Botulism in Argentina

INSTRUCTOR’S VERSION

Original investigators: Rodrigo G. Villar, MD,1 Roger L. Shapiro, MD,1 Silvina Busto, MD, MPH,2 Clara Rive-Posse, MD, MPH,3 Guadalupe Verdejo MD, MPH,4 Maria Isabel Farace, DVM,5 Francisco Rosetti, MS,5 Jorge A. San Juan, MD,6 Carlos Maria Julia, MD, MPH,3 John Becher, RPh,1 Susan E. Maslanka, PhD,1 David Swerdlow, MD1

1Centers for Disease Control and Prevention, 2Ministero de Salud-Region V, 3Ministero de Salud y Accion Social, 4Pan American Health Organization, 5Departamento de Bacteriologia, Instituto Nacional de Enfermedades Infecciosas, 6Hospital F. J. Muñiz, Buenos Aires, Argentina

Case study and instructor’s guide created by: Jeanette K. Stehr-Green, MD

NOTE: This case study is based on a real-life outbreak investigation undertaken in Buenos Aires, Argentina, in 1998. Some aspects of the original outbreak and investigation have been altered, however, to assist in meeting the desired teaching objectives and allow completion of the case study in less than 3 hours.

Students should be aware that this case study describes and promotes one particular approach to foodborne disease outbreak investigation. Procedures and policies in outbreak investigations, however, can vary from country to country, state to state, and outbreak to outbreak.

It is anticipated that the epidemiologist investigating a foodborne disease outbreak will work within the framework of an “investigation team” which includes persons with expertise in epidemiology, microbiology, sanitation, food science, and environmental health. It is through the collaborative efforts of this team, with each member playing a critical role, that outbreak investigations are successfully completed.

Please send us your comments on this case study by visiting our website at http://www.phppo.cdc.gov/phtn/casestudies. Please include the name of the case study with your comments.

April 2002

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control and Prevention
Atlanta, Georgia 30333
**Target audience:** epidemiologists and other persons with knowledge of basic epidemiologic concepts and experience in data collection and analysis who are interested in learning specific skills for investigating infectious disease outbreaks

**Trainee prerequisites:** working knowledge of descriptive epidemiology, epidemic curves, measures of association, stratified analysis, study design, outbreak investigation. The student will also benefit from having some familiarity with food microbiology and environmental investigation techniques but will be likely to rely heavily on others with greater expertise in these areas in a real-life outbreak situation.

**Teaching materials required:** graph paper, calculator

**Time required:** approximately 2 hours and 30 minutes

**Language:** English

**Level of case study:** Basic ____ Intermediate **X** Advanced ____

**Materials borrowed from:**
“Foodborne Illness Investigation and Control Reference Manual”, Massachusetts Department of Public Health, Division of Epidemiology and Immunization, Division of Food and Drugs, and Division of Diagnostic Laboratories (1997)

“Guidelines for the Investigation and Control of Foodborne Disease Outbreaks”, World Health Organisation, Food Safety Unit Division of Food and Nutrition and Division of Emerging and Other Communicable Diseases Surveillance and Control (DRAFT, 1999)

**Reviewed by:**
Richard Dicker, MD, MPH, Centers for Disease Control and Prevention
Steve Luby, MD, Centers for Disease Control and Prevention
Rob Tauxe, MD, MPH, Centers for Disease Control and Prevention
Chris Zahniser, RN, MPH, Centers for Disease Control and Prevention
David Swerdlow, MD, Centers for Disease Control and Prevention
Sharon McDonnell, MD, MPH, Centers for Disease Control and Prevention
Thomas Grein, MD, MPH, World Health Organization
Allison Hackbarth, MPH, Massachusetts Department of Public Health
Denise Werker, MD, MHSc, FRCPC, Laboratory Centre for Diseases Control, Health Canada
John Sarisky, RS, MPH, Centers for Disease Control and Prevention

**Cover art by:** Barbara Orisich, MS

**Training materials funded by:** the Centers for Disease Control and Prevention (National Center for Infectious Diseases, Food Safety Initiative, Public Health Practice Program Office, and Epidemiology Program Office/Division of International Health)
INSTRUCTOR’S VERSION
Botulism in Argentina

Learning objectives:

After completing this case study, the student should be able to:
1. describe outbreak situations in which acute control measures should be undertaken
2. communicate information on an outbreak or outbreak investigation and write a press release
3. given the leading hypothesis(es) in an outbreak, develop a questionnaire
4. given details on the origin, distribution, and preparation of an implicated food item, identify critical points for the control of contamination and microbial survival and growth
5. discuss possible barriers to implementation of specific interventions following an outbreak investigation
6. describe measures that can be used to monitor the success of an intervention
7. describe the occurrence, signs and symptoms, and control of foodborne botulism

PART I - OUTBREAK DETECTION

Foodborne botulism is a severe illness that results from the ingestion of a preformed toxin produced by a bacterium, Clostridium botulinum, in contaminated food. Death can occur in up to 60% of untreated cases; supportive care and prompt administration of antitoxin have reduced mortality in the United States to less than 10%. Outbreaks of botulism have been linked to improperly preserved vegetables, fruits, and meats including fermented fish products, sausages, smoked meat, and seafood.

On January 13, 1998, an infectious diseases physician at a Buenos Aires hospital telephoned the Directorate of Epidemiology of the Argentine Ministry of Health (MOH) to report two possible cases of botulism. The patients, both men, presented with drooping eyelids, double vision, difficulty swallowing, and respiratory problems. One patient had onset of symptoms on January 5 and the other on January 6. The physician had drawn sera and collected stool specimens from the men to test for botulinum toxin but no results were available.

Question 1: As a public health practitioner in Argentina, what are the major concerns raised by these two possible cases of botulism in Buenos Aires?

Because cases of foodborne botulism can be very severe and result from ingestion of contaminated food that may still be available to cause illness in others, a single case of foodborne botulism represents a public health emergency that might herald a larger outbreak.
Public health actions resulting from a suspected case of botulism include an immediate search for other possible cases and identification of suspected food exposures, as well as confirming the diagnosis. Diagnostic testing of both case specimens and foods should be performed as needed.

