



Two-family outbreak of botulism associated with the consumption of smoked ribs in Sichuan Province, China



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ARTICLE INFO

Article history:

Received 13 July 2014

Received in revised form 1 October 2014

Accepted 25 October 2014

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:

Botulism
Outbreaks
Smoked ribs

SUMMARY

Background: On September 22, 2013, two patients from Sichuan Province, China presented with symptoms of food-borne botulism, a rare but fatal illness caused by the consumption of foods containing *Clostridium botulinum* neurotoxins.

Methods: Investigators reviewed the medical charts and food consumption histories, and interviewed patients and family members. Food samples and clinical specimens were tested for botulinum toxin and neurotoxin-producing *Clostridium* species by standard methods.

Results: The first two index cases presented with cranial neuropathies and flaccid paralysis, and required mechanical ventilation. There were 12 confirmed outbreak-associated cases. Botulinum toxin type A was identified in the smoked ribs, and all of the patients had consumed the smoked ribs from the same local restaurant. The smoked ribs contained no added salt, sugar, or preservative. Botulinum toxin production likely resulted from the cold-smoking preparation method and inappropriate refrigeration.

Conclusions: Smoked ribs produced by a local restaurant, contaminated with type A botulism, was the contributor to this outbreak. The supervision of food safety should be strengthened to prevent future outbreaks in China.

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1. Introduction

Botulism is an acute life-threatening neurological disorder caused by the neurotoxin of *Clostridium botulinum*. Botulinum neurotoxins (BoNTs) are considered the most toxic substances currently known.¹ BoNTs have traditionally been categorized into seven serotypes – BoNT/A to BoNT/G – and BoNTs A, B, E, and more rarely F, are known to cause botulism in humans.²

Food-borne botulism is caused by the consumption of food containing a BoNT produced by the bacterium *C. botulinum*. The spores of these bacteria are dormant in the environment, but germination and toxin production occur under anaerobic, low sugar, and low salt conditions at non-refrigeration temperatures (temperatures >10 °C for most strains).^{3,4} Food-borne botulism can arise from home-canned foods with a low acid content, such as carrot juice, green beans, and corn. Therefore, low-acid foods should be boiled before consumption.

BoNTs bind irreversibly to the presynaptic nerve endings and inhibit the release of acetylcholine, resulting in symmetric cranial

nerve palsy and secondary symmetric descending flaccid paralysis, which may progress to respiratory arrest.^{5,6} Clinical symptoms of botulism appear after an incubation period and vary greatly according to the serotype and degree of exposure to the toxin.⁷ The death rate among patients with botulism is 3–10%.^{4,8,9}

Here we describe an outbreak of food-borne botulism affecting 12 people in two families; the outbreak occurred on September 22–23, 2013 in Sichuan Province, China. Clinicians immediately took proper therapeutic measures and notified the public health officials. An epidemiological investigation was initiated to identify persons exposed to the implicated product and to collect clinical specimens and food samples for laboratory testing. The epidemiological and microbiological investigations showed that smoked ribs made in the same local restaurant were the only source of the outbreak.

2. Methods

2.1. Epidemiological investigation

We interviewed the first two patients and their families and reviewed their contacts with regard to food exposure to common items. We defined a case as illness in a patient with any objective

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neurological findings consistent with botulism, who had consumed the smoked ribs in the local restaurant before illness onset. A confirmed outbreak-associated case had to meet one of the following three criteria: (1) A clinically diagnosed patient with laboratory-confirmed botulism (BoNT and/or *C. botulinum* identified in a clinical specimen), known to have consumed the implicated product; the remnants of the product were contaminated with BoNT. (2) A patient with laboratory-confirmed botulism who was reported to have consumed the implicated product. (3) A patient with clinically-diagnosed botulism who was reported to have consumed the implicated product.

2.2. Traceback and investigation of the smoked ribs

The smoked ribs were identified as the sole food item that all patients reported having eaten. Investigators examined the kitchen equipment, interviewed the chef about how much they were processed, and collected food samples for testing.

