

Original Article**Foodborne Botulism: A Study of 57 Cases in Northwest Iran**Rahim Nejadrahim¹, Mohammad Delirrad^{*2}

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ABSTRACT

Background: Foodborne botulism (FBB) is a rare paralyzing disease caused by ingestion of foods contaminated with botulinum neurotoxin (BoNT). It is still a health concern in many countries. This study aimed to investigate the epidemiology of FBB in West Azerbaijan Province, Iran.

Methods: Botulism surveillance data of the Health Deputy of Urmia Medical Sciences University, northwestern Iran was used in this retrospective survey from 2010 to 2014. Demographics, clinical features, bioassay results, implicated foods and outcome were analyzed. Clinically, patients were classified to three groups (mild, moderate, and severe).

Results: During the five years, 57 cases of FBB were identified involving 36 men (63.2%) and 21 women (37.8%). Mean age was 22.35 ± 17.84 yr. Most events were sporadic (86%). Bioassay confirmed serotype A of BoNT in 19 patients (33.3%). The most implicated food was locally-made cheese ($n=10$). Average incubation period was 24.35 ± 12.35 h. Clinically, mild, moderate and severe forms were observed in 68.4%, 19.3% and 12.3% of patients, respectively. All cases were hospitalized and treated with trivalent antitoxin. Mean hospital stay was 7.71 ± 3.94 days. Eight patients (14%) required mechanical ventilation and three of them deceased (5.3%). Median annual incidence rate of FBB was 0.37 cases per 100,000 population in this study.

Conclusion: Comparing other part of the world, FBB has a medium incidence rate in our province. Unpasteurized dairy products are the main causative foods and BoNT/A is the most commonly recognized toxin type. Promoting correct preservation and appropriate cooking are recommended for preventing FBB.

Keywords: Botulinum Neurotoxin, Botulism, Foodborne, Epidemiology, Iran.

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INTRODUCTION

Botulism is a rare paralyzing disease caused by the potent neurotoxin of the bacterium *Clostridium botulinum*. "The disease was first described in consumers of sausages (Latin: botulus) in Europe at the 18th century" [1, 2]. "The causative organism was discovered by Emile Van Ermengem at 1897, and the toxin was isolated during the 1910s" [3, 4].

C. botulinum is an anaerobic, gram-positive, spore-forming bacillus [5]. Its hardy spores are ubiquitously spread in soil and aquatic sediments and commonly contaminate foods [2, 6]. Spores are heat-resistant and survive in standard cooking practices that do not exceed 100 °C [7]. Bacterial growth is inhibited by refrigeration below 4 °C, heating above 121 °C, acidity (pH <4.5) or high water activity [2]. On the other hand, toxin

production may occur in special conditions that include an anaerobic milieu, low-acidity (pH of >4.6), low-salt and sugar contents, no preservatives, and a proper temperature (4 °C–120 °C) [2, 8, 9]. Toxin is destroyed by heating to 85 °C for at least 5 min while spores are inactivated by pressure heating to 121 °C for at least 20 min [10].

Botulinum neurotoxin (BoNT) is considered the most lethal known substance [10, 11]. It has been estimated to cause death in humans in doses as small as 0.05–0.1 µg [4]. Since the discovery of the toxin about 100 years ago, eight immunologically distinct BoNTs have been identified and designated by letters A through H [3, 12, 13]. Almost all human cases of botulism are caused by one of three serotypes (A, B, or E) [1, 2, 5, 9, 14].

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The toxin interferes with presynaptic release of acetylcholine in the parasympathetic and the sympathetic systems as well as the neuromuscular junctions [1, 7, 10, 15]. A clinical pattern of acute symmetrical descending flaccid paralysis is characteristic for botulism. Other common presentations include abdominal pain, vomiting, constipation, blurred vision, and diplopia [1, 7, 10, 11, 14, 16].

To date, six clinical forms of the disease have been described: 1) classic or foodborne botulism; 2) wound botulism; 3) infant botulism; 4) adult intestinal or hidden botulism; 5) iatrogenic or inadvertent botulism; and 6) inhalational botulism [4, 10, 17, 18].

Food-borne botulism (FBB) is the most common form of the disease in developing countries, including Iran, and results from ingestion of a food contaminated with BoNT. Worldwide, traditional and native foods are the most frequent cause of FBB and have caused many outbreaks; for example, homemade fermented beans in China [1, 14], canned vegetables in USA [1, 19], fish and fish eggs in Canada and Alaska [17, 20], cream cheese in Italy [21] and sea foods (smoked salted fish, fermented fish, spawn) and locally-made dairy products (cheese, whey) in Iran [1, 9, 14, 22, 23].

