265,000 in July of 1997.)

*The incidence is a measure of **the frequency with which an event** (e.g., a new case of a disease or isolation of a pathogen) **occurs in a population over a period of time**. The numerator is the number of events occurring during a given time period. The denominator is the population at risk.*

incidence = number of events /population at risk

incidence (lab-confirmed salmonellosis) = 109 isolates per 1,265,000 people per year
= 0.0000862 isolates per person per year
= 8.6 isolates per 100,000 persons per year

NOTE TO INSTRUCTORS:

- *Incidence (instead of raw numbers) is used to compare the occurrence of disease in different populations because it is a rate and accounts for differences in population sizes.*
- *In a rate, a time period must be specified. In this analysis, the time period is 1997.*
- *The event should be clearly defined. For this analysis, the event is the isolation of Salmonella from blood or stool of a resident of Trinidad or Tobago. It excludes isolates obtained from visitors.*
- *The denominator should only include persons at risk of acquiring the illness. Although there is a vaccination for Salmonella Typhi, it does not impact the occurrence of other serotypes. Previous infections with Salmonella will not protect from subsequent infections. Therefore, for this analysis, it would be reasonable to use the entire Trinidad and Tobago population for the denominator.*
- *Some students have difficulty with decimal places when calculating incidence. They need to realize that if they divide the number of events by the population estimate, the resulting number equals the number of events per person in the population. Because it is difficult to think of incidence in these terms (i.e., the number will be very small), the student should calculate how many events would be expected among a larger group of people (typically 100,000) by multiplying by that larger number.*

Question 7B: The annual incidence of laboratory-confirmed *Salmonella* infections in Trinidad and Tobago is approximately 9 per 100,000 population. Assume that: 1) approximately one in every 10 people with diarrhea go to the doctor, 2) doctors request submission of a stool specimen from approximately one in every 10 patients with diarrhea that they see, and 3) approximately two in every three stool specimens are properly tested for *Salmonella* and are reported through the surveillance system.

Given these assumptions, what is the true burden of *Salmonella* in Trinidad and Tobago?

To answer this question, it is useful to look at the "Burden of Foodborne Disease Pyramid" and create multipliers for key sections on the pyramid. We can multiply the incidence of laboratory-confirmed salmonellosis by these multipliers to estimate the overall incidence of Salmonella per year in Trinidad and Tobago (i.e., the" true" estimate of burden).

Burden of Foodborne Diseases Pyramid

**Burden of Illness Pyramid courtesy of FoodNet (http://www.cdc.gov/foodnet, April 22, 2004)*

Based on the information provided above, you can create the following multipliers:

- *1 in 10 people with diarrhea go to the doctor (labeled "Person seeks care" in pyramid) = 1/10 or 0.10 →* ***the multiplier will be the inverse of 0.10 (or 10)***
- *Of those consulting a doctor, 1 in 10 are requested to submit a stool specimen (labeled "Specimen obtained" in pyramid) = 1/10 or 0.10 →* ***the multiplier will be the inverse of 0 .10 (or 10)***
- *Two out of every three stool specimens are properly tested for Salmonella and are reported through the surveillance system (labeled "Lab tests for organism" and "Reported to Health Department" in pyramid) = 2/3 or 0.667→* ***the multiplier will be the inverse of 0.67 (or 1.5)***

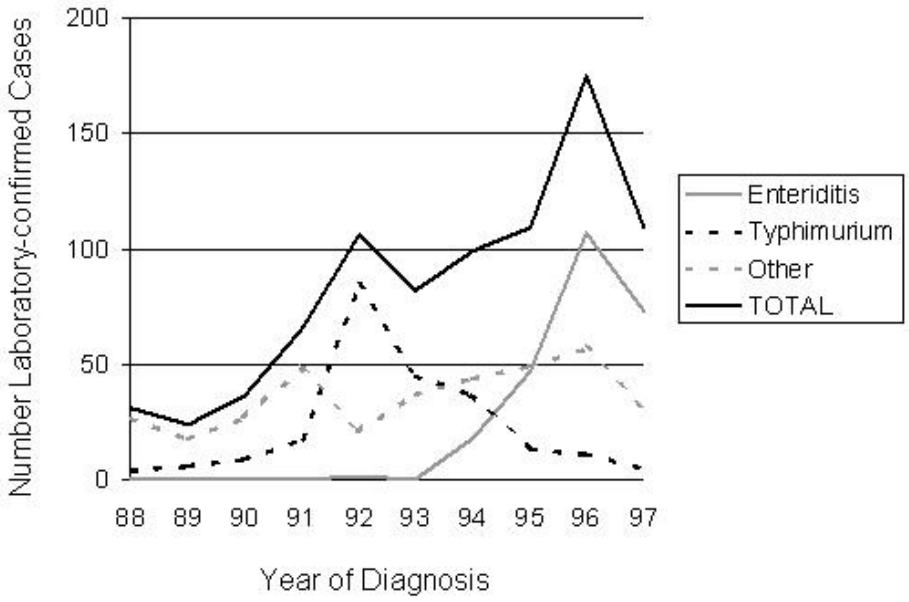
To estimate the true number of Salmonella cases in Trinidad and Tobago:
Step 1: *Multiply the multipliers together. This is 10 x 10 x 1.5 = 150. This is your* ***final multiplier****.*
Step 2: *Multiply the incidence of laboratory-confirmed cases by the final multiplier to obtain the estimate of the true incidence of Salmonella cases in Trinidad and Tobago. This is 9 laboratory-confirmed Salmonella cases per 100,000 population times 150 which equals an* ***estimated 1,350 cases of Salmonella per 100,000 population each year in Trinidad and Tobago*** *(or 17,078 Salmonella infections).*