(NOTE: In this outbreak, another public health issue became apparent. As will be seen, the affected individuals were bus drivers. They continued to drive their routes although they had double vision and drooping eyelids. These vision difficulties could have resulted in unsafe driving practices and, ultimately, motor vehicle accidents.)

The clinical syndrome of botulism is dominated by neurologic signs and symptoms. Dryness of the mouth, drooping eyelids, and blurred and double vision are usually the earliest neurologic complaints. These initial symptoms may be followed by disturbances in speech, difficulties swallowing, and peripheral muscle weakness. If respiratory muscles are involved, ventilatory failure and death may result unless supportive care is provided. The average incubation period for botulism is 18-36 hours, but symptoms can occur as early as six hours or as late as 10 days after exposure.

Because botulism is rare, many physicians are unfamiliar with its presentation. As a result, patients with botulism can be misdiagnosed as other illnesses (e.g., stroke, myasthenia gravis, Guillain-Barré syndrome) delaying the administration of life-saving botulinum antitoxin for days and increasing the mortality rate among cases.

**Question 2:** How might you go about swiftly determining if there are other cases of botulism associated with the cases in Buenos Aires?

**Additional cases of botulism may be identified through the following means:** (list is in order of likely productivity)

- talk with patients to identify common meals/foods and other persons who may have shared them
- talk with family members and friends of cases who may have shared meals with the cases and ask if any have signs/symptoms of botulism
- talk with co-workers who may have shared meals with the cases and ask if any have signs/symptoms of botulism
- contact local hospitals and emergency rooms, describe signs/symptoms suggestive of botulism, and ask that all similar cases of acute neurologic illness be reported to the MOH
- review admission and emergency room logs at local hospitals for patients with admitting diagnoses suggestive of an acute neurologic disease (e.g., stroke, myasthenia gravis, Guillain-Barré syndrome) and follow-up suspicious patients to determine if botulism is a possibility
• contact local physicians (especially neurologists), describe signs/symptoms suggestive of botulism, and ask that all similar cases of acute neurologic illness be reported to the MOH
• contact area laboratories that do testing for botulism and ask to be notified of all requests for botulism testing (e.g., demonstration of toxin in serum, stool, gastric aspirate, and food or culture of C. botulinum from gastric aspirate or stool) (NOTE: Few laboratories do testing for botulism; therefore, this may be an unlikely source for identifying cases.)
• notify the public of the outbreak through various forms of media (e.g., newspapers, radio, television)

The Directors of the National Laboratory and the Environmental Health and Sanitation Program were notified of the possible cases of botulism. The two patients, still in the hospital, were interviewed by an MOH epidemiologist.

Upon questioning, it was learned that both patients were drivers for the same bus company and drove the same route and shift. The patients knew each other but worked on different days of the week. They had not eaten together in more than a month.

To find additional cases, the MOH contacted all employees of the bus company with the ill drivers to see if any had symptoms suggestive of botulism. Hospitals in the area of Buenos Aires, where the two cases occurred, were asked to report any patients with acute neurologic illnesses that could be botulism. Family members of cases were questioned about whether they also had symptoms of botulism. Additionally, the MOH developed a press release for distribution to the local news media.

**Question 3A:** What key points would you include in the press release?

_In writing a press release, it is important to consider the purpose of the release and who will be reading it. As a start, one might want to identify the 2-3 messages to be covered. One would then want to outline the “what”, “who”, “when”, “where”, and “why” of the story/issue._

_In this situation, the purpose of the press release is to identify additional cases of botulism which might be connected to the two cases reported by the infectious diseases physician. One might want to include the following items in the press release:_

• the fact that two cases of a potentially fatal disease were diagnosed in the local community (and the dates of illness onset and location within the city)
• the need to identify additional cases so that the individuals can be adequately treated
• the need to find additional cases so an investigation can be undertaken, the source of the outbreak can be determined, and the implicated product can be recalled and destroyed
• signs and symptoms of botulism and the most likely sources of intoxication_
• a contact name (and telephone number) who can answer questions
• where to go if a person thinks s/he is a case

NOTE: A “Question and Answer Sheet” (“Q & A”) is often prepared in concert with a press release to help handle the influx of calls and questions.

Question 3B: Who should be involved in developing the press release or notified before its distribution?

In developing a press release, it is important to consider both in-house procedures and the politics of the broader public health and medical community. The MOH should not unilaterally distribute a press release but should consult with a variety of persons and agencies to make them aware of the situation, take advantage of their expertise and resources, and gain their support in addressing the outbreak, if necessary. Persons/agencies to notify include:
• the Minister of Health (and other appropriate supervisors within the Ministry)
• communications/public relations staff at the MOH
• staff at the public health laboratory who may be involved in testing of clinical or food specimens
• the local health department where the terminal stop of the bus route is located
• management from the bus company
• professional groups (e.g., educational bodies, university staff)
• national and international public health authorities and reference laboratories (e.g., the World Health Organisation [WHO], the Centers for Disease Control and Prevention [CDC])
• food regulatory agencies (e.g., U. S. Food and Drug Administration [FDA])

On January 14, the MOH distributed the following press release:*

On Monday, January 13, two bus drivers, Pablo Esteban and Juan Rojas, from south central Buenos Aires were diagnosed with botulism by an infectious diseases physician at Hospital F. J. Muñiz. The men had been ill for several days before the diagnosis was made. The attending physician, Dr. Jorge San Juan reported that botulinum antitoxin was requested from the U. S. Centers for Disease Control and Prevention last night.

Because botulism is potentially fatal if untreated, the Argentine Ministry of Health is working with local public health officials and health care providers to

*This is not the press release distributed by the Ministry of Health but was developed specifically for this exercise.
identify the source of the botulism. Officials worry that other persons may have been exposed and need treatment or that the source may still exist and cause more people to become ill.

Botulism is a rare but serious paralytic illness caused by a nerve toxin produced by a bacterium, *Clostridium botulinum*. The symptoms of botulism can easily be confused with other illnesses and include diplopia, ptosis, dysphagia, dysarthria, and muscle weakness. If untreated, these symptoms may progress to cause paralysis of the arms, legs, trunk and respiratory muscles, and ultimately death. If diagnosed early, botulism can be treated with an antitoxin which blocks the action of the toxin circulating in the blood.

