CLINICAL PRACTICE ARTICLE

REVISED Suspected botulism outbreak after the consumption of vegetarian pâté in the south of Viet Nam [version 4; peer review: 3 approved with reservations]

Le Quoc Hung¹, Vo Ngoc Anh Tho^{id1}, Do Thi Ngoc Khanh¹, Vo Thi Thanh Hien¹, Jeremy N. Day^{id2,3}, Nguyen Ngoc Sang¹, Hua Thoai Tam¹, Ho Thi Chi Thanh¹, Nguyen Thi Thuy Ngan^{id1,2}

¹Department of tropical diseases, Cho Ray hospital, Ho Chi Minh city, Ward 5, 70000, Vietnam

²CNS/HIV group, Oxford University Clinical Research Unit, Ho Chi Minh city, Ward 5, 008428, Vietnam

³Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine,, University of Oxford, Oxford, Oxford, UK

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Abstract

Botulism and other botulinum neurotoxins-producing clostridia are potentially life-threatening diseases caused by toxins produced by *Clostridium botulinum*. Here we reported a case series of six patients who presented with botulism following ingestion of commercially made pâté. The key features of presentation were acute onset of bilateral cranial nerve palsies and symmetrical descending weakness in the absence of fever resulting in the need for mechanical ventilation in all six patients. The clinical diagnosis of botulism was confirmed through the identification of *C. botulinum* from the suspected food source. Given that botulinum antitoxin was not available in Vietnam at the time, and their severe status, all patients received a trial of plasma exchange therapy, but no clear benefit was seen.

Due to its rarity, diagnosing botulism is a challenge, demanding high clinical suspicion. Successful outcomes depend upon early recognition and rapid initiation of specific treatment with botulinum antitoxin. There is a need to improve global access to antitoxin. These cases, the first in Viet Nam, serve as a reminder of the need to maintain the highest possible food hygiene and preservation practices.

Keywords

Botulism, botulinum toxin, Clostridium botulinum, pâté

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1. Michel Popoff^{id}, Institut Pasteur, Paris, France
Veterinary Academy of France, Paris, France
2. John W Austin^{id}, Bureau of Microbial Hazards, Food Directorate, Health Products and Food Branch, Ottawa, Canada
3. Fabrizio Anniballi^{id}, Higher Institute of

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Health, Rome, Italy

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Corresponding author: Nguyen Thi Thuy Ngan (nganntt@oucru.org)

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REVISED Amendments from Version 3

I changed the title of the paper as reviewer's suggestion to accurately reflect the content of the paper. Additionally, I provided more information on the microbiology as reviewers' requests. I also included a row of respiratory failure condition in Table 1.

Any further responses from the reviewers can be found at the end of the article

Introduction

Botulism and other botulinum neurotoxins (BoNTs)-producing clostridia are neurotoxin-mediated illnesses caused by the gram positive, anaerobic, spore-forming bacillus *Clostridium botulinum* (*C. botulinum*), which occurs naturally in soil and sediments. Foodborne botulism follows the ingestion of food contaminated with one of a number of the described toxin subtypes, and is the commonest form of human botulism^{1,2}. Typically, foodborne botulism occurs following the consumption of meat products, domestically canned, low acid-containing foods³. Examples of such home-canned foods include vegetables, seafood (fermented fish and smoked fish), and dairy products⁴. However, botulism outbreaks have also been described where the source has been commercial food products⁵. Less frequently, botulism can arise following injuries which result in wound inoculation or contamination with *C. botulinum*. Here, unlike in food-borne botulism, toxin production occurs within the human host. Rarely, infant botulism occurs due to colonization of the gut by *C. botulinum*, again resulting in endogenous toxin production.

The botulinum toxin exerts its effects within neurons by inhibiting the fusion of acetyl choline containing pre-synaptic vesicles with the cell membrane, thus preventing the release of the neurotransmitter⁶. This presents clinically as a flaccid paralysis, and the classical manifestation of botulism is described as an acute onset of bilateral cranial neuropathies with a symmetrical descending paralysis. Fever is not a feature. Botulism diagnosis is mainly a clinical diagnosis, made based upon a typical presentation and a contact history. Definitive diagnosis depends upon the detection of botulinum toxin, or isolation of *C. botulinum*, from both clinical and source (food) samples. The laboratory confirmation is essential to exclude all diseases included in the differential diagnosis for the definitive diagnosis. Foodborne botulism has been documented in Europe since the eighteenth century⁷. However, in Viet Nam, botulism has not previously been described. This may be genuine, perhaps due to food practices, or represent under-reporting – the diagnosis may be missed due to low index of suspicion or overlap of symptoms with other neurological syndromes. Importantly, the specific treatment for botulism – antitoxin – is not always available in Viet Nam. Delay in diagnosis and lack of specific treatment are likely to result in worse clinical outcomes. Here, we report six patients who presented to our hospital with symptoms suggestive of botulism following consumption of a commercially produced vegetarian pâté. To our knowledge, these are the first cases of botulism reported from

Viet Nam, and also the first outbreak associated with vegetarian pâté.

Case presentation

On July 24, 2020, case 1, a Taiwanese 36-year-old male, working as a hotel staff presented to our Department of Tropical Diseases, Cho Ray hospital, Ho Chi Minh City. He had been referred from his local hospital in Khanh Hoa province, south central coast of Viet Nam where he had been admitted 4 days previously with a one-day history of nausea and vomiting. There was no history of fever. Over the ensuing four days at Khanh Hoa he developed progressive dizziness, blurred vision, dysphagia and bilateral ptosis. On arrival at Cho Ray hospital, he was found to have dysarthria and complained of mild breathlessness. There was no recent travel. He reported consumption of seafood one day prior to the onset of the original symptoms (mussels, clams and obtuse horn shells). On physical examination, he was alert, breathing spontaneously, and had bilateral ptosis, worse on the right side. His pupils appeared normal. Limb power was normal. Due to his history of seafood ingestion, the initial differential diagnosis was saxitoxin, brevetoxin or tetrodotoxin poisoning, with consideration also given to Guillain-Barré syndrome.

The second case was the wife of case 1. She was a Vietnamese 36-year-old female factory worker in the second trimester of pregnancy and had accompanied her husband to our hospital. She had consumed the same seafoods at the same time as her husband, and had had similar gastrointestinal symptoms one day later. She had not previously received a hospital assessment. However, as her husband was being assessed she mentioned she now had blurred vision and dysphagia. On