International Journal of Infectious Diseases 24 (2014) 20–22

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Case Report

Food-borne botulism in Japan in March 2012

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ARTICLE INFO

Article history:
Received 3 October 2013
Received in revised form 17 January 2014
Accepted 17 January 2014

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Clostridium botulinum
Food-borne botulism
Japan

SUMMARY

In March 2012, two patients were transported urgently to the hospital in Tottori Prefecture, Japan, because of symptoms suggestive of botulism. Botulinum neurotoxin type A was detected in the clinical specimens and the food consumed by the two patients (vacuum packed adzuki-batto, a sweet adzuki bean soup containing noodles). We were able to make a prompt diagnosis of food botulism associated with the consumption of adzuki-batto, from which the causative pathogen *Clostridium botulinum* Ab was cultured.

1. Introduction

Botulism is recognized as one of the most serious infectious hazards. Foodborne botulism, a notifiable disease in Japan, the outbreak is recently very rare for several decades. Here we report a foodborne botulism case occurred in Tottori prefecture, Japan in March, 2012.

2. Case presentations

Early in the morning of March 24, 2012, two married patients, a 69-year-old male (patient 1) and a 69-year-old female (patient 2), were admitted to the hospital in Yonago City, Tottori Prefecture, in Japan. Both patients were able to communicate upon arrival. However, at initial medical examination, the patients exhibited bilateral, symmetrical flaccid weakness affecting the facial and ocular muscles, followed by dysarthria progressing to diaphragmatic paralysis, which led to respiratory arrest (Figure 1). The patients were placed on respiratory support and developed autonomic features, such as paralytic ileus and labile blood pressure. A blood test showed no abnormal signs (normal leukocyte and erythrocyte counts and normal liver function tests; Table 1) and a computed tomography (CT) scan also showed no abnormalities. The patients were subsequently hospitalized for over 1 year.

3. Laboratory investigations

3.1. Clinical samples

Fecal and serum samples were taken at admission and transported to our laboratories. These were immediately cultured anaerobically for the isolation of *Clostridium botulinum*. The samples were then homogenized in 0.2% gelatin/phosphate buffered saline (PBS) and filtrated with a 0.45-μm syringe filter. After incubation with trypsin (0.2 mg/ml) for 1 h at 37 °C, a 0.5-ml portion of the filtrate and serial dilutions of the filtrate were injected intraperitoneally (IP) into BALB/c mice (15–20 g body weight) to determine the presence of botulinum neurotoxins (BoNTs), as described by Kondo et al.[1] and in accordance with the Manual for Laboratory Diagnostics of Pathogens: Botulism (http://www.nih.go.jp/niid/images/lab-manual/botulism121207.pdf, in Japanese). An antibody neutralization assay was conducted simultaneously to determine the toxin type using equal amounts

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http://dx.doi.org/10.1016/j.ijid.2014.01.014
1201-9712