Previous outbreaks of botulism in Argentina have been caused primarily through eating improperly preserved vegetables and meats. Implicated foods include ham, red and green peppers, vigcacha (an Andean rodent), eggplant, cucumbers, palm hearts, tomatoes, peaches, spinach, and a type of cheese with onions. It is not yet known what specific food may have caused the botulism in this outbreak but both men are bus drivers for the same company, Arriba Bus, Inc., and drive the same route and shift.

Persons who feel they or their relatives might have symptoms of botulism are asked to contact the Directorate of Epidemiology of the Argentine Ministry of Health at xxxx-xxxx.

**Question 4:** Critique the press release. How might the press release impact the outbreak investigation?

*A press release should be brief (1-2 double spaced pages) and simply written; it should be written at a level understandable to the general public (i.e., 5-8th grade reading level), not include jargon or technical terminology, or assume that the reader has any prior knowledge of the subject that is being discussed. A press release does not need to read like a finely crafted article, but it should be written to get the reporter’s interest and provide the facts necessary for a subsequent article to be developed. To grab the reporter’s attention, one should probably put the most important information up front in the release; details (or the explanation of the most important points) should follow.*

**Critique of above press release:**

- The press release is relatively long. Deletion of a number of sentences would make it more readable without interfering with its goals.
• The use of patient names in the first paragraph compromises their confidentiality. Agency confidentiality policies should be considered before releasing patient names and other identifiers.

• The statements about getting botulinum antitoxin from CDC and the description of MOH activities are good. It reassures the community that something is being done.

• The use of medical terminology (e.g., diplopia, ptosis, dysphagia) and jargon will prevent many readers from understanding the common symptoms of botulism.

• The press release, if published, could introduce several biases in future studies:
  Selection bias:
  - Bus drivers from Arriba Bus, Inc. (particularly, those from the morning shift) might be more likely to seek medical attention and be diagnosed with botulism than other cases.
  - Physicians might be more likely to test for botulism among bus drivers from Arriba Bus, Inc. than persons in other occupations who have similar symptoms.
  Information bias:
  - Persons diagnosed with botulism might be more likely to recall eating the food items mentioned as sources of botulism in the news release.

• Directing all calls to the MOH could be a mistake. The signs and symptoms of botulism are non-specific and could be consistent with a number of different neurologic maladies in the community. Such a request could result in a deluge of calls to the MOH which may not be in a good position to examine the individuals or arrange for treatment. It might be better to have individuals consult their physicians who can make the diagnosis, initiate appropriate care, and report to the MOH. It would be appropriate to direct calls from the media (i.e., reporters) to the MOH if clarification of the press release is needed.

Seven additional patients with neurologic signs consistent with botulism were identified. Five of the patients had sought medical attention and four were hospitalized. Working diagnoses for these patients at the time the initial two cases were discovered included myasthenia gravis (1), Guillain-Barré syndrome (2), stroke (1), and diabetic complications (1).

Botulinum toxin was identified in sera and/or stool from three patients, including one of the original cases reported on January 13.

All patients were drivers from the same bus company as the original cases and drove the same route. From initial reports, all had eaten at a home located at the terminal stop of the bus route where the drivers stopped during their breaks. Approximately 58 bus drivers worked this route; 27 in the morning shift, 16 in the afternoon shift, and 15 in the evening shift.
Question 5: Would you initiate any control measures at this time? What criteria would you consider in implementing control measures so early in an investigation?

Epidemiologists often want to delay implementation of specific control measures until more definitive information on the source of the outbreak and mode of transmission are available (e.g., results from a case-control or cohort study). Although usually a wise approach, obvious control measures should not be delayed because investigations are still underway. If sufficient information is available to prevent additional cases of a foodborne disease, then one must act!

Information which might lead the outbreak investigation team into taking action when data are suggestive but insufficient to make a definitive call include:

- the severity of the disease (e.g., E. coli O157:H7, botulism) (i.e., one may be moved to act more quickly with a very serious or potentially fatal disease than one which is mild or self-limiting)
- the population at risk (e.g., infants, immunocompromised persons, the elderly) (i.e., if the population at risk includes persons who are highly likely to have poor outcomes from the infection/intoxication, then one may be moved to act more quickly)
- whether exposure is suspected to still be occurring
- how easily control measures can be implemented (e.g., does control require a nationwide recall of a commercially distributed product or temporary removal of one foodhandler?)

In this outbreak, one might argue that the severity of the illness increases the urgency for action. At a minimum, one would want to follow-up on the 58 bus drivers to make sure no other drivers were ill and that ill persons were receiving appropriate treatment. In addition, one would probably want to follow-up on the terminal home of the bus route, the owners, its workers, and other customers to see if they were ill. Given the nature of the outbreak (i.e., a very serious disease for which exposure might be ongoing), one should consider suspending commercial food services at the home (or asking the home to voluntarily suspend services) until more information can be collected.
PART II - DESCRIPTIVE EPIDEMIOLOGY AND HYPOTHESIS GENERATION

Staff from the local health department where the terminal stop of the bus route was located were invited to participate in the investigation.

Physicians attending the cases of botulism were asked to provide demographic and clinical information on their patients. (Table 1)

Table 1. Characteristics of cases of botulism, Buenos Aires, January 1998.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Work shift</th>
<th>Onset of neuro­logic symptoms</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>M*</td>
<td>Morning</td>
<td>January 6</td>
<td>blurred vision, double vision, drooping eyelids, upper and lower extremity weakness, respiratory difficulty, fatigue</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>M</td>
<td>Morning</td>
<td>January 5</td>
<td>blurred vision, double vision, drooping eyelids, upper and lower extremity weakness</td>
</tr>
<tr>
<td>3</td>
<td>23</td>
<td>M</td>
<td>Morning</td>
<td>January 9</td>
<td>blurred vision, drooping eyelids, upper extremity weakness, fatigue</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>M</td>
<td>Morning</td>
<td>January 8</td>
<td>drooping eyelids, difficulty speaking</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>M</td>
<td>Morning</td>
<td>January 5</td>
<td>blurred vision, double vision, drooping eyelids, difficulty speaking, respiratory difficulty</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>M</td>
<td>Morning</td>
<td>January 10</td>
<td>blurred vision, drooping eyelids, difficulty speaking</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>M</td>
<td>Morning</td>
<td>January 15</td>
<td>blurred vision, double vision, drooping eyelids, upper and lower extremity weakness, respiratory difficulty, fatigue</td>
</tr>
<tr>
<td>8</td>
<td>44</td>
<td>M</td>
<td>Morning</td>
<td>January 14</td>
<td>respiratory difficulty, fatigue, drooping eyelids</td>
</tr>
<tr>
<td>9</td>
<td>24</td>
<td>M</td>
<td>Morning</td>
<td>January 12</td>
<td>drooping eyelids, fatigue</td>
</tr>
</tbody>
</table>

*M=male gender
Question 6A: Summarize the demographic and clinical information on these patients and draw an epidemic curve.

