Outbreak of Type A Botulism and Development of a Botulism Surveillance and Antitoxin Release System in Argentina

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Botulism is a potentially fatal, neuroparalytic illness resulting from toxins produced by the bacterium Clostridium botulinum.1,2 Human illness occurs as infant botulism, botulism from intestinal colonization and wound infections, and foodborne botulism.3 Symptoms appear approximately 12 to 36 hours after toxin exposure beginning with bulbar and autonomic nervous system disturbances. Descending, symmetrical skeletal muscle weakness, paralysis, and respiratory failure may also follow,4,5 which require prompt, supportive therapy including mechanical ventilation.6,8 Botulism antitoxin, the only specific therapy available to ameliorate illness, can help prevent progression of paralysis, reduce duration of illness, and decrease fatality rates.7

Supportive care and prompt administration of antitoxin have reduced mortality in the United States to less than 10%,8 and prompt identification of suspect food vehicles during outbreaks has prevented additional cases and reshaped our understanding of the epidemiology of botulism. Global antitoxin supplies are limited and antitoxin has been unavailable for immediate use in several recent outbreaks (eg, Italy and Egypt).9,10 In the United States, the Centers for Disease Control and Prevention established a botulism surveillance and antitoxin release system that provides more timely distribution of antitoxin to patients and may serve as a model for other nations.11

Context Botulism is an important public health problem in Argentina, but obtaining antitoxin rapidly has been difficult because global supplies are limited. In January 1998, a botulism outbreak occurred in Buenos Aires.

Objectives To determine the source of the outbreak, improve botulism surveillance, and establish an antitoxin supply and release system in Argentina.


Main Outcome Measure Occurrence of botulism and implication of a particular food as the vehicle causing this outbreak.

Results Nine (43%) of 21 bus drivers developed botulism, presenting with gastroenteritis, symptoms of acute cranial nerve dysfunction including ptosis, dysphagia, blurred vision, and motor weakness. One driver experienced respiratory failure. Type A toxin was detected from 3 of 9 patients’ serum samples. All drivers received botulism antitoxin; there were no fatalities. Consumption of matambre (Argentine meat roll) was significantly associated with illness. Among 11 persons who ate matambre, 9 developed illness, compared with none of those who did not eat it (P<.001). The matambre had been cooked in water at 78°C to 80°C for 4 hours, sealed in heat-shrunk plastic wrap, and stored in refrigerators that did not cool adequately. Subsequently, a botulism surveillance and antitoxin release system was established.

Conclusions Insufficient cooking time and temperatures, storage in heat-shrunk plastic wrap, and inadequate refrigeration likely contributed to Clostridium botulinum spore survival, germination, and toxin production. A rapid-response botulism surveillance and antitoxin release system in Argentina should provide more timely distribution of antitoxin to patients and may serve as a model for other nations.

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Health officials identified 5 other drivers who drove the same route and morning shift. Both men were respiratory difficulties and notified the hospital observed 2 men presenting with ptosis, diplopia, dysphagia, and who were well who drove the morning shift of the bus route where the drivers stopped during their breaks.

Epidemiologists from the Argentine Ministry of Health called the Foodborne and Diarrheal Diseases Branch, National Center for Infectious Diseases, at the CDC to report the outbreak and request botulism antitoxin to treat these patients and possibly others exposed to an unknown contaminated food source. In this article, we report the outbreak investigation and discuss how the preparation, storage, and packaging of the implicated food may have permitted C. botulinum spores to germinate.

In addition to investigating the outbreak and reviewing the epidemiology of botulism in Argentina, the Argentine Ministry of Health and the CDC used this opportunity to establish a local system for botulism surveillance and antitoxin distribution. The goals of the system are to reinforce surveillance, to deliver antitoxin to patients in Argentina more rapidly and efficiently, and to serve as a model for other nations.

METHODS
Case Identification and Cohort Study
To identify cases, all employees of the bus company with the ill drivers were contacted. Hospitals in the areas of Buenos Aires where cases occurred and where a suspected food was produced were asked to report any patients with acute neurologic illness that could be botulism. Additionally, the outbreak was reported by the local news media.