Compare:
Laboratory-confirmed Salmonella isolates in 1997: ***109 isolates or 9/100,000 population***
Estimated Salmonella infections in 1997: ***17, 078 infections or 1,350/100,000 population***

Question 8: Create a line graph of the number of *Salmonella* isolates by serotype by year of diagnosis for Trinidad and Tobago from 1988 to 1997. Interpret the graph.

NOTE TO INSTRUCTORS: Divide class into groups of 2-4 students each. Have each group create the line graph for total isolates or one serotype. After 5-10 minutes, reassemble the class. Have one member from each group present their line graph to the rest of the class.

Salmonella isolates by serotype and year of diagnosis, Trinidad and Tobago, 1988-1997.



Instructors should spend time on the appropriate construction of the line graph as well as its interpretation. A graph should be able to "stand alone". Viewers should be able to quickly discern the information conveyed by the graph and not need additional explanations from another source.

In critiquing a graph, one should ask the following questions:

- *Does the graph have a title?*
- *Does the title describe the content, including subject, person, time, and place?*
- *Is each axis labeled clearly and concisely?*
- *Are the specific units of measurement included as part of the label?*
- *Are the scale divisions on the axes clearly indicated?*
- *Are the scales for each axis appropriate for the data?*
- *Does the y-axis start at zero?*
- *Are the plots drawn clearly?*
- *If more than one series of data or components are shown, are they clearly distinguishable on the graph?*
- *Is each series of data or components labeled on the graph, or in a legend or key?*
- *Are all codes, abbreviations, or symbols explained?*

Interpretation of graph:
Overall, the isolation of Salmonella increased dramatically from 1988 to 1997 in Trinidad and Tobago. (This increase may be due, in part, to implementation of new surveillance methods described in Part II).

The distribution of isolates by serotype also changed during this time period. In the early 1990s, S. Typhimurium was the most prevalent Salmonella serotype in Trinidad and Tobago. The isolation of S. Enteritidis, however, increased from 1 (<1%) of 106 Salmonella isolates in 1992 to 73 (67%) of 109 isolates in 1997. As a result, S. Enteritidis surpassed S. Typhimurium to become the most frequent Salmonella serotype causing diarrheal illness on the two islands. (The shift in serotype distribution cannot be attributed to the implementation of new surveillance strategies.)

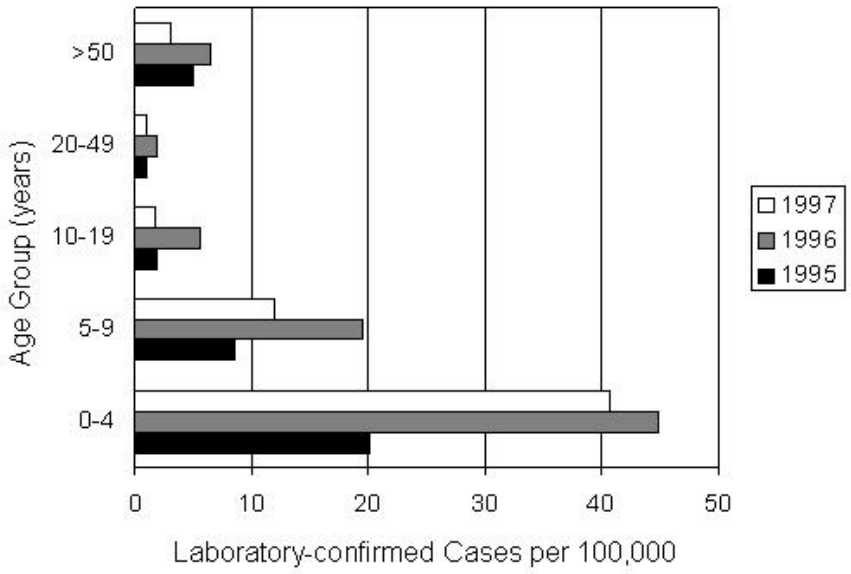
NOTE TO INSTRUCTORS: At this point, the class should pause and reflect. Would these trends have been detected through the original communicable disease surveillance system? How useful would the overall isolation rates of Salmonella have been as opposed to the serotypes?