All patients were male and all were bus drivers from the same company working on the morning shift of the same route. The median age of cases was 42 years (range: 23-54). All patients had drooping eyelids (ptosis). Six patients had blurry vision; five had fatigue; four each had double vision (i.e., diplopia), upper extremity weakness, and respiratory difficulty; three had lower extremity weakness. Onset of neurologic symptoms occurred from January 5 through January 15. Cases did not peak at any particular point during the 11-day period.

Figure 1. Onset date of neurologic symptoms among patients with botulism in Buenos Aires, Argentina, January 1998. (N=9)

![Epidemic Curve Graph]

Question 6B: When was the most likely period of exposure among these cases?

As noted above, the average incubation period for botulism is 18-36 hours, but symptoms can occur as early as six hours or as late as 10 days after exposure. The following two methods can help determine the time of exposure if dates of onset of symptoms are known:

Method #1: If a point source is suspected and the organism and time of onset of illness are known, 1) identify the last known case in the outbreak and count back the maximum incubation
period and 2) identify the first case and count back the minimum incubation period. Ideally, the
dates should be similar and represent the probable period of exposure. Using this technique, the
period of exposure for this outbreak would be January 4-5. However, at this point, it is
unknown if the exposure occurred on one day or over a more extended period of time.
Therefore, one would consider a slightly longer period of exposure in exploring possible sources
of the outbreak.

Method #2: If a point source is suspected and the organism and time of onset of illness are
known, count back the median incubation period from the peak of the outbreak. Looking at the
epidemic curve for this outbreak, cases do not cluster at any obvious point in time; therefore, it
might be more difficult to use this technique to identify the exposure period.

In hypothesis-generating interviews with cases and other bus drivers, being a driver on the
morning shift of the bus route and eating at the terminal home of the route were the only
common exposures among cases. No cases of botulism occurred among bus drivers from the
afternoon or evening shift of the route. Bus drivers from those shifts did not usually eat at the
terminal home because it was only open for lunch.

The investigators hypothesized that the outbreak was limited to morning shift bus drivers and
resulted from eating or drinking something at the home at the terminal stop of the particular bus
route between January 3 and 7.

Question 7: What type(s) of study would you use to investigate this hypothesis? Why?

To test the hypothesis, one would want to compare specific exposures among persons who
became ill with those among persons who did not become ill. One could use either a cohort or
case-control study to explore this hypothesis. Because the outbreak is confined to a well-defined
and relatively small group of individuals (i.e., 27 bus drivers on a particular route and shift), a
cohort study would seem most logical. A cohort study would also permit investigators to
calculate attack rates.

In addition to epidemiologic studies, other studies may be of use (e.g., collection and testing of
food samples, environmental investigations, or exploration of food preparation methods at the
terminal home). Results from each of these studies could provide important information on the
source of the outbreak and the necessary means to control it.

Should one undertake the epidemiologic study first or the other studies? The epidemiologic
investigation can often focus subsequent investigations; however, on certain occasions, other
studies must be initiated first or simultaneously (e.g., the collection of food specimens which
might be discarded). Therefore, the order of various investigations will depend largely on the
specifics of the outbreak.
PART III - DESIGNING AN EPIDEMIOLOGIC STUDY TO TEST THE HYPOTHESIS

To identify the source of the outbreak, investigators undertook a retrospective cohort study among bus drivers who drove the morning shift of the bus route. Data were collected from January 15-19.

Investigators defined a confirmed case of botulism as a bus driver from the morning shift of the bus route with a serum or stool sample that demonstrated botulinum toxin or yielded *Clostridium botulinum* with onset of symptoms between January 5 and 15. A probable case was defined as acute cranial nerve dysfunction (e.g., blurred vision, double vision, drooping eyelids, problems swallowing) with no laboratory confirmation in this group of drivers during the same period. The comparison group consisted of all bus drivers from the morning shift of the implicated bus route who had no acute neurologic symptoms suggestive of botulism.

After consultation with the local health department where the terminal stop of the bus route was located and the bus company management, investigators developed a structured questionnaire for the epidemiologic study.

**Question 8:** What general types of information would you include in the questionnaire?

- **Identifying information** (e.g., name, medical record number, code number). This information allows the investigator to identify the patient and update the questionnaire as more information becomes available. It also prevents duplicate entry of records.

- **Demographic information** (e.g., age, gender, race/ethnicity, location). This information is basic to all descriptive epidemiology allowing one to characterize the population at risk and examine possible confounders.

- **Clinical information** (e.g., signs/symptoms, time of onset of illness, laboratory results, whether the patient saw a doctor or was hospitalized). This information allows one to confirm the diagnosis, characterize the disease, and chart the course of the outbreak.

- **Risk factor information** (e.g., foods eaten, underlying medical illnesses, routine medications). This information allows one to explore the source of the outbreak.

- **Source of the information** (e.g., patient, parent, spouse, physician). This information will provide some insight into the accuracy of the information obtained.
Question 9: What steps would you take to develop the questionnaire for this investigation.