We conducted a cohort study among bus drivers who were ill and also those who were well who drove the morning shift of the bus route. On January 18-19, 1998, we conducted face-to-face interviews and administered a written questionnaire to the drivers. We asked drivers about demographic characteristics, symptoms, and food exposures during the probable exposure period. We defined a confirmed case of botulism as a serum or stool sample that demonstrated botulinum toxin or yielded C. botulinum from a bus driver on the morning shift who ate or drank at the home terminal stop between January 3 and 7. Probable illness was defined as acute cranial nerve dysfunction in this group of drivers during this period. We excluded drivers from other shifts because none reported illness and they did not eat at the terminal stop or share any other common food exposures with the morning shift drivers.

Data were analyzed and relative risks with Fisher exact P values and 95% confidence intervals were calculated using Epi Info version 6.04a (Centers for Disease Control and Prevention, Atlanta, Ga) and Exact Software (the Netherlands). Values of P<.05 and 95% confidence intervals that did not include 1 were considered significant.

Environmental Investigation
We interviewed the family living in the house where the drivers ate, asked what foods and drinks were served, and inspected the food preparation and storage facilities. We also inspected the supermarket where the foods were purchased and the facility where a suspected food was produced.

Microbiologic Investigation
Serum specimens were collected from all patients between January 13 and 16, and stool specimens were obtained from patients between January 18 and 20 for both toxin and culture testing for C. botulinum. Assays were conducted in the diagnostics and food laboratory for botulism of the Instituto Nacional de Enfermedades Infecciosas, Buenos Aires. Approximately 10 to 40 g of patients’ feces with an equivalent volume of diluent gelatin were ground in sterile mortars. The solution reposed for 12 to 18 hours at 4°C to allow any toxin present to transfer into the liquid medium. It was then centrifuged at 12 000 rpm for 20 minutes.

Toxicity and neutralization assays were conducted in duplicate using white mice weighing 18 to 20 g. For each mouse, 0.1 mL of monovalent an-
titoxin (BioMerieux, Institute Pasteur, Paris, France) was combined with either 0.5 mL of fecal supernatant or patient serum and allowed to incubate at 37°C for 1 hour before being injected intraperitoneally. Antitoxin types A, B, and E were tested individually (only 1 type of antitoxin was injected into each mouse and no polyvalent or mixed-valence antitoxins were assayed). For detection of toxin types B and E, the assay was conducted with 1 tube of the fecal supernatant heated to 100°C for 10 minutes, and 1 tube containing 0.5 mL of supernatant mixed with trypsin (9 parts supernatant and 1 part trypsin, 1:250 to 1%, incubated at 37°C for 30 minutes). Normal rabbit (nonimmunized) serum mixed with diluent gelatin was used for control assays.

After the sediment and supernatant from the fecal material were resuspended, 2 samples of each clinical specimen were inoculated in a chopped meat medium. One of these tubes was heated to 80°C for 10 minutes to select for spore-forming organisms. Culture tubes were incubated at 31°C for 5 to 10 days; supernatant from these cultures was tested for the presence of botulism toxin using the mouse assay as described. If toxin presence was confirmed, isolation of _C botulinum_ was attempted on a solid egg-yolk medium.

### RESULTS

#### Case Identification and Cohort Study

In total, 27 bus drivers worked the same route and shift; all were men. Of these, 22 were interviewed. The other 5 were unavailable for interviews, but either family members or the bus company reported that they were well. One driver, who was not ill, denied eating or drinking anything at the home and was excluded from further analysis. The 21 drivers reported that the home terminal stop was the only location where they had eaten together between January 3 and 7. Local hospitals and health departments did not identify other cases of botulism during this period.