Due to the increase in *S*. Enteritidis in Trinidad and Tobago, CAREC focused their analyses on this serotype. The following data are for *S*. Enteritidis only.

From 1995-97, 227 laboratory-confirmed cases of *S*. Enteritidis infection were reported in Trinidad and Tobago. Approximately, 76 cases were reported each year for an annual incidence of 6 per 100,000 population. In general, the geographic distribution of patients with *S*. Enteritidis infection reflected population distributions on the two islands. The largest numbers of cases were reported from the most populous counties of St. George and Victoria.

A similar proportion of *S*. Enteritidis infections occurred among males (48%) and females (52%). However, the distribution of cases varied by age group (Figure 4) and month of diagnosis (Figure 5).

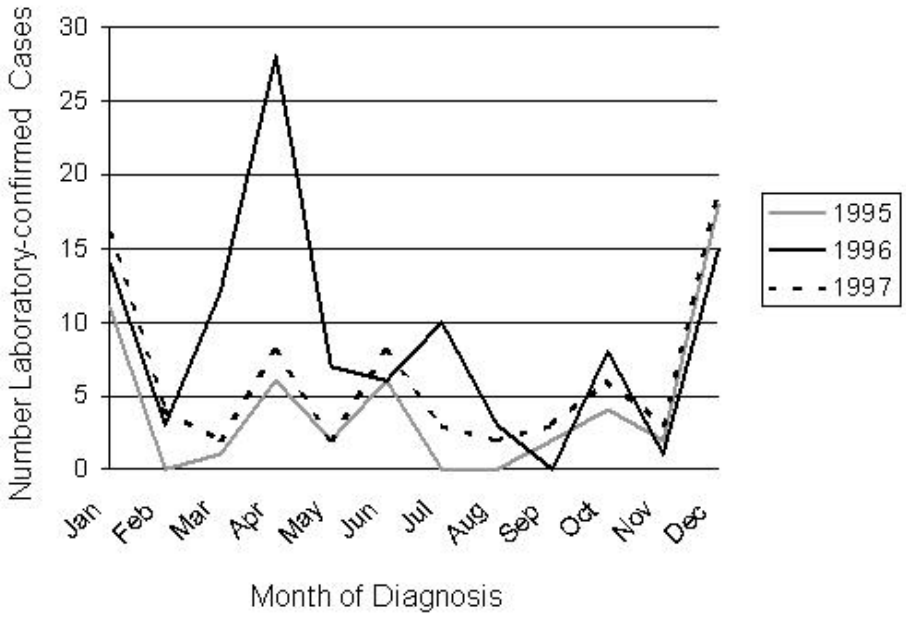
Figure 4. Laboratory-confirmed cases of *Salmonella* Enteritidis (per 100,000 population) by age group and year of diagnosis, Trinidad and Tobago, 1995-1997.



Question 9: Interpret the grouped bar chart of laboratory-confirmed *S.* Enteritidis cases by age group. What age group(s) is at highest risk for infection?

From 1995 to 1997, children 0-4 years of age had the highest rates of infection (with rates of 20-45 per 100,000), followed by children 5-9 years of age (with rates of 9-20 per 100,000).

Figure 5. Laboratory-confirmed cases of *Salmonella* Enteritidis by month and year of diagnosis, Trinidad and Tobago, 1995-1997.



Question 10: Describe the occurrence of *S.* Enteritidis infection in Trinidad and Tobago by month of diagnosis?

S. Enteritidis infections increased each year in December and January. The cases that occurred during these two months accounted for approximately 40% of the cases for each year. In addition, there was a large increase in cases of S. Enteritidis in March and April of 1996.

A characteristic distribution of cases (i.e., repeated pattern) that changes through the year, such as in this example, is called ***seasonality****. Seasonality may suggest hypotheses about the mode of transmission, behavioral factors that increase risk, or other contributors to the disease or condition. For example, it is possible that the increase in S. Enteritidis infections in Trinidad and Tobago in December and January is somehow related to the Christmas-New Year holiday season.*

NOTE TO INSTRUCTORS: Be ready to speculate on the increase in cases in March and April of 1996. How might students explore the nature of that increase? (e.g., geographic distribution, age and sex distribution)

Part IV – Case-Control Study of *S.* Enteritidis in Trinidad and Tobago

To explore risk factors for *S.* Enteritidis infection in Trinidad and Tobago, a matched case-control study was undertaken from March 1998 - May 1999. A case-control study design was used because the cases did not arise from a well-defined group of people and were distributed across the entire country.