2.3. Laboratory investigation

Clinical specimens were tested by the Academy of Military Medical Sciences in Beijing, China, which has developed immuno-colloidal gold kits to screen serum and stool specimens for BoNT type A.¹⁰ First, a colloidal gold solution was prepared by constant mixing of 0.01% (w/v) chloroauric acid hydrate (gold chloride) and 0.01% (w/v) sodium citrate for about 10 min at boiling temperature. The pH of the solution was then titrated to 8.5 with K₂CO₃. Next, anti-BoNT-type A antibody was dialyzed and added to the colloidal gold solution. The antibody was labeled with colloidal gold after incubation for 30 min at room temperature (RT). Bovine serum albumin (BSA; 2% (w/v)) was added, and the solution was incubated for 15 min at RT. To remove large debris, the solution was centrifuged for 10 min at 500 g, and to enrich the labeled probe, another centrifugation was performed (6000 g, 20 min). The probe was then re-suspended in blocking solution (1% (w/v) BSA in phosphate buffered saline (PBS) (pH 7.4)). Test strips were constructed for BoNT type A detection using instruments HGS201 and HGS101 (JN-Bio, Shanghai, China), in accordance with the manufacturer's protocol. Stool specimens were cultured anaerobically for the isolation of *C. botulinum* by use of standard culture techniques.^{11,12} Patient serum specimens, stool specimens, and the smoked ribs were examined for BoNT according to the study protocol approved by the Human Research Ethics Committee of West China Hospital of Sichuan University; all participants gave their written informed consent.

3. Results

3.1. Descriptive epidemiology and clinical findings

A total of 12 cases were considered as suspected outbreak-associated cases. On September 22, 2013, the first two patients were admitted to West China Hospital of Sichuan University with similar symptoms of diplopia, ptosis, dizziness, dysphagia, and dysarthria, preceded by gastrointestinal symptoms. One day after hospital admission, the conditions of these patients deteriorated rapidly. Food-borne botulism was suspected based on clinical manifestations. During interviews, it was found that they came from the same household and had consumed smoked ribs in a local restaurant on September 21, 26 h before illness onset. During days 1 and 2 of hospitalization, descending paralysis progressed quickly to quadriplegia and respiratory arrest, requiring intubation and mechanical ventilation. On September 23, 1 day after the index cases were hospitalized, five patients from the same household also exhibited signs and symptoms of botulism; the ocular muscles

were affected, followed by dysarthria progressing to muscle weakness. These patients, together with the first two patients, had consumed a meal on September 21 that included the smoked ribs.

The five patients from family 2 had illness onset on September 23, 2013. They had consumed a dinner together in the same local restaurant on September 21. Similar to the patients from family 1, they had ordered the smoked ribs. They were admitted to the country hospital. The symptoms reported by the five patients were dry mouth, feeling of a 'thick tongue', tiredness, dysarthria, dysphagia, diplopia, ptosis, and subjective muscle weakness. Although their clinical symptoms matched with botulism, the doctors of the country hospital did not recognize a suspected botulism outbreak at that time. Due to the restriction of medical condition, botulism anti-toxin A was not administered to these patients in the country hospital. On October 1, all five patients were transferred to the West China Hospital of Sichuan University for further treatment. Botulism anti-toxin A was administered based on the patients' clinical manifestations in combination with the epidemiological connection, without waiting for laboratory confirmation.

The date of illness onset ranged from September 22 to 23. The median age of the patients was 37 years (range 4–61 years); six (50%) were male and six were female. All 12 patients were admitted to hospital and treated with 100 000 units of botulism anti-toxin A by intravenous drip. Botulism anti-toxin A was administered immediately when a patient was suspected to have botulism. Bowel cleaning therapy, including enemas with 5% sodium bicarbonate, was also given. The median duration of hospitalization was 8 days (range 5–19 days). Two patients from the first family had severe paralysis, and mechanical ventilation support was required; however no deaths occurred. Patients presented the following neurological symptoms: blurred vision (50% of patients), diplopia (41.7%), ptosis (58.3%), dysphonia (50%), dysphagia (50%), and muscle weakness (75%). Patients also exhibited varying degrees of gastrointestinal symptoms, including nausea (25%), abdominal pain (8.3%), vomiting (16.7%), and diarrhea (8.3%). Two patients from the first family experienced predominantly gastrointestinal symptoms of nausea and vomiting, which preceded the neurological symptoms (Table 1).

3.2. Laboratory investigation

Serum and stool specimens from all 12 patients were examined. For the seven patients from family 1, BoNT type A was identified in serum samples collected 2 days after illness onset by use of immuno-colloidal gold kits. BoNT type A was also identified in four stool samples and confirmed as type A by ELISA; *C. botulinum* strains were isolated and identified using standard culture techniques. For the five patients from family 2, four serum samples tested positive for BoNT type A and one serum sample, collected 3 days after admission to the country hospital, tested negative. Stool specimens collected on days 3 and 4 were negative for BoNT and no neurotoxin-producing *C. botulinum* strains were isolated.