Nine of the 21 bus drivers had illness meeting the case definition for botulism. Three of these patients were confirmed cases and 6 were probable cases. Their median age was 42 years (range, 23-54 years). Seven experienced gastroenteritis 12 to 48 hours after eating food from the terminal stop between January 3 and 5. Two patients were originally hospitalized for severe vomiting and abdominal pain. Reported onset of neurologic symptoms occurred between January 5 and 15 (5-day median incubation period; range, 0-10 days) (FIGURE). All 9 patients reported experiencing ptosis. Neurologic and gastrointestinal symptoms are listed in Table 1. One patient experienced respiratory insufficiency and required mechanical ventilation. Trivalent botulism antitoxin (anti-A, B, and E) (Connaught Laboratories Inc, Swiftwater, Pa) was administered to all patients on arrival at the infectious diseases hospital (January 13-15). There were no fatalities.

Among foods consumed between January 3 and 7, only _matambre_, a traditional meat roll prepared from meat, vegetables, spices, and eggs, was significantly associated with illness. Among those who ate the _matambre_, 9 of 11 became ill, compared with none of 10 who did not eat it (relative risk, undefined; 95% confidence interval, 4.37-∞; _P_ < .002). No other foods, including solid ham, processed ham, and hot dogs, were significantly associated with illness (Table 2).

#### Environmental Investigation

The terminal stop was identified as a home used by the drivers as a resting point and a place to eat while they awaited their scheduled time to resume driving. Although the home was not formally equipped as a restaurant, the owners provided the bus drivers with meals. The drivers and occasionally the homeowner and his family were the only persons who consumed these meals. Perishable foods, including the _matambre_, were kept in 2 large refrigerators inside the home. Although the refrigerators were set at the coldest possible settings, temperatures measured inside the refrigerators were 9°C and 10°C.

The homeowner reported the most recent _matambre_ served at the home weighed approximately 4 kg. It had been purchased on January 3, was cut into about 15 slices, and was served in sandwiches to the drivers between January 3 and 6. No other condiment or ingredients were added to the sandwiches. Only this _matambre_ was served at the home during the period when persons became ill. No _matambre_ remained in the home at the time of inspection.

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, we discovered from customers that _matambres_ had recently been sold at reduced prices because of power outages.

### Table 1. Symptoms of Patients (n = 9) With Botulism

<table>
<thead>
<tr>
<th>Symptom</th>
<th>No. of Patients</th>
</tr>
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<tbody>
<tr>
<td>Ptosis</td>
<td>9</td>
</tr>
<tr>
<td>Dry mucous membranes</td>
<td>8</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>7</td>
</tr>
<tr>
<td>Blurry vision</td>
<td>7</td>
</tr>
<tr>
<td>Diplopia</td>
<td>4</td>
</tr>
<tr>
<td>Upper extremity weakness</td>
<td>4</td>
</tr>
<tr>
<td>Respiratory difficulty</td>
<td>4</td>
</tr>
<tr>
<td>Respiratory insufficiency</td>
<td>1</td>
</tr>
<tr>
<td>Nausea</td>
<td>7</td>
</tr>
<tr>
<td>Stomach pain</td>
<td>6</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>5</td>
</tr>
<tr>
<td>Constipation</td>
<td>5</td>
</tr>
</tbody>
</table>

### Table 2. Case Identification and Cohort Study

No. of Patients | Symptom   | No. of Patients |
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>12345678910</td>
<td>Probable Dates</td>
<td>January 1998</td>
</tr>
<tr>
<td>1</td>
<td>Probable Dates</td>
<td>January 1998</td>
</tr>
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<td>1</td>
<td>Probable Dates</td>
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</tr>
<tr>
<td>1</td>
<td>Probable Dates</td>
<td>January 1998</td>
</tr>
</tbody>
</table>

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The market purchased *matambres* from a small-scale, commercial producer who made *matambres* and processed hams at 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. This meat roll was placed into a rectangular stainless steel pan to keep ingredients inside during cooking. Between 10 and 15 *matambres* in individual steel pans were immersed together into water heated to 78°C to 80°C (never boiling), and cooked for approximately 4 hours. After cooking, the water was drained and the producers reported they checked each *matambre* to ensure an internal temperature of about 68°C. The producer placed each warm *matambre* in heat-shrinked plastic wrap, squeezed out the air, and sealed the plastic with heat. The plastic-wrapped *matambres* 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. The producer reported making *matambres* every 2 weeks in batches of 15 to 20 each time; the last batch produced before the outbreak was made in early December. The producer was unable to provide receipts or a distribution list with names of locations where his products were sold. He reported that most of his clients distributed his products in the western greater Buenos Aires area. After an inspection by local food safety officials, the facility was closed.