Cases were patients with laboratory-confirmed *S.* Enteritidis infection who were reported through the communicable disease surveillance system. Cases were enrolled prospectively, shortly after diagnosis. Controls were persons with no diarrheal illness in the previous 4 weeks who lived in the same neighborhood as cases and were similar in age. Investigators attempted to enroll two controls for each case.

Using a standardized questionnaire, investigators collected information from cases about foods and beverages consumed, recent travel, and food handling practices in the 3 days before they became ill. Controls were asked about these exposures during the same 3-day period as the matched case. The questionnaire was administered to both cases and controls by one of the investigators in face-to-face interviews.

Forty-five patients and 92 controls were enrolled in the case-control study. The investigators analyzed the results of the case-control study.

Question 11: What is the measure of association in a case-control study? How is it interpreted?

*The **odds ratio** is the measure of association for a case-control study (matched or unmatched). It is the ratio of two odds: the odds of exposure to a factor among cases and the odds of exposure to the factor among controls. An odds ratio tells us how many times higher the odds of exposure is among cases compared to controls.*

Odds ratios are always between 0 and infinity. An odds ratio of:

- ***Less than 1.0** means that the **odds of exposure among cases is lower** than the odds of exposure among controls. The exposure may be protective against the health problem.*
- ***One (or close to 1.0)** means that the **odds of exposure among cases is the same** as the odds of exposure among controls. The exposure is not associated with the health problem.*
- ***Greater than 1.0** means that the **odds of exposure among cases is greater** than the odds of exposure among controls. The exposure may be a risk factor for the health problem*

*Tests of statistical significance (e.g., chi-square, Fisher exact test) must be used to determine the probability that an observed odds ratio could have occurred due to chance alone. This probability is called the **p-value**. A very small p-value means that you would be unlikely to observe a particular outcome due to chance alone, if there were no association between the exposure and the disease. If the p-value is less than some predetermined cut-off (usually 0.05 or a 5 in 100 chance), the association is then said to be statistically significant.*

In the Trinidad and Tobago case-control study, cases and controls were similar to each other in terms of age, sex, ethnic distribution, and place of residence. Exposure to potential sources of *Salmonella*, however, differed between cases and controls (Table 2).

Table 2. Potential sources of exposure to *Salmonella*, Trinidad and Tobago Case-Control Study, March 1998 – May 1999.

Exposure*	**Matched Odds Ratio**	**p-value**
Ate chicken	0.5	0.4
Ate shell eggs	8.8	<0.001
Ate dishes that contained raw or undercooked eggs	18.9	0.001
Ate ground beef	1.3	0.6
Ingested powdered milk	1.5	0.2
Exposed to live chickens	1.3	0.4
Bought refrigerated eggs	0.1	<0.001
Refrigerated eggs at home	0.03	<0.001

*in the 3 days before onset of illness in the associated case

Question 12: Interpret the odds ratios for the above exposures. What exposures appear to be risk factors for *S*. Enteritidis infection in Trinidad and Tobago?

The following interpretations can be gleaned from Table 2:

- *The odds of eating shell eggs was almost 9 times higher among cases than controls. (The probability that this finding was due to chance alone was less than one-in-a-thousand.)*
- *The odds of eating dishes that contained raw or undercooked eggs was almost 20 times higher among case than controls. (The probability that this finding was due to chance alone was one-in-a-thousand.)*
- *The odds of purchasing refrigerated eggs was one-tenth as common among cases as controls. (The probability that this finding was due to chance alone was less than one-in-a-thousand.)*
- *The odds of refrigerating eggs after purchase was less than one-tenth as common among cases as controls. (The probability that this finding was due to chance was less than one-in-a-thousand.)*
- *The odds of eating chicken, beef, or powdered milk, or having been exposed to live chickens was similar among cases and controls.*

The findings of the case-control study suggest that consumption of shell eggs, particularly raw or undercooked eggs or foods containing them, was a ***significant risk factor*** *for sporadic S. Enteritidis infection in Trinidad and Tobago. Purchase of refrigerated eggs or storage of eggs in the refrigerator at home was a* ***protective factor****.*

The specific raw egg-containing foods that were implicated by the case patients' food histories included homemade eggnog, cake batter, homemade ice cream, punch a crème (i.e., a drink similar to eggnog), and stout and eggs. The implicated food items correlated with the predominance of cases in December and January as many of these foods are consumed more frequently in the holiday season.

Samples of the implicated foods were collected from patients, from the places where patients had originally purchased the foods, or both and were cultured for *Salmonella*. *S.* Enteritidis isolates from patients and food were phage-typed at the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia.

S. Enteritidis samples from 34 patients were selected for phage typing. Of these, 30 (88%) were found to be phage type 4 and 4 (12%) were found to be phage type 1. *S*. Enteritidis was isolated from 15 (45%) of the 33 food samples implicated by the patients' food histories. Nine of the 15 food isolates were phage typed; all nine were phage type 4.