In a crude estimation, about 1,000 cases of FBB are reported annually around the world [24]. If FBB is not detected early, it can be fatal and also lead to large outbreaks [6]. Therefore, early detection and timely management can save patients' lives [18]. In sporadic cases and even in small outbreaks, diagnosis is frequently missed, partly due to rarity of the disease with which most clinicians are unfamiliar [1, 10, 11, 18].

Routine laboratory tests are not useful for diagnosis of botulism but, clinical presentation is highly distinctive [18]. Confirmation rests on demonstration of the toxin in related specimens (serum, vomitus or gastric secretions, stool and suspected foods) or by isolation of the causative organism from suspected food [19]. However, laboratory tests require approximately 2-4 days for final results [10, 11]. Accordingly, all clinical decisions for management and initial public health interventions are solely determined on the basis of clinical diagnosis [10].

Mainstays of therapy are meticulous intensive cares with mechanical ventilation, if needed, and administration of antitoxin [2, 7, 10,

11]. Timely antitoxin administration may arrest the progression of paralysis and decrease the duration of illness [10, 11].

Considering the relative rarity of FBB, investigations may provide useful information about implicated foods and conditions resulting in toxin formation in each region [17]. So, every attempt to increase our understanding concerning the epidemiology of botulism is important for future development of preventive strategies against the disease [7].

The main aim of this study was to investigate the epidemiology of botulism in West Azerbaijan Province of Iran.

MATERIALS AND METHODS

This study was performed in West Azerbaijan Province, north-west Iran. According to last national census (2012), the province has 3,080,576 inhabitants (about 4.1% of country population). The province has 17 cities and Urmia is its center. Health department in every city administers all health services and is responsible for city health problems. Physicians are required by law to report all suspected cases of botulism to the health department. Then, experts start investigation about the suspected case and complete a standardized checklist, which included patient demographics, illness history, and clinical characteristics for each patient. Appropriate samples are collected and sent to Pasteur Institute, Tehran, Iran for toxin detection using mouse bioassay and culture for *C. botulinum*. In the province level, all suspected botulism cases are reported to Health Deputy of Urmia Medical Sciences University (HD-UMSU) by health departments.

This study was approved by the Ethics Committee on Research of Urmia Medical Sciences University.

This retrospective descriptive study was performed on the submitted data regarding suspected and confirmed botulism cases to HD-UMSU in a five-year period from January 1, 2010 until December 31, 2014. Variables concerning number of cases, age and gender of patients, living place (urban, rural), implicated foods (traditional dairy product, canned food, fish, meat, homemade vegetable pickle, etc.), clinical presentations, time interval between consumed food and onset of symptoms (incubation period),

length of hospital stay and patients' outcome were studied.

The diagnosis of botulism was verified by epidemiological data, a clinical score of severity and laboratory confirmation. The previously proposed clinical symptoms and signs were used to establish a clinical score of severity which permitted classification of the disease into three forms: mild, moderate, and severe. These signs and symptoms were: 1) problems of accommodation (visual impairment); 2) dryness of the mouth (dry syndrome); 3) retarded intestinal transit; 4) dysphagia with liquid and/or solid meals; 5) persistent constipation; 6) urinary dysfunction; 7) asthenia, 8) respiratory paralysis; and 9) peripheral paralysis and impossibility of oral feeding. Each symptom or sign was scored for 1 point. The presence of presentations 1-7 led to classification as mild (score < 3) or moderate (score 4-7) forms; and the presence of score 8-9 or any including the last two signs defined severe disease.

A patient was considered to have botulism if medical records indicated that this was the final diagnosis. An event was defined as the occurrence of a sporadic case or an outbreak of botulism. An outbreak was defined as two or more cases of botulism caused by consuming a common source contaminated food. A food was considered as the source of an outbreak if it tested positive for botulinum toxin and was eaten by a case-patient, or if an epidemiologic investigation linked a food to a botulism event, if the food was not tested.

Statistical analysis was performed using a Microsoft Excel spreadsheet. Descriptive statistics were presented as mean \pm standard deviation (SD) and percentage, where appropriate, to summarize the demographic characteristics, clinical features and outcomes of the cases.