**Laboratory Investigation**

The mouse assay for botulism toxin presence was performed with serum from each patient; 3 were positive for toxin type A. None of the stool cultures yielded *C. botulinum*. No implicated *matambre* was available for testing.

**COMMENT**

We reported an outbreak of botulism among bus drivers in Buenos Aires, Argentina, caused by consumption of *matambre*, a food that has not been described previously as a vehicle for botulism. A cohort study determined that all ill persons ate slices of a single *matambre*; no other foods were associated with illness. Type A toxin was identified from 3 of 9 patients’ serum samples. Unfortunately, no implicated *matambre* was available for laboratory analysis.

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. *Matambres* produced by licensed, commercial facilities use nitrates, acidifiers, or other preservatives to prevent bacterial growth; the implicated *matambre* lacked these. Insufficient cooking, vacuum packaging in heat-shrinked plastic wrap, and inadequate refrigeration may have provided conditions for live spores to germinate and produce toxin. To our knowledge, this is the first time heat-shrinked plastic wrap has been linked to botulism, emphasizing concern about the safety of this packaging method.

The *matambre* that is believed to be the cause of this outbreak was cooked at relatively low temperatures (78°C-80°C) over a time period (approximately 240 minutes) too short to kill all *C. botulinum* spores. These spores are difficult to destroy using conventional cooking techniques; temperature survival curves have demonstrated *C. botulinum* survival at 80°C for more than 270 minutes. In fact, a nonkilling heat shock and the lack of preservatives or acidifiers may enhance germination and toxin elaboration by *C. botulinum* spores. To safely prepare foods intended for canning or long-term storage, the US Department of Agriculture recommends that all low-acid foods (foods with pH >4.6, including red meats, seafood, poultry, milk, and fresh vegetables) be sterilized at temperatures of 116°C to 121°C in pressure canners operated at 0.68 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 to 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. The time needed to safely process low-acid foods in boiling water canners ranges from 7 to 11 hours.

The time and temperatures used to cook the *matambre* in this outbreak were insufficient to kill all botulism spores. Reduced oxygen packaging of foods can increase the risk of certain foodborne illnesses if other safety barriers have not been applied. Although vacuum packaging may inhibit the growth of some types of bacteria in foods, several *Clostridium* species can grow and produce toxin in this environment. To reduce the risk of botulism from foods in reduced oxygen packaging, the US Food and Drug Administration requires documented employee training in plants that use reduced oxygen packaging, stringent refrigeration, specific labeling with storage temperature instructions and use-by dates, and plant approval for reduced oxygen packaging of fish, soft cheeses, meats, and poultry.

**Table 2. Foods Consumed by Bus Drivers (n = 21) in Buenos Aires, Argentina, in January 1998**

<table>
<thead>
<tr>
<th>Food Item</th>
<th>No./Total (%)</th>
<th>Relative Risk (95% Confidence Interval)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matambre</td>
<td>9/11 (82)</td>
<td>0/10 (0)</td>
<td>Undefined (4.37-∞)</td>
</tr>
<tr>
<td>Bologna</td>
<td>1/1 (100)</td>
<td>8/20 (40)</td>
<td>2.50 (1.46-4.28)</td>
</tr>
<tr>
<td>Mate</td>
<td>4/8 (50)</td>
<td>5/8 (63)</td>
<td>1.30 (0.49-3.45)</td>
</tr>
<tr>
<td>Hot dog</td>
<td>1/2 (50)</td>
<td>8/19 (42)</td>
<td>1.19 (0.27-5.23)</td>
</tr>
<tr>
<td>Salami</td>
<td>1/2 (50)</td>
<td>8/19 (42)</td>
<td>1.19 (0.27-5.23)</td>
</tr>
<tr>
<td>Solid ham</td>
<td>2/5 (40)</td>
<td>7/16 (44)</td>
<td>0.86 (0.26-2.85)</td>
</tr>
<tr>
<td>Processed ham</td>
<td>2/5 (40)</td>
<td>7/16 (44)</td>
<td>0.86 (0.26-2.85)</td>
</tr>
</tbody>
</table>