Question 13: Discuss possible interpretations of the same phage type among *Salmonella* isolated from patients with salmonellosis and suspect food samples.

Bacteriophages *(i.e., phages) are groups of viruses that infect bacteria. Each bacterial strain will exhibit resistance to some phages and be susceptible to others. The profile of resistance and susceptibility to a standardized battery of phages is called the* ***phage type****.*

Phage typing can be used to distinguish between bacteria within a particular serotype. Identification of a common phage type among patients infected with the same serotype or between patients and a potential vehicle of infection (e.g., food item) can help establish epidemiological linkages.

In this investigation, the identification of phage type 4 among most of the patients and all of the food items suggests that the implicated foods were likely to be the source of the patients' infections. We cannot, however, rule out that the implicated food item may have actually been contaminated by the patient himself/herself or that the implicated food item was cross-contaminated by another food item that was the source of infection for the patient.

Of note: phage type 4 is more virulent than other S. Enteritidis phage types and is remarkable for its ability, once introduced into poultry, to cause marked increases in human illness. Phage type 4 has been dominant in Europe since the 1980s and emerged in the United States in the mid-90s. The high prevalence of phage type 4 in Trinidad and Tobago suggests that S. Enteritidis might have been introduced through imported breeder flocks, chicks for layer flocks, or hatching eggs.

Question 14: What control measures would you consider at this point?

At this point, control measures will be directed primarily at consumers, food service establishments, and foodhandlers. Control measures include recommendations to:

- *Buy refrigerated eggs.*
- *Keep eggs refrigerated after purchase and until the time of use.*
- *Discard cracked or dirty eggs.*
- *Wash hands and cooking utensils with soap and water after contact with raw eggs.*
- *Eat eggs promptly after cooking. Do not keep eggs warm for more than 2 hours.*
- *Refrigerate unused or leftover egg-containing foods.*
- *Avoid eating raw eggs (as in homemade ice cream, eggnog, or stout).*
- *Avoid restaurant dishes made with raw or undercooked, unpasteurized eggs. Restaurants should use pasteurized eggs in any recipe (such as Hollandaise sauce or Caesar salad dressing) that calls for use of raw eggs.*

Part V – Study of Eggs in Trinidad

To further investigate the role that eggs may play as a source of *Salmonella* infections in Trinidad, a microbiologic survey of shell eggs was undertaken. Ten egg-producing farms across the country were selected, nine of which were the largest and most popular commercial table egg farms in Trinidad. Their total production accounted for approximately 75% of the country's egg supply.

Twenty-five freshly laid eggs were collected from each farm on three different occasions for a total of 750 eggs. Each set of 25 eggs was cultured for *Salmonella* in pooled batches.

Eggshells were cultured separately from egg contents. The eggshell samples were prepared by swabbing the shell surface of each of the 25 eggs with cotton wool tips moistened with lactose pre-enrichment broth. The eggs were not washed prior to swabbing. The eggs were then sanitized using U.S. Food and Drug Administration eggshell disinfection procedures: each egg was cleaned with a stiff wire brush, hand washed under running water with antibacterial soap, and patted dry with a paper towel. The eggs were then placed in a wire basket and immersed in 70% alcohol for 30 minutes followed by distilled water for 10 minutes. The eggs were then allowed to air dry. The contents were removed aseptically, pooled together, and homogenized in a blender.

Question 15: Why were the eggshells cultured separately from the egg contents? Why were the eggs sanitized before the contents were cultured?

Shell eggs can become contaminated with Salmonella in two ways:

1) ***external fecal contamination of shells*** *– Salmonella (from the intestinal tract of the laying hen or in the environment from another source) contaminates the shell of the egg after it has been laid. This external contamination can penetrate into the egg through cracks in the shell. Stringent procedures for disinfecting the exterior of the eggshell and rejecting cracked eggs have decreased this route of transmission of Salmonella.*
2) ***transovarian transmission*** *– An ovarian infection in the laying hen contaminates the contents of the egg during its formation (i.e., before the eggshell is formed), resulting in an egg that is intact, unbroken, and normal looking but colonized with Salmonella. Disinfection of the eggshell surface and rejection of cracked eggs* ***do not prevent*** *this route of Salmonella contamination. Only prevention of infection in laying hens can prevent transovarian transmission.*

For this study of shell eggs from egg-producing farms in Trinidad, eggshells were tested separately from the egg contents to determine the relative contribution of these two sources of contamination. Because egg contents can become contaminated from the eggshell when the egg is cracked, the eggshells were disinfected before removing the contents.

Salmonella was detected more often in shell cultures (4.6% of samples) than in content cultures (1.2% of samples). *S*. Typhimurium was the most prevalent serotype found on the eggshells and *S*. Enteritidis was the most prevalent serotype isolated from the egg contents (Table 3).