Although there are no set steps for developing a questionnaire for an epidemiologic study, one would probably go through the following thought processes:

1) Identify the primary and secondary hypothesis for the source of the outbreak.
2) List the discrete pieces of information needed to accept or reject the primary and/or secondary hypotheses. This includes clinical information to distinguish cases from controls and exposure information. (For this outbreak, it would be useful to obtain detailed menus of foods served or talk with the chef to determine what was truly served.)
3) List information that is needed for the logistics of the study (e.g., subject name, address, study number, interviewer name) or to control for potential confounders (e.g., age, race, sex, location).
4) Identify how the collected information will be used to test the hypothesis (i.e., plan analysis including dummy tables of key variable).
5) Write the questions to collect the information. (One will need to consider the most appropriate format and wording of the questions. It might also be helpful to consult with other jurisdictions that have had similar outbreaks and obtain questionnaires they used.)
6) Organize the questions into questionnaire format and develop introductions/closings and possible responses for close-ended questions. (One may need to consult with key informants or conduct focus groups to determine the most appropriate responses for the setting and community.)
7) Pre-test the questionnaire.
8) Revise the questionnaire.
9) Train interviewers, if used.

Question 10: Using information on foods served at the terminal stop home from January 3-7 (Appendix 1), draft questions for food exposure for this study.

Two important issues to consider in developing these questions:

1) Multiple exposures during the 5-day period. Some persons may have eaten at the terminal home on more than one occasion. To address this, one might wish to:
   • collect information on foods eaten each day during the period of interest
   • ask whether a particular item was eaten at any time during the period of interest
   • ask “how many times” a particular item was eaten during the period of interest

   (In some situations, it may be appropriate to ask “how much” was eaten since risk may increase with dose.)
Responses to multiple choice or “Yes/No” questions. Responses should include a “Don’t know” category. This allows investigators to distinguish between the respondent not knowing the answer to a question and the respondent overlooking or purposefully skipping a question. To encourage study subjects to answer questions, many investigators use the following instructions (or a close facsimile), “We know it can be difficult to remember what you ate more than a few days ago. Please make your best guess as to whether you ate any of the following food items. It might be useful to look at a calendar to help you remember what foods you have recently eaten.”

The following questions were used by the study investigators:

Did you eat or drink any foods at the terminal house on your route between Saturday, January 3 and Wednesday, January 7?

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
<th>Do not know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If NO, finish the questionnaire...</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If YES....</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

On Saturday, January 3, at the terminal house on your route, did you eat or drink ...

<table>
<thead>
<tr>
<th></th>
<th>YES</th>
<th>NO</th>
<th>Do not know</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. black coffee?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b. coffee with milk?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c. tea?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d. soda pop?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e. mate?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>f. ham sandwich?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g. processed ham sandwich?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h. matambre sandwich?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. Did you eat the filling in the matambre?</td>
<td>YES</td>
<td>NO</td>
<td>Do not know</td>
</tr>
<tr>
<td>j. hot dog?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k. salami?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l. bologna?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m. sauce?</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n. other foods?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These questions were repeated for each day from January 3 - January 7.
Investigators conducted interviews with each of the drivers of the morning shift of the bus route to complete the questionnaires.

**Question 11:** If the investigators had decided on self-administration of the questionnaire, what changes might need to be made to it?

*Method of administration plays a large role in both the wording and organization of a questionnaire. For a self-administered questionnaire (or one in which you use a person less familiar with the issues to collect the information [e.g., a translator]), no one will be available to define vocabulary, provide guidance on navigation through the questionnaire, or encourage the subject to complete the entire questionnaire. Therefore, one might want to focus attention on the following:*

- The wording of each question should be easily understood and free of technical words or jargon.
- Use of abbreviations should be limited to those readily known to all study subjects.
- When creating potential responses for closed-ended questions, anticipate and prepare for all possibilities. Make sure responses are mutually exclusive and easily understood. Be sure to include a “Don’t know” category.
- Means of entering/selecting responses should be as simple as possible (and explained if necessary) (e.g., some subjects may not know how to enter a date into six blanks).
- Skip patterns should be kept to a minimum and should be clearly described.
- The questionnaire should be as short as possible.

*(NOTE: Most of the above are also important issues for other forms of questionnaire administration but may be more critical with self-administered questionnaires or ones in which you use a person less familiar with the issues or epidemiology to gather the data.)*

*Pretesting the questionnaire with persons who are similar to study subjects will help identify and correct potential problems before it is too late.*
PART IV - ANALYSIS AND INTERPRETATION OF EPIDEMIOLOGIC RESULTS

The following food exposure information was collected through the cohort study. On January 19, the information was tabulated by epidemiologists from the Argentine MOH. (Table 2)

Table 2. Foods eaten by ill and well bus drivers at the home at the terminal bus stop, January 3-7, 1998. (N=21)

<table>
<thead>
<tr>
<th>Food item</th>
<th>Ate item</th>
<th>Did not eat item</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ill</td>
<td>Well</td>
</tr>
<tr>
<td>Bologna</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hot dog</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Matambre*</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Mate**</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Processed Ham</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Sauce</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Salami</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Solid ham</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

*Matambre is a traditional meat roll in Argentina.  
**Mate is green tea.

Question 12: Calculate the appropriate measures of association for these exposures.

The appropriate measure of association for a cohort study is the relative risk. The relative risk (RR) reflects the excess risk (of disease) in the exposed group compared with the unexposed group.
relative risk = \frac{\text{attack rate among persons who ate item}}{\text{attack rate among persons who did not eat the item}}

= \frac{\# \text{ of ill persons who ate item/total \# of persons who ate item}}{\# \text{ of ill persons who did not eat item/total \# of persons who did not eat item}}

= \frac{a/(a+b)}{c/(c+d)}

**NOTE:**
A relative risk of 1.0 means the risk of disease is similar in the exposed and unexposed group and exposure is not associated with disease.

A relative risk of greater than 1.0 means the risk of disease is greater in the exposed than the unexposed group and the exposure could be a risk factor for the disease.

A relative risk of less than 1.0 means the risk of disease is less in the exposed group than the unexposed group and the exposure could be a protective factor.

Tests of statistical significance (such as the chi-square or Fisher exact test) are used to determine how likely it is that the observed relative risk could have occurred by chance alone, if exposure was not actually related to the disease. This probability is the p-value. A very small p-value means that one would be unlikely to observe such an outcome if indeed there was no association between the exposure and the disease. If the p-value is smaller than some predetermined cutoff (usually 0.05 or 5 in 100 chance), the association is then said to be “statistically significant”.