RESULTS

This study comprised 57 patients including 19 laboratory confirmed and 38 suspicious cases of botulism during the five-year period. The study population included 36 (63.16%) males and 21 (36.84%) females with a mean age of 22.35 ± 17.84 yr. According to the area of residence, 32 patients (56.1%) lived in urban and 25 patients (43.9%) in rural areas of the province.

All botulism events were suspected or confirmed to be foodborne and 52 events were identified including 49 sporadic cases and 3

outbreaks including 2, 3 and 3 members of three different families, respectively. The median number of cases per event was 1 (range 1-3) and the median number of events per year was 9.8. The median annual incidence rate of FBB in West Azerbaijan Province was calculated as 0.37 cases per 100,000 population.

Mouse bioassay using toxin types A, B and E had been reported positive for the specimens of 19 patients (33.3%) including 14 foods, 3 stools, 1 food and stool, and 1 serum samples. The identified toxin was serotype A (BoNT/A) in all samples. Implicated foods in these cases were locally-made cheese (n=10, 17.5%); homemade canned fruits (n=3, 5.3%); homemade vegetable pickle (n=3, 5.3%); canned tuna fish (n=1, 1.8%), canned black olives (n=1, 1.8%) and canned beans (n=1, 1.8%). In other cases (n=38, 66.7%) the causative foods were not distinguished.

Average incubation period for all cases was 24.35 ± 12.35 hours. Summary of clinical findings of the patients are shown in Table 1.

Table 1. Clinical findings of 57 cases of foodborne botulism in West Azerbaijan Province of Iran from 2010-2014.

Symptoms or Signs	No	%
Ocular	51	89.5
Blurred vision	27	47.4
Diplopia	18	31.6
Ptosis	21	36.8
Bucco-Pharyngeal	48	84.2
Dry mouth	44	77.2
Dysphagia	26	45.6
Dysphonia	14	24.6
Gastrointestinal	43	75.4
Nausea \pm Vomiting	26	45.6
Constipation	30	52.6
Miscellaneous	47	82.5
Urinary retention	3	5.3
Respiratory problem	7	12.3
Asthenia/Weakness	44	77.2

According to the clinical criteria, 7 patients (12.3%) were classified as suffering from severe disease (score 8-9), 11 (19.3%) from moderate illness (score 4-7) and 39 (68.4%) from a mild form (score ≤ 3). All cases were hospitalized and 8 patients (14%) had to be admitted to the intensive care unit and required ventilator assistance. All patients were treated with trivalent antitoxin containing antitoxins to serotypes A, B and E and 54 (94.7%) recovered without sequelae. The mean

hospital stay duration was 7.71 ± 3.94 days. Three patients deceased and in-hospital fatality rate was 5.3%.

DISCUSSION

Botulism is one of the most frightening diseases to afflict humankind. The occurrence of FBB has been dramatically declined in industrialized countries in recent decades while it is still a health problem in some developing countries. Our findings showed that FBB is still an important health problem in the West Azerbaijan Province of Iran. A previous national study performed between 2003 and 2007, reported only 4 cases from the province [25]. This increasing number of identified FBB may be related to improvement of surveillance system in recent years and/or mandatory reporting of clinically suspected cases of FBB to health authorities.

Annual incidence rate of FBB is stated as number of cases per 100,000 population. However, few countries report the incidence. Of those that do, Georgia has the highest nationally reported rate in the world (0.9 per 100,000 population during 1991 to 2002) [6]; other countries include Russia (0.3 per 100,000 population in 1998) [7]; Canada (0.03 per 100,000 population during 1985-2005) [17]; USA (0.01 per 100,000 population during 1990-2000) [2]; Romania (0.05 per 100,000 population during 1999-2007) [7]; and European Union (generally <0.1 per 100,000 population) [7]. Given the 57 patients during five years in current study and the population of our province, an annual incidence rate of 0.37 cases per 100,000 population was calculated for the West Azerbaijan Province of Iran, indicating the continuing need for uninterrupted surveillance and public health interventions.

In our study, most affected patients were males with M:F ratio of 1.7:1. Some other studies from Iran [18], Poland [17], France [19], and Romania [7], also reported men to be affected more than women. This higher rate of FBB in men may have roots in their different behaviors; men are more willing to eat canned foods and are less likely to heat foods. However, no significant gender differences were reported in other studies [6, 11, 17, 25].