Table 3. *Salmonella* serotypes isolated from the 750 pooled eggshells and egg contents from 10 egg-producing farms, Trinidad, 1998-1999.

	Percent positive for serotype*	
***Salmonella* serotype isolated**	**Pooled eggshells**	**Pooled egg contents**
S. Typhimurium	3.06	0.4
S. Enteritidis	0.67	0.8
S. Ohio	0.27	-
S. Cerro	0.27	-
S. Infantis	0.27	-
S. Heidelberg	0.13	-
Total	4.6	1.2

*Because *Salmonella* isolates are generally present in very low numbers in eggs, it was assumed that each isolate came from one positive egg and the percentage was based on a denominator of 750.

The isolation rates of *Salmonella* on shell surfaces and in egg contents varied among the 10 egg-producing farms. At least one serotype of *Salmonella* was isolated from eggshells at all 10 of the farms. *Salmonella* was isolated from egg contents at only three of the farms.

An environmental health assessment was undertaken at each of the farms by a food safety officer from the Trinidad and Tobago Ministry of Agriculture to identify factors that could have contributed to the contamination of eggshells and contents with *Salmonella*.

Question 16: What specific activities would you undertake as part of an environmental health assessment of the egg-producing farms?

An environmental health assessment should focus on critical points where:

- ***laying chickens could become infected with <u>Salmonella</u>*** *(e.g., brood chickens that produce laying hens, rodent infestations, nesting boxes, poultry houses, feed, water, litter)*
- ***egg shells could become contaminated with <u>Salmonella</u>*** *between the time they are laid until they are shipped to market (e.g., rodent infestations, nesting boxes, handling by humans, conveyor belts, containers in which eggs are stored)*
- ***growth of <u>Salmonella</u> already present on or in eggs could occur*** *(e.g., how quickly eggs are collected after laying, whether eggshells are cleaned/disinfected before storage, what temperatures eggs are held at and for how long)*

The food safety officer should examine the general sanitation of the poultry houses and farms including presence of rodents and source of water, feed, and litter for the chickens. The officer should talk with farm managers and employees about standard operating procedures, observe egg-handling activities, and draw a flow diagram for egg production. The officer should measure temperatures to which the eggs are exposed (and how long they are likely to be held at those temperatures) and collect environmental specimens. The food safety officer should clarify the system of chicken rearing including the nursery the laying hens came from and where that source got its fertile eggs. The food safety officer should then search for antecedents for the conditions that could lead to infection of laying chickens, egg contamination, and growth of <u>Salmonella</u> on eggs.

The food safety officer inspected the farms and collected information about the system of chicken rearing, quality control measures, feed and litter type, egg cleanliness, and other management practices.

At four of the farms, the environment and immediate surroundings were generally clean with dry litter surfaces and clean drinking water, poultry houses, nesting boxes, and equipment. Proper egg-handling techniques and good farm practices were also employed. The eggs collected from these farms appeared clean with little or no fecal matter on their surfaces.

In contrast, the surroundings of the other six egg-producing farms generally appeared unsanitary: litter surfaces were wet on most occasions. Egg belts, poultry houses, and nesting boxes were dirty and there were rodents and flies. These farms were also characterized by odor build-up, such as ammonia, and the eggs collected from them frequently had feces and sometimes blood on the shells. In general, these farms had higher *Salmonella* isolation rates from pooled eggshells and egg contents than the other farms.

None of the 10 farms had routine microbial monitoring of their flocks or eggs.

Question 17: What food safety practices at the egg-producing farms might help prevent or reduce the risk of salmonellosis from the consumption of eggs from these farms?

- *monitor breeder flocks that produce egg-laying chickens and destroy infected flocks*
- *monitor egg-laying flocks for infection and remove infected flocks from the egg supply*
- *when infected breeder flocks or egg-laying flocks are identified, undertake traceback and trace forward investigations to find out where the chickens were obtained and which other farms may have used the same source (and, therefore, also are likely to have the problem)*
- *obtain new laying flocks only from breeder flocks that are known to be free of S. Enteritidis*
- *use Salmonella free feed for egg-laying and breeder flocks*
- *increase sanitation measures at egg-producing farms including drinking water, poultry houses, nesting boxes, and equipment*
- *control rodents on egg-producing farms*
- *refrigerate eggs from the producer to the consumer*
- *use a Hazard Analysis Critical Control Point (HACCP) system on egg-producing farms to identify potential problematic areas in the production of eggs*

Part VI - Prevention and Control

Following release of the results from the *S.* Enteritidis case-control study, the microbiologic survey of shell eggs, and environmental health assessments of egg-producing farms, the Trinidad and Tobago Ministries of Health and of Agriculture initiated a farm-to-table approach to *Salmonella* prevention and control strategies. These strategies combined public health education of consumers, food service establishments, and food workers (on the risks associated with eating raw and undercooked eggs and using unrefrigerated eggs) and strategies for reduction of *Salmonella* infections among egg-laying flocks and breeder flocks.