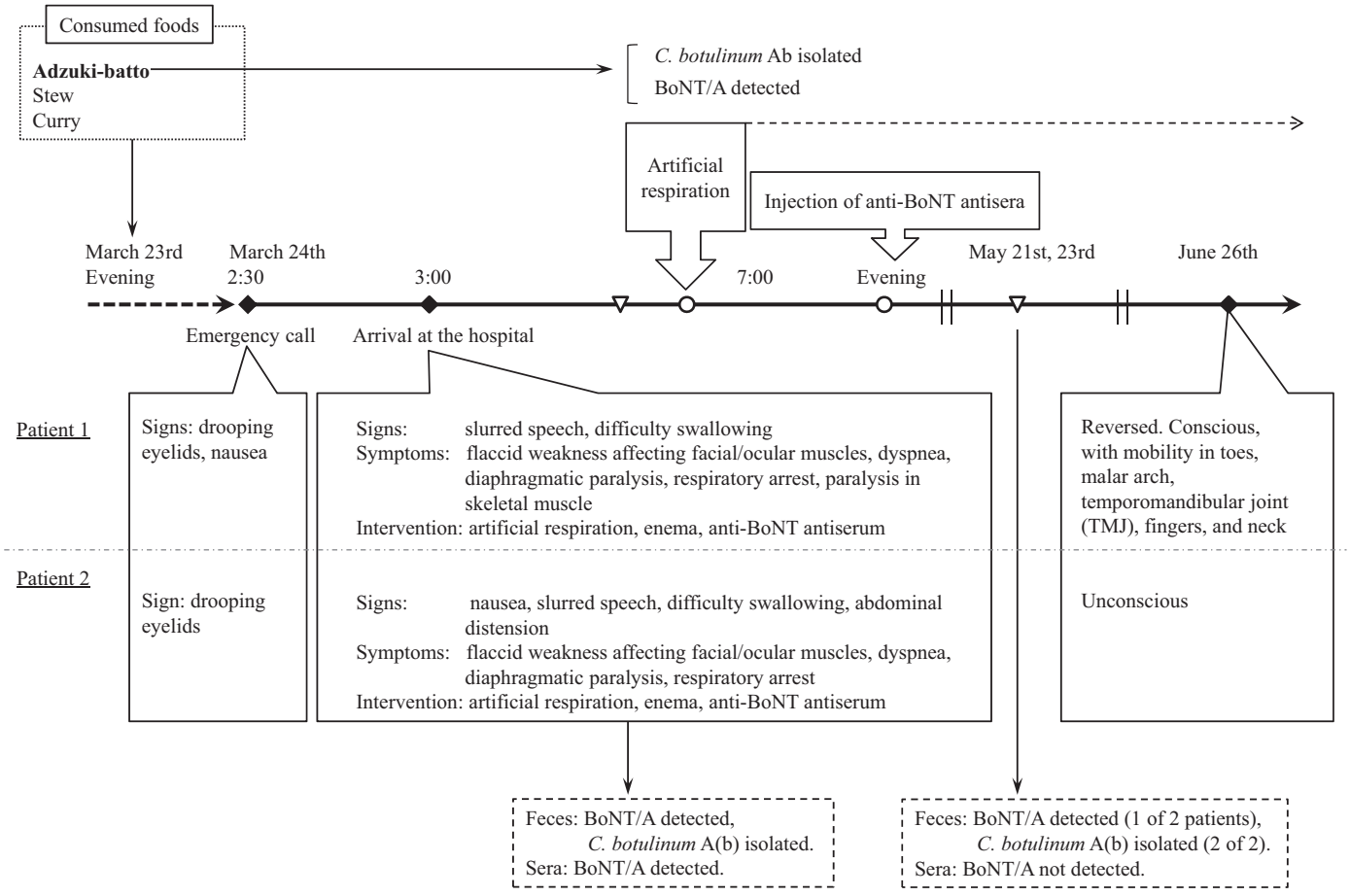

Figure 1. Time course illustrating the conditions of the patients, clinical interventions, and specimen collection. Closed squares, time points at which the conditions of the patients were noted; open circles, time points of clinical intervention; open triangles, time points of fecal and serum sample collection.

of anti-BoNT/A, B, C, D, E, and F antiserum (1%3Fndash;10 IU/ml, produced in-house), as described previously.[1] The animal experiments were performed under the guidelines for animal care and use of our institutes. Finally, BoNT/A was detected in both samples and BoNT/A-producing *C. botulinum* was isolated from fecal samples. The two patients were administered multivalent sera against BoNT/A, B, and F at the hospital. Consequently, no toxin activity was observed in the serum samples of either patient thereafter. Nonetheless, 2 months later (May 2012), *C. botulinum* was still present in the fecal samples of both patients and BoNT/A was detected in a fecal sample from one of the two patients (patient 2).