To calculate a chi-square using above data layout and notation: (optional)

\[ X^2 = \frac{t(ad-bc)^2 - t/2}{(a+b)(c+d)(a+c)(b+d)} \]

**NOTE:** The instructor may wish to divide up the calculations among the students to limit the amount of time this exercise takes. One student can be asked to explain how s/he calculated their relative risk. The remaining students can just share their answers.
The calculations for the food items served at the terminal home of the bus route are:

<table>
<thead>
<tr>
<th>Food item</th>
<th>Ate item</th>
<th>Did not eat item</th>
<th>Relative Risk</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ill Well</td>
<td>Attack Rate Ill</td>
<td>Well Attack Rate</td>
<td></td>
</tr>
<tr>
<td>Bologna</td>
<td>1 0</td>
<td>100%</td>
<td>8 12 40%</td>
<td>2.5</td>
</tr>
<tr>
<td>Hot dog</td>
<td>1 1</td>
<td>50%</td>
<td>8 11 42%</td>
<td>1.2</td>
</tr>
<tr>
<td>Matambre</td>
<td>9 2</td>
<td>82%</td>
<td>0 10 0%</td>
<td>undefined</td>
</tr>
<tr>
<td>Mate</td>
<td>4 4</td>
<td>50%</td>
<td>5 3 63%</td>
<td>0.8</td>
</tr>
<tr>
<td>Processed Ham</td>
<td>2 3</td>
<td>40%</td>
<td>7 9 44%</td>
<td>0.9</td>
</tr>
<tr>
<td>Sauce</td>
<td>7 2</td>
<td>78%</td>
<td>2 10 17%</td>
<td>4.6</td>
</tr>
<tr>
<td>Salami</td>
<td>1 1</td>
<td>50%</td>
<td>8 11 42%</td>
<td>1.2</td>
</tr>
<tr>
<td>Solid ham</td>
<td>2 3</td>
<td>40%</td>
<td>7 9 44%</td>
<td>0.9</td>
</tr>
</tbody>
</table>

NOTE: The relative risk for matambre is undefined. This is because among persons who did not eat the matambre, no one became ill (i.e., the attack rate is zero among persons not eating matambre.) For the calculation of the relative risk, this would mean the denominator is “0” and division by “0” is undefined.

Question 13: Interpret the results. What further data analysis/information might help?

[Unlike the original investigation and real life, we have included “sauce” as a menu item that has an elevated relative risk.]

Among the foods consumed between January 3 and 7, only matambre, a traditional meat roll (prepared from meat, vegetables, spices, and eggs) and “sauce” were significantly associated with illness. All ill persons ate matambre. Furthermore, 82% of persons eating matambre became ill, whereas none of the 10 persons who did not eat matambre became ill. Almost all of the persons who ate sauce became ill (78%); however, two ill persons did not eat the sauce.

The question, at this point, is whether both the matambre and the sauce were contaminated with botulinum toxin, if one cross-contaminated the other, or if only one of the items was contaminated but both were routinely served together (and rarely apart). It would be of interest to ask how the sauce and matambre were usually prepared and served. One would also want to
collect any leftover specimens of both items to search for botulinum toxin. A stratified analysis, to examine only individuals who ate one of these items but not the other, might also be of interest. (However, numbers may be very small.)

As it turns out, the sauce was not contaminated but just served routinely with the implicated matambre.
PART V - ENVIRONMENTAL STUDIES AND FOOD INVESTIGATION

Matambre is a traditional Argentine dish prepared from meat, vegetables, spices, and eggs. In a traceback of the implicated matambre, the MOH discovered that it originated from a small scale producer located not far from the terminal stop of the bus route. The matambre was purchased at a local market on January 3 by the owners of the home that served the matambre. The MOH initiated an environmental health assessment of the matambre to identify production factors that could have contributed to the occurrence of botulism.

A complete environmental health assessment is not a cursory inspection of operations and sanitary conditions as is performed for the licensing of a food establishment, but focuses on the suspect food or meal and follows it from its raw ingredients to consumption by the customer. The objective of the environmental health assessment is to identify critical points where the implicated food could have become contaminated or microbial survival and growth in the food could have occurred, determine why these conditions existed, and identify appropriate interventions. The factors in Table 3, often found in these assessments, have been associated with an increased risk of foodborne disease.

Table 3. Factors that commonly contribute to outbreaks of foodborne diseases, from Bryan et al., 1987.

<table>
<thead>
<tr>
<th>Contamination</th>
<th>Survival</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>raw foods that are contaminated</td>
<td>inadequate cooking</td>
<td>inadequate refrigeration</td>
</tr>
<tr>
<td>infected foodhandler</td>
<td>infected re-heating</td>
<td>improper cooling</td>
</tr>
<tr>
<td>unclean equipment</td>
<td>inadequate acidification</td>
<td>inadequate hot-holding</td>
</tr>
<tr>
<td>cross-contamination</td>
<td></td>
<td>preparation too far in advance of serving</td>
</tr>
<tr>
<td>contaminated foods eaten raw or lightly cooked</td>
<td></td>
<td>use of leftover foods</td>
</tr>
<tr>
<td>inappropriate container for food unsafe sources</td>
<td></td>
<td>inadequate acidification</td>
</tr>
<tr>
<td>added poisonous chemicals</td>
<td></td>
<td>high water content</td>
</tr>
<tr>
<td>natural toxicant</td>
<td></td>
<td>inadequate curing salt</td>
</tr>
<tr>
<td>poor dry-storage practices</td>
<td></td>
<td>environment that provides favorable conditions for pathogen (e.g., anaerobic packaging)</td>
</tr>
</tbody>
</table>
Question 14: What types of activities do you think you would undertake as part of an environmental health assessment on the matambre? What equipment would you want to have?