Regional workshops were held in November 2002 for egg producers on production and food safety. "Good Agricultural Practices" for hatchery sanitation and egg production were developed from the proceedings. Drafts were widely distributed for review and comment. Final copies were distributed to all egg-producing farms under the coordination of the Inter-American Institute for Cooperation on Agriculture. The Ministry of Agriculture, responsible for the regulation of food safety in Trinidad and Tobago, made staff available on an ongoing basis to answer questions from producers on the "Good Agricultural Practices" and help them to explore and solve problems.

Through public and private partnerships and networking, Ministry of Agriculture officials developed a protocol to identify and remove infected flocks from the egg supply and increase quality assurance and sanitation measures at egg-producing farms. The procedures included the following steps:

- Both eggs and chickens from commercial egg-producing farms will be tested for *Salmonella* on a quarterly basis.
- Any flocks that test positive for *Salmonella* on routine exam will be re-tested.
- If a second sample is positive, traceback investigations will be undertaken to identify breeder flocks.
- Infected breeder flocks (those that produced the egg-laying chickens) will be slaughtered.
- Eggs from infected egg-laying chickens will be pasteurized instead of being sold as shell eggs.
- Non-infected flocks from farms at which infected flocks have been detected will be tested more frequently (i.e., every 4 weeks).

The Ministry of Agriculture implemented the above procedures in Trinidad and Tobago in 2003.

Question 18: In addition to the testing of eggs and flocks for *Salmonella*, how might you monitor the impact of *Salmonella* control measures in Trinidad and Tobago?

In addition to testing eggs and flocks for Salmonella, public health officials should:

- *monitor the incidence of human salmonellosis by serotype, characterizing cases by time, place, and person*
- *investigate clusters of cases to identify risk factors/sources of infection*
- *undertake periodic environmental health assessments of egg-producing farms*

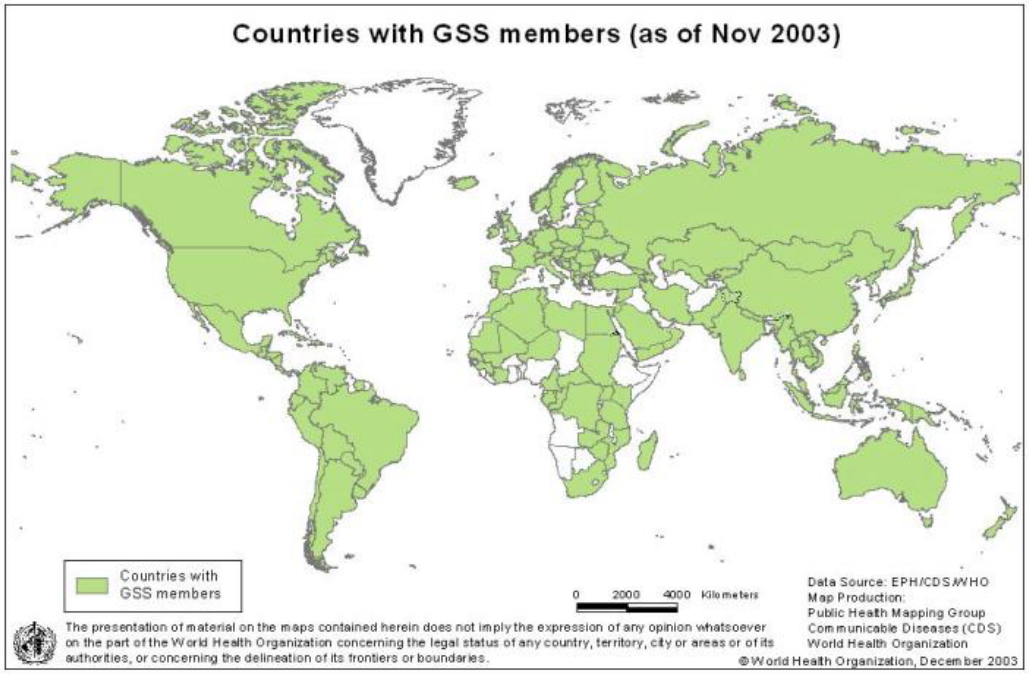
Epilogue

Serotyping of *Salmonella*, as used in the investigation of *Salmonella* Enteritidis in the Caribbean, is a common subtyping procedure used throughout the world. In a 1997 survey of World Health Organization (WHO) member states, 69 of the responding 104 countries conducted *Salmonella* serotyping as part of public health surveillance for salmonellosis. Serotyping was performed in all six WHO regions; however, surveillance was limited in time or scope for some countries. Access to serotyping reagents varied by country and some countries reported only serogroup results.