*Matambre* is the traditional meat roll in Argentina and mate is green tea.
The matambre in this outbreak was packaged in a reduced oxygen environment and lacked other safety barriers needed to prevent Clostridium spores from proliferating.

Inadequate refrigeration also provided a favorable growth environment for C. botulinum. The refrigerators (at temperatures between 9°C-10°C) used to store the matambre at the terminal stop were not cold enough for safe storage of perishable foods. The US Food and Drug Administration recommends that refrigerator temperatures be 5°C or colder to prevent the growth of C. botulinum in foods stored in reduced oxygen packaging. Maintenance of adequate storage temperatures for the matambre also was not documented by the producer or the market where it was sold.

The clinical characteristics of botulism among patients in this outbreak differed in several respects from those in other reports. Typically, most patients with botulism develop severe muscular weakness and mild gastroenteritis. In this outbreak, the majority of patients experienced severe gastroenteritis, and only 1 patient developed severe muscular paralysis. In US cases of type A botulism, the reported median incubation period was 1 day (range, 0-5 days). In the Argentine outbreak, the incubation period was longer; the median time between food exposure and onset of neurologic symptoms was 5 days (range, 0-10 days). Several reported outbreaks of botulism from Europe have described patients with primarily mild to moderate symptoms, but most of these outbreaks have been caused by type B toxin. It is not known why some strains of C. botulinum cause milder illness than others, although the numbers of spores present and amount of toxin produced and ingested may influence clinical manifestations. The severity of gastroenteritis experienced by these patients could have also occurred if the matambre had been contaminated by other bacteria or toxins.

The outbreak investigation had several limitations. All of the implicated matambres from the supermarket where the implicated meat was purchased were discarded before the investigation began. Although no matambres were available for laboratory analysis, epidemiologic data and information gained from the investigation implicated the product. Furthermore, receipts were unavailable from the supermarket or the matambre producer to document dates of purchase or exact distribution of the product. However, no additional cases of type A botulism were detected.

The use of heat-shrunked plastic wrap and vacuum packaging for cooked foods followed by inappropriate refrigeration may provide an ideal environment for the growth of C. botulinum, and the safety of these packaging methods should be further investigated. Education of food manufacturers and distributors, including the importance of adequate cooking time and temperatures to kill C. botulinum spores, adding acidifiers or preservatives to foods intended for prolonged shelf life and refrigerating foods properly, may help prevent such outbreaks.

**Enhancing Surveillance and Establishing an Antitoxin Release System**

Botulism is an important public health problem in Argentina. Data maintained by the Argentine Ministry of Health indicate that during the years 1979-1997, 277 cases of botulism were reported in Argentina (Ministry of Health, Republic of Argentina, unpublished data, 1998). Between 1987 and 1997, the CDC released 54 vials of antitoxin to Argentina (CDC, unpublished data, 1998). In 1997, 23 patients with suspected foodborne botulism were reported in Argentina, about the same number of laboratory-confirmed cases per year as in the United States, which has a population approximately 10 times that of Argentina. In addition, 11 cases of culture-confirmed infant botulism were reported in Argentina that year. Only 1 case of wound botulism has been described in Argentina. Of 89 laboratory-confirmed cases reported between 1991 and 1997, 83 (93%) were identified as toxin type A, 4 (5%) as toxin type E (all occurred in a single outbreak), and 2 (2%) as toxin type B (Ministry of Health, Republic of Argentina, unpublished data, 1998). In the United States, toxin types A, B, and E were identified in 60%, 30%, and 10% of cases, respectively.