The local public health team carried out an intensive investigation including a comprehensive food consumption history. The smoked ribs had been served by a local restaurant during a family meal 2 days before the first two patients were admitted to hospital. The smoked ribs had been eaten by the first two patients who were placed on respiratory support, and had been tasted by the other patients from the household. The five patients from the second family had also consumed the smoked ribs in the same restaurant on the same day. BoNT type A was identified in the smoked ribs obtained from the refrigerator of the local restaurant. *C. botulinum* strains from the food samples were isolated and then verified as type A.

Table 1
Demographic characteristics, time intervals between key clinical events, and clinical outcomes of patients who consumed the smoked ribs

	Group 1	Group 2	Group 3
Patient	Patients 1 and 2 from family 1	Patients 3, 4, 5, 6, and 7 from family 1	Patients 8, 9, 10, 11 and 12 from family 2
Date of consumption	September 21, 2013	September 21, 2013	September 21, 2013
Date of symptom onset	September 22, 2013	September 23, 2013	September 23, 2013
Public health officials notified	September 22, 2013	September 23, 2013	October 1, 2013
Symptom onset to anti-toxin treatment	22 h	16 h	186 h
Clinical severity	Required mechanical ventilation support	Hospitalized	Hospitalized
Symptom onset to intubation	26 h for patient 1 78 h for patient 2	-	-
Duration of intubation	12 days for patient 1 9 days for patient 2	-	-
Clinical outcome 6 months after symptom onset	Rehabilitation facility, not ventilator-dependent	Fully recovered	Fully recovered

3.3. Traceback and investigation of the smoked ribs

On October 8, 2013, the restaurant was temporarily closed to cooperate with the investigations into the process of producing the smoked ribs. In brief, the process used was as follows: First, the cure mixtures were prepared, which contained salts, sugar, and spices. Usually, 1 g salt was used for 0.4 kilograms of ribs and the mixture was rubbed all over the ribs. The salt-rubbed ribs were then transferred to a large bag and placed in the refrigerator for 5 days. Next, the ribs were removed from the bag 5 days later, rinsed thoroughly under cold water, and dried on a towel uncovered in the refrigerator overnight. Following this 'cold smoking' was done over a smoldering fire (below 85 °C) for 12–24 h. Finally, the ribs were cooled in the refrigerator overnight before serving. The sterile cubicle used to sterilize containers of the implicated products showed signs of improper operation and maintenance. Additionally, the smoked ribs were usually stored at room temperature. However, there was no firm indication of how the smoked ribs had become contaminated.

All of the implicated products that were still in the restaurant were withdrawn by the local authorities, and the restaurant helped to identify consumers who had eaten the smoked ribs. The implicated product was reported in a press release accompanied by a photograph of the smoked ribs. Warnings were issued to the public and to the medical institutions about the clinical symptoms of botulism. Emergency procedures were put in place to ensure that physicians could obtain botulism anti-toxin A if required.

4. Discussion

Food-borne botulism has mainly occurred in the northern areas of China, particularly in Hebei Province¹⁰ and the Qinghai–Tibet plateau.¹³ It has rarely been reported in the southwest of China, including Sichuan Province. The outbreak discussed here was the first report of *C. botulinum* linked with smoked ribs in China.

Botulism is an acute paralytic disease caused by a neurotoxin produced by an anaerobic bacterium, *Clostridium botulinum*. Food-borne botulism occurs when food containing BoNT is eaten. BoNT is produced in contaminated food before digestion, and the food has usually been subject to inadequate cooking, poor production hygiene, and improper storage.¹⁴ Because the smoked ribs contaminated with *C. botulinum* type A accounted for this outbreak, we assumed that there was a failure to maintain a temperature below 10 °C. The smoked ribs were prepared and stored at room temperature, which may have provided the conditions required for the growth of *C. botulinum* and for toxin production.¹⁵ BoNT production may have occurred due to incorrect handling, for example no acidifying preservative, salt,

or sugars were added to the smoked ribs, and also the duration and temperature of the smoking process were inadequate to kill the spores. Heating for 30 min at 121 °C is required to kill the spores, and BoNT can be decomposed by boiling or heating to 80 °C for 10 min. In accordance with the guidelines of the US Food and Drug Administration (FDA), the cold-smoking method is not recommended by most food scientists because of the inherent risks, since rapid microbial growth can occur in the temperature zone used (40–140 °C). Additionally, the cold-smoked foods should be cooked to an internal temperature of 160 °C before they are eaten.