Table 1
Blood test results of the patients at initial medical examination

Test item	Patient		Unit
	1	2	
Blood biochemistry			
Total protein	7.6	7.2	g/dl
Aspartate aminotransferase	15	16	IU/l
Alanine aminotransferase	10	20	IU/l
Lactate dehydrogenase	295	153	IU/l
Na	142	145	mEq/l
K	4.5	4.2	mEq/l
Cl	104	106	mEq/l
Blood urea nitrogen	12	8	mg/dl
Creatinine	0.76	0.58	mg/dl
C-reactive protein	0.06	0.17	mg/dl
Complete blood counts			
White blood cell count	17.7	9.1	$\times 10^9$/l
Red blood cell count	4.56	4.96	$\times 10^{12}$/l
Hemoglobin	14	15.1	g/dl
Hematocrit	41.9	44.6	%
Platelets	344	207	$\times 10^9$/l

3.2. Food samples

Suspected causative foods were examined simultaneously for the detection of BoNTs and *C. botulinum*, including adzuki-batto, stew, and curry, which were the leftovers at the patients' home. No pathogenic bacteria were isolated from the stew or curry. However, both BoNT/A neurotoxin and the organism producing it, *C. botulinum*, were detected in the adzuki-batto, a sweet adzuki bean soup containing flat wheat noodles made in Miyako, Iwate Prefecture in Japan. Medical interview revealed that the two patients had eaten this dish for lunch on March 23, 2013. The toxin titer in the adzuki-batto was estimated to be 75 000 IP LD_{50}/g of the food sample using mouse assays.[1]

3.3. Characterization of the isolates

The in vivo antibody neutralization assays using anti-BoNT/A, B, E, and F antiserum revealed that the organisms isolated from both the patients and the food produced only BoNT/A, whereas PCR assays for the detection and characterization of BoNT genes (BoNT/A–G) using multiplex primer sets[2] in combination with the primer sets from Takara Bio, Shiga, Japan (Code No. S021-S027), showed that they were positive for both BoNT/A and BoNT/B genes. Correspondingly, the bacterial isolates were also positive for the

ha33 and *p47* genes, which are representative markers for the BoNT/A and BoNT/B gene clusters, respectively.[3] These observations confirmed that the cases of infection were associated with the intake of adzuki-batto contaminated with *C. botulinum* Ab.

4. Discussion

Since the first case of food-borne botulism was reported in 1951 in relation to the consumption of 'Izushi', a fish fermented in rice served with malted rice and vegetables, several food-borne cases have been reported in Japan.[4] The main cause of the pathogenicity of *C. botulinum* infection is BoNT, which is largely classified into types A to G.[5] While type E botulism was dominant until the 1980s, the numbers of cases of type A and type B botulism have increased in recent years.[6] Our rapid response to this emergency case thus provided data supporting clinical treatment with the epidemiological conclusion.

These cases showed an unusual clinical aspect: *C. botulinum* and BoNT/A were continuously detected in the feces of the patients. One possible explanation is that the patients consumed a large amount of the causative food, since neither patient had an underlying immunosuppression or gut abnormalities; also, high titers of BoNT/A (75 000 IP LD_{50}/g) were detected in the causative food. It is considered that the BoNT-dependent paralytic ileus further prolonged the accumulation of *C. botulinum* and its toxin.

Vacuum packing provides an atmosphere that allows the growth of anaerobes but not *Enterobacteriaceae*,[7] which poses further potential risks for infection with *Clostridium* bacteria through the consumption of these food types. We concluded that these cases occurred in association with the consumption of vacuum-packed adzuki-batto accidentally contaminated with *C. botulinum* Ab, which overgrew and produced the neurotoxin.

Acknowledgements

This work was supported financially in part by grants from the Ministry of Health, Labour and Welfare, Japan (H25-shokuhin-ippan-010, Shokkenpi-H24).

Conflict of interest: No conflict of interest to declare.

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