WHO Global Salm-Surv, an international, foodborne disease surveillance network, was created by WHO in partnership with the Danish Institute for Food and Veterinary Research, the Centers for Disease Control and Prevention, Institut Pasteur International Network, Health Canada, and the Animal Sciences Group (ID-Lelystad) in the Netherlands. The goal of WHO Global Salm-Surv is to reduce foodborne diseases through enhancement of laboratory-based surveillance (including serotyping and antimicrobial resistance testing) and outbreak detection and response techniques. Components of the network that help promote this goal include international training courses, an external quality assurance system, and country and region-specific projects. The network also offers a moderated list serv, web-based annual *Salmonella* summary data from member institutions, and a website, and provides services such as reference testing and identification of reliable sources of antisera for countries.

As of November 2003, WHO Global Salm-Surv had members from 138 countries including the Bahamas, Barbados, Belize, Dominican Republic, Jamaica, Saint Lucia, Suriname, and Trinidad and Tobago in the Caribbean. Participation in WHO Global Salm-Surv has provided critical information to investigate outbreaks such as the one described in this case study and has led to local interventions that have reduced the human health burden of *Salmonella* and other foodborne diseases globally.

Figure 6. WHO Global Salm-Surv Country Membership



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Indar-Harrinauth L, Daniels N, Prabhakar P, Brown C, Baccus-Taylor G, Comissiong E, Hospedales J. Emergence of *Salmonella enteritidis* phage type 4 in the Caribbean: Case-control study in Trinidad and Tobago, West Indies. Clinical Infectious Diseases 2001;32:890-6. (See Appendix for abstract.)

Indar L, Baccus-Taylor G, Commissiong E, Prabhakar P, Reid H. Salmonellosis in Trinidad: evidence for transovarian transmission of *Salmonella* in farm eggs. West Indian Med J 1998;47:50-3. (See Appendix for abstract.)

Orrett FA and Shurland SM. Susceptibility patterns and serotypes of non-typhoidal *Salmonella* in Trinidad. Saudi Med J 2001;22:852-5.

Appendix: Abstracts from Original Investigations

Indar-Harrinauth L, Daniels N, Prabhakar P, Brown C, Baccus-Taylor G, Comissiong E, Hospedales J. Emergence of *Salmonella enteritidis* phage type 4 in the Caribbean: Case-control study in Trinidad and Tobago, West Indies. Clin Infect Dis 2001;32(6):890-6.

A prospective case-control study involving 46 case patients and 92 age- and neighborhood-matched control subjects was conducted in Trinidad and Tobago (T&T) between March 1998 and May 1999 to determine the etiology, sources, and risk factors for *Salmonella enteritidis* (SE) infection. SE infection in T&T was found to be associated with the consumption of shell eggs, and in particular raw or undercooked eggs. SE isolates from 30 (88%) of 34 patients and from 9 implicated egg or egg-containing food samples were phage type 4. Homemade eggnog and ice cream, cake batter, and egg-containing beverages were the main raw egg-containing foods, reflecting the cultural practices of the people of T&T. Public health education on the risks of eating raw or undercooked eggs, thorough cooking of all egg dishes, and refrigeration of shell eggs and egg dishes; studies tracing infected eggs to their sources; and testing of flocks of layer chickens for SE are needed to reduce the incidence of this infection.

Indar L, Baccus-Taylor G, Commissiong E, Prabhakar P, Reid H. Salmonellosis in Trinidad: Evidence for transovarian transmission of *Salmonella* in farm eggs. West Indian Med J 1998;47(2):50-3.

The aim of this study was to determine whether the contents of farm eggs in Trinidad are contaminated with *Salmonella* and if transovarian transmission occurs. 750 fresh eggs from 10 farms supplying 75% of the country's eggs were cultured for *Salmonella*. *Salmonella* was found on the egg shells' surfaces from all farms, and in the egg contents from three farms. Isolates were obtained from the cultures of the contents and shells of nine (1.2%) and 35 (4.66%) eggs, respectively ($p < 0.005$). Serotypes found in the contents were *S. enteritidis* (0.8%; deduced to be contaminated by transovarian transmission) and *S. typhimurium* (0.4%); those isolated from the shells (contaminated by faecal transmission) were *S. typhimurium* (3.06%), *S. enteritidis* (0.67%), *S. ohio* (0.27%), *S. cerro* (0.27%), *S. infantis* (0.27%) and *S. heidelberg* (0.13%). This study provides the first evidence for *Salmonella* and, more importantly, *S. enteritidis*, in eggs in Trinidad. This is of major public health significance because *S. enteritidis* infected eggs appear normal and the organism is difficult to detect and control. The consumption of these eggs may increase the risk of *Salmonella* infection. Food safety practices, particularly the thorough cooking (> or = 70 degrees C) of all egg dishes and the refrigeration (< 10 degrees C) of shell eggs and egg dishes, are recommended.