Botulism tends to occur as sporadic cases or small outbreaks that affect a few people [10, 14]. In 15 different outbreaks reported from Iran

between 2004 and 2010, only two to five persons were involved in each outbreak [1]. Majority of our cases were also sporadic and there were only three outbreaks involving 2 or 3 persons.

The mean age of our studied patients was 22.35 ± 17.84 yr. In other studies from Iran, the reported mean ages were between 30 and 50 yr [11, 14, 26]. However, the range is wide and botulism may affects any age groups from neonatal to elderly.

Some authors reported slightly higher incidence of FBB in rural areas [5, 7, 19, 27, 28]. In our study, 56.1% of patients were residents of urban areas, but, this may still consistent with the results of other studies because according to the last national census, more than 62.7% of our province's inhabitants live in urban areas.

The mean incubation period in this study was about one day (range, 12 hours to 4 days). Generally, the incubation period of FBB varied from 1 to 3 days (range, 6 hours to 8 days) [18, 29]. In our study, the observed clinical presentations (Table 1) were consistent with previous reports [10, 11, 14, 18, 23, 30]. The initial period was short and characterized by gastrointestinal symptoms followed by bilateral and symmetrical paralytic ocular manifestations (blurred vision, diplopia and ptosis) associated with dry mouth and oropharyngeal symptoms (dysphagia and dysphonia) [10].

Most cases of FBB in our study were related to ingestion of traditional dairy products, a major factor in production of the BoNT/A. In a previous study, 341 cases of FBB were reported from Iran during 2003-2007; of which 31% were related to consumption of sea foods (salted fish, smoked fish, spawn), especially in the northern seaside provinces of Iran (Gilan, Mazandaran and Golestan); and 9.7% were due to traditional dairy products (non-pasteurized locally made cheese or whey) in other mountainous provinces such as the West Azarbaijan [23, 25].

In spite of introducing some new laboratory methods [5], mouse bioassay has still been the standard reference method for detection of the toxin in biological specimens and suspected food samples. In Iran, toxin detection using mouse bioassay is only performed in the Pasteur Institute, Tehran. A delay for more than two days between ingestion of the toxin and sample collection, decreases the chances for toxin detection as low as 30% and only 36% of stool cultures will be

positive after 3 days [4]. For these reasons, positive results for toxin detection were only 33.3% in our study. More cases of botulism will be detected or confirmed if mouse bioassay is available in other regions of the country.

All patients of this study received antitoxin. Three types of botulinum antitoxin are exist in Iran as: 1) monovalent type A, type B, type E; 2) divalent type A+B; and 3) trivalent type A+B+E [31]. Since the decision for treatment is based on clinical diagnosis and epidemiologic information, without laboratory confirmation, the trivalent type antitoxins have been administered for all suspected patients of this study after collection of required samples. The antitoxin neutralizes toxin molecules that are not yet bound to nerve endings and may arrest the progression of paralysis and decrease the duration of illness [2, 18].

With adequate supportive cares and antitoxin therapy, the mortality rate of botulism is about 3%-6% in developed countries such as United States and Canada [4, 10, 17]. In Iran, the national guideline for botulism surveillance estimated the mortality rate about 7%-10% (2007) [32]. The mortality rate of FBB in our study was 5.3%.

Our study had some limitations as follows. Because of the retrospective nature of the survey, we only reviewed the previously collected data. All botulism cases in this study were foodborne botulism. However, we think other types of the disease, such as infant botulism, wound botulism or even adult intestinal botulism might also occur in our province, but, due to lack of availability of screening or confirmatory laboratory methods in the region, many of these cases remain undetected and/or unconfirmed.

CONCLUSION

Foodborne botulism, if not detected, may cause large outbreaks with high morbidity and mortality. In northwest of Iran unpasteurized dairy products such as cheese, whey or buttermilk (dough) are important causative foods and toxin type A producing clostridium species are more prevalent. Diagnosis of FBB in sporadic cases and even in small outbreaks is frequently missed. Clinicians should suspect botulism if a patient simultaneously reveals acute onset of cranial nerves involvements and gastrointestinal problems. However, negative laboratory results

cannot exclude the diagnosis. If suspected, botulinum antitoxin should be given immediately, particularly in the first 24 h of onset of symptoms. Serum samples should be obtained immediately and always before administration of antitoxin. Prophylaxis is very important through increasing public awareness about proper cooking and canning of home-made foods.

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