Previous outbreaks in Argentina have been caused primarily through the consumption of improperly preserved vegetables and meats. Although C. botulinum spores are ubiquitous, they are found most frequently in soil samples. The level of Clostridium species contamination on vegetables, particularly those harvested directly from soil, is generally higher than that found in meats and may in part explain why preserved vegetables are more frequently implicated.

Because of the relatively high incidence of botulism in Argentina, the Ministry of Health and the CDC collaborated to establish, in January 1998, an antitoxin release and surveillance system for botulism in Argentina based on the US program. The system components include (1) establishment of a local stock of antitoxin, (2) a mechanism for antitoxin distribution within Argentina, (3) emergency notification and response for suspect cases, and (4) laboratory confirmation of suspect cases. The critical element that ensures both rapid response and effective surveillance is the maintenance of a centralized antitoxin supply and a single emergency release telephone number. The system is briefly described below.

**Antitoxin Supply.** Botulism antitoxin was supplied to Argentina through a modification of the existing agreement between Pan-American Health Organization and the CDC. Ultimately, the Argentine Ministry of Health may contract directly for botulism antitoxin from pharmaceutical manufacturers. Such a contract would guarantee annual supply of product and would simplify maintenance of Argentina’s antitoxin supply. The emergency stock is currently maintained by the Ministry of Health.

(Continued on page 1340.)
Antitoxin Distribution. Patients with suspected botulism are identified based on clinical signs and symptoms, food history, and supportive data from ancillary diagnostic testing. Reporting of cases initiates the process of antitoxin release. To request antitoxin, treating physicians or provincial epidemiologists consult with an epidemiologist at the Directorate of Epidemiology at the National Ministry of Health using a 24-hour emergency telephone number. The epidemiologist from the Ministry of Health obtains information from the treating physician using a botulism case report form, requests a copy of the patient’s medical chart, and requests that diagnostic specimens be submitted to the laboratory. Consultation between treating physicians and the Ministry of Health epidemiologist may assist in differentiating botulism cases from other illnesses, thus preventing unnecessary antitoxin administration. If the epidemiologist and treating physician determine that botulism is a likely diagnosis, the epidemiologist arranges the release of 10-mL vial of antitoxin for each patient.

Emergency Notification and Response. To ensure investigation of potential additional cases of botulism, the epidemiologist notifies provincial health authorities by telephone. Inspection of the patient’s home and/or investigation of suspected food vehicles are then initiated. The epidemiologist also alerts national food safety authorities to assist with commercial product recalls if necessary or if the vehicle is unknown.

Laboratory Confirmation. Ministry of Health epidemiologists will provide physicians and provincial epidemiologists with information about specimen collection and will direct specimens to laboratories located in Buenos Aires and Mendoza. These 2 laboratories will generate an annual summary of specimen testing, confirmation method, toxin type, and suspected food vehicle (if applicable). The Ministry of Health epidemiologist also will record whether antitoxin was released for the patient and provide additional information about suspected foods and patient outcome. The data base can then be reviewed by the Ministry of Health.

Argentina’s System as a Model for Other Nations

Establishment of a system for antitoxin release and surveillance should improve the management of botulism in Argentina. The system is based on the US model, but tailored to the specific needs of Argentina. It maintains the principle of centralized antitoxin release to improve the efficiency of emergency reporting, allow rapid delivery of antitoxin to patients, and enhance laboratory confirmation and surveillance for botulism. The system also improves coordination between public health authorities and food safety agencies to facilitate investigation of outbreaks and prevent additional cases, and it provides a mechanism to track changes in the epidemiology of the disease.

The adoption of this system by Argentina serves as a model for other nations. Centralized antitoxin release, tightly linked to preventive measures and surveillance, provides an effective mechanism for managing this rare but deadly disease. In addition, the creation of a cooperative, rapid-response botulism surveillance network may provide a useful framework for a future surveillance system to monitor the emergence of new and resurgent infectious diseases throughout the world.

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REFERENCES