In the investigation of the matambre, one would want to do the following:

- **Describe the matambre (e.g., all raw materials and ingredients used, source of ingredients, physical and chemical characteristics such as pH, intended use).**
- **Observe procedures from receipt of ingredients to finished product (e.g., cleaning methods, temperature history of the ingredients or product as it was stored, transported, cooked, heat-processed, held warm, chilled, or reheated).**
- **Talk with foodhandlers, managers, and others who may be familiar with the food preparation process and facility. Collect information on any aspect of the establishment, facilities, equipment, food, water, and processes that may have contributed to the outbreak. Record observations as well as reported information concerning economics, social and cultural beliefs, and management decisions that may have influenced or altered the food preparation process.**
- **Make appropriate measurements (e.g., time and temperature conditions to which the food was exposed, water activity, moisture, and pH of suspect food, size of containers used in procedures and depth of food in containers).**
- **Draw a flow diagram of the operations (e.g., exact flow of operations for the suspect food, name of persons performing operations, equipment used, results of measurements taken). It may be necessary to develop flow diagrams for each food preparer and/or shift.**
- **Collect appropriate specimens (e.g., leftover samples of the implicated food, samples of the ingredients, environmental testing of food preparation surfaces or equipment).**
- **Collect copies of invoices for the implicated food item or its ingredients (e.g., including information about the source of the items, batch or lot numbers, dates shipped and received, quantities received).**

To do the above, one would probably need a pencil/pen and paper, several thermometers (−17 to 104°C or 0 to 220°F), a thermocouple, a ruler, sterile sample containers, sterile sample collection implements (e.g., spoons, scoops, spatula, swabs), waterproof labels/tags, sample forms, refrigerants (e.g., ice packs, heavy plastic bags, insulated containers), and a camera.

*Clostridium botulinum* is a spore-forming obligate anaerobic bacterium (i.e., it cannot grow in the presence of oxygen). The spores are widespread in soil and dust worldwide. The toxin is produced in improperly canned, low-acid or alkaline foods and in pasteurized and lightly cured foods held without adequate refrigeration, especially in airtight containers. The toxin is destroyed by boiling; inactivation of spores requires much higher temperatures.
Food safety inspectors from the local health department initiated the environmental health assessment on January 20. The home at the terminal stop of the bus route was not formally licensed or equipped as a restaurant. Perishable foods, such as the matambre, were kept in two large refrigerators inside the home. Although the refrigerators were set at the coldest possible setting, temperatures measured inside the refrigerators were 9°C and 10°C (48°F and 50°F, respectively).

The home owner reported the most recent matambre served in the home weighed approximately 4 kg. The matambre was cut into about 15 slices and was served in sandwiches, usually with a spicy sauce; no other condiment or ingredient was added to the sandwiches. No matambre was available for testing.

The implicated matambre was bought at a local market where it had been stored in a refrigerator. The market had no temperature records or sales receipts; however, some customers reported that the matambre had recently been sold at reduced prices because of power outages.

The market purchased matambre from a small scale commercial producer who made matambre and processed hams in his home. To make matambre, the producer placed a slab of raw beef (1- to 3-cm thick) on a stainless steel table. Ingredients included raw sliced carrots, hard-boiled eggs, salt, red pepper flakes, dried oregano, and commercial potato flour. The meat was rolled up around the vegetables and eggs to make an approximately 10 x 30 cm cylinder. The meat roll was placed into a rectangular stainless steel pan to keep ingredients inside during cooking. Between 10 and 15 matambre in individual steel pans were immersed together in water heated to 70 to 80°C (158 to 176°F) and cooked approximately 4 hours. The matambre was never brought to a boil. After cooking, the water was drained and the temperature was checked to ensure an internal temperature of about 68°C (154°F). The producer placed each warm matambre in plastic wrap, squeezed out the air, and sealed the plastic with heat. The plastic wrapped matambre were allowed to cool, placed in a walk-in refrigerator, and were stored for up to 2 weeks before being sold to either supermarkets or directly to consumers.

**Question 15:** Identify the food handling practices for the matambre which were most likely to contribute to the development of botulism.

*Students should consider which of the many problems in the production of the matambre are critical in the survival of botulinum spores and/or toxin. Given the microbiologic characteristics of *C. botulinum*, the following factors will put one at higher risk for botulism:*

- inadequate cooking
- inadequate reheating
- long storage time between preparation and consumption
- anaerobic packaging
- low acid and high water content
These points are shaded in gray in the diagram below. Instructors may wish to draw Figure 2 on the board as the above description is being read or share copies with the students.

Figure 2. Flow diagram for preparation and serving of matambre, Botulism Outbreak in Buenos Aires, January 1998.

The producer reported making matambre every 2 weeks in batches of 15-20 each time. The last batch produced before the outbreak was made in early December. No matambre were available from that batch.

Question 16: What control measures would you initiate at this time? What difficulties might you encounter?

Although testing of the matambre for botulism toxin is not possible, the following actions should be undertaken based on the epidemiologic results and supporting environmental investigation:

1) Recall and destroy all matambre made by this producer
2) Stop further production of matambre by this producer until its safety can be assured
3) explore whether other food items made by this producer could also be problematic (and, if so, stop production and recall products as necessary)

4) assess other facilities preparing matambre to determine if problematic practices are more widespread

To correct identified deficiencies, one would want to provide education on appropriate foodhandling procedures to the matambre producer and his employees, persons at the market where the electrical outages occurred, and the owner(s) and foodhandlers at the home at the terminal stop of the bus route. One would also want to ensure that all equipment at these facilities was in proper working order and apply public health engineering methods where appropriate. Temporary closure of the three facilities or more thorough inspections to ensure proper foodhandling procedures would also need to be considered.

Possible problems:

- Identification and recall of remaining matambre produced by this facility could be problematic. It is likely that much of the matambre (from the December and subsequent batches) has already been sold and consumed; that which remains is likely to be in a number of private homes and food service establishments around the area. Unless the product is clearly labeled (and distinguishable from other locally produced matambre) or the producer has good records on distribution and sales, a general recall may not be feasible or productive (and could result in widespread panic or damage to the reputations/businesses of unimplicated producers).

- Closure of the facilities could cause local hardships. For instance, the home at the terminal stop of the bus route could be the only food service establishment available to bus drivers and other persons visiting the area. Closure would mean that patrons (such as the bus drivers) would need to find other sources of food or carry food with them. Cessation of matambre production alone would probably not cause the same level of local hardship other than the loss of revenue by the producer.

- Closure of these sites could be an extensive process, depending on local regulations and policies. Local health officials may not have the authority to close the establishments without due process and approval by another local decision making authority (e.g., local board of health or city/county council). Facility inadequacies would need to be fully documented and presented with other evidence to the implicated facilities, themselves, and the local decision making authority. Hearings might be needed and opportunities for appeals made. Given the serious nature of this outbreak, these hearings would presumably occur after the facility was closed. Again, cessation of matambre production alone will not be as problematic as closing the facilities.
• More thorough inspection of the facilities may overburden local public health resources. All over the world, local health departments struggle to complete inspections needed for the purposes of licensing and annual renewals. Increasing the time spent during routine visits to the facilities may greatly strain local public health resources.