It is important to keep in mind that food-borne botulinum is still a very real possibility, with serious results.¹⁶ Many physicians are unfamiliar with the disease in China, and disease symptoms can easily be confused with those of other more common illnesses. Even in this outbreak, a mistaken preliminary diagnosis was made; the index cases were first diagnosed as myasthenia gravis. Other causes of flaccid paralysis must be excluded.¹¹ An incremental response to repetitive stimulation in electrophysiological studies may be helpful in distinguishing botulism from myasthenia gravis.¹⁷ Further, computed tomography scans are useful to identify a space-occupying lesion, cerebrospinal fluid (CSF) findings can help to identify infections of the central nervous system, and CSF protein is usually increased in Guillain–Barré syndrome but normal in botulism.¹⁸

The incubation period for the first family was 32 h after exposure and the incubation period for the second family was 2 days. These findings are consistent with previously reported botulism case descriptions, and the median incubation period for patients with type A botulism is usually 12–36 h following the consumption of BoNT.^{18,19} However, the longest incubation period reported is 12 days.^{20–22} In addition, the first two index cases were characterized by rapid onset and protracted paralysis. These two patients had consumed large amounts of the smoked ribs according to the detailed history-taking. This may explain the rapid progression and requirement for mechanical ventilation.

Prompt anti-toxin therapy is particularly important for these patients. The administration of botulinum anti-toxin is the only specific treatment for botulism, and anti-toxin should be given to patients as soon as possible after clinical diagnosis, ideally at <24 h after the onset of symptoms. Normally, if anti-toxin is given at >3 days after the onset of symptoms, it is less likely to be of benefit, and anti-toxin is not given if a patient is identified with neurological symptoms consistent with botulism at >7 days after disease onset and if symptoms are not progressing. However, it has been reported that the administration of anti-toxin 11 days after exposure to contaminated food is helpful for patients with

botulism.^{20,23} In another study, two patients were found to be toxemic 13 days and 46 days, respectively, after the onset of symptoms, and their conditions improved with anti-toxin treatment.²⁴ In this outbreak, all patients were treated with 100 000 units of botulism anti-toxin A. We take the following factors into consideration: toxemia may occur for 7 days after symptom onset and the patients may have consumed a high dose of toxin. Before receiving anti-toxin, patients should take an allergy test for sensitivity. In our cases, the skin test was positive in one patient who was severely paralyzed and dependent on respiratory support. This patient was desensitized over several hours before the full dose of anti-toxin was administered and no allergic reactions were observed during the entire treatment.

The timing of sample collection is important as the sensitivity of detection decreases rapidly with time. BoNT is detected in <25% of serum specimens obtained after 3 days²⁵ and <20% of serum samples taken after 6 days.² In this outbreak, BoNT type A was detected in all serum samples collected 2 days after illness onset for the first family. For the second family, clinical specimens were obtained and refrigerated by the country hospital; these were submitted to the botulism laboratory for the specific diagnosis and confirmation of botulism after the botulism outbreak was suspected. BoNT type A was present in the serum samples of four of these patients, obtained 5 days after toxin consumption. Although the persistence of BoNT in the bloodstream has been reported at 25 days after symptom onset,²⁴ ingested BoNT is normally not demonstrable in serum at 1 week after ingestion.²⁶ Stool specimens collected 6 days after toxin consumption from these four patients with toxemia were negative for BoNT and did not contain *C. botulinum*, suggesting that there was no *in vivo* toxin production due to an intestinal colonization. Issues that need to be considered are that only 36% of stool samples are positive for BoNT after 3 days,²⁷ and *C. botulinum* is present in stool samples of >70% patients within 2 days and 40% after 10 days.²⁵ In addition, some patients in the first family presented gastrointestinal symptoms initially, including diarrhea, vomiting, nausea, and abdominal pain, and this phenomenon may partly explain why only four out of seven patients in the first family had stool samples positive for BoNT type A.

With the help of the local health authority, the Centers for Disease Control and Prevention, and the National Health and Family Planning Commission, the response to this outbreak was timely. Even though botulism is a rare disease, it should be considered in every patient with neuromuscular deficits who has a history of suspicious food consumption. Profound, protracted paralysis may be associated with the consumption of high levels of toxin, and timely and sufficient anti-toxin treatment is required. This outbreak of botulism suggests that the supervision of food safety should be strengthened in China to avoid this potentially severe health hazard.

Conflict of interest: The authors declare that there are no actual or potential conflicts of interest.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (grant number 81301093).

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