NONE OF THE ABOVE PROBLEMS SHOULD PREVENT ONE FROM TAKING THE MOST APPROPRIATE ACTION. However, for a control effort/intervention to contribute to the public’s health, it needs to be acceptable to those implementing it and able to be implemented with the given resources.

At this point, students should be encouraged to insert the matambre production date into the epidemic curve drawn in Question 6A.
PART VI - CONTROL

After inspection by local food safety officials, the facility producing the matambre was closed. The producer was unable to provide receipts or a distribution list with locations where his products were sold. He reported that most of his clients distributed his products in the western greater Buenos Aires area. The producer’s matambre was not labeled in any way to indicate the source or date of production, so a recall of any remaining matambre was not deemed feasible.

Based on data maintained by the MOH, botulism is not an uncommon occurrence in Argentina. During the years 1979-97, 277 cases of botulism were reported; for most, the source of the exposure was undetermined. In 1997, 23 patients with suspected botulism were reported (of which 13 [57%] died); about the same number of laboratory-confirmed cases were identified in the United States, which has 10 times the population of Argentina.

Question 17: Given the scope of the problem, what longer term interventions might help decrease the number of botulism cases in Argentina?

Many possible interventions exist:
1) more thorough and rapid investigation of individual cases of botulism to learn more about usual sources and limit the number of persons exposed through any one source
2) improved diagnostic capabilities (e.g., increased provider knowledge, improved laboratory resources) to increase the speed of diagnosis and the initiation of further investigations
3) placement of more resources into food production facility education, licensing, and monitoring, especially for high risk foods
4) consultation with food scientists (or targeted research) to identify safe ways to produce matambre
5) education of the general public about high risk foods and means to prevent botulism

Because of the relatively high incidence and case fatality ratio for botulism in Argentina, the MOH and Centers for Disease Control and Prevention (CDC) collaborated to establish a botulism surveillance and antitoxin release system in Argentina. The system components included: 1) the establishment of a local stock of antitoxin, 2) a mechanism for antitoxin distribution within the country, 3) emergency notification and response for suspect cases (including urgent epidemiologic investigation of the possible food source), and 4) laboratory confirmation of suspect cases.
Question 18: What is the rationale behind the components of this control effort?

The centralized antitoxin supply and single emergency release number at the MOH are aimed at both a rapid response to each case of botulism and more effective surveillance of cases over time. Upon receiving a request for antitoxin, consultation between the treating physician and the MOH will help in differentiating botulism from many other illnesses, thus preventing unnecessary antitoxin administration. If the MOH consultant and the treating physician determine that botulism is a likely diagnosis, locally held antitoxin can be swiftly dispatched resulting in more rapid treatment of patients and, hopefully, decreased morbidity and mortality. The required consultation ensures that a search for additional cases of botulism and investigation of a food vehicle is quickly initiated. This will enable the early diagnosis of some exposed persons and prevent other cases from occurring. The laboratory confirmation of suspect cases and other surveillance data will allow public health officials to better understand trends in botulism in Argentina and allow the identification of more outbreaks and development of more effective interventions and prevention programs in the future.

Question 19: How might you monitor the Argentine botulism surveillance and antitoxin release system over time to determine if it is effective?

The following trends may help evaluate the success of the Argentine botulism surveillance and antitoxin release system:

- number of cases of botulism
- number of cases of botulism for which a food is implicated
- average number of cases associated with each implicated food item
- number of cases associated with commercially prepared foods
- time from diagnosis to administration of antitoxin
- sequella and complications among cases
- case-fatality rate
- number of unsafe foods and food practices identified

With a strong surveillance system, decreases in each of these numbers over time would suggest at least limited success of the program. Paradoxically, as one implements improved surveillance and outbreak investigation, the number of identified cases will initially rise. With consistent data collection and the implementation of appropriate interventions, however, the numbers will be expected to decrease over time.

Again, students should be encouraged to note the time at which various control measures/interventions were implemented (e.g., closure of the matambre production facility, initiation of surveillance system) on their epidemic curves. (Figure 3)
Figure 3. Onset date of symptoms among patients with *Botulism in Buenos Aires, Argentina, December 1997 - January 1998*. (N=9)
EPILOGUE

Although consumption of matambre is an established tradition in Argentina, it is usually consumed fresh and is not generally intended for pickling or long-term preservation. Matambre produced by licensed, commercial facilities use nitrites, acidifiers, or other preservatives to prevent bacterial growth; the implicated matambre lacked these. Insufficient cooking, vacuum packing in heat-shrunked wrap, and inadequate refrigeration may have provided conditions for live spores to germinate and produce toxin.

The matambre that is believed to be the cause of the outbreak was cooked at relatively low temperatures (78-80°C or 158-176°F ) over a time period of approximately 240 minutes, too short to kill all C. botulinum spores. These spores are difficult to destroy using conventional cooking techniques. In fact, nonkilling heat shock and the lack of preservatives or acidifiers may even enhance germination and toxin elaboration.

To safely prepare foods for canning or long-term storage, the U.S. Department of Agriculture recommends that all low-acid foods (i.e., foods with a pH > 4.6, including meats, seafood, poultry, milk, and fresh vegetables) be sterilized at temperatures of 116 to 121°C (240-250°F) in pressure canners operated at 0.66 to 0.97 atm (10-15 lb/in²). At these temperatures, the time needed to destroy bacteria in low-acid canned food ranges from 20-100 minutes. The exact time depends on the kind of food being canned, the way it is packed, and the amount of food being cooked.
REFERENCES


APPENDIX 1

Foods served to bus drivers in home at terminal stop of bus route in the first week of January:

Bologna  
Hot dogs  
Matambre*  
Mate**  
Processed ham  
Sauce  
Salami  
Solid ham

*Matambre is a traditional meat roll in Argentina. At the terminal stop it was sliced and served in sandwiches, usually with a spicy sauce.  
**Mate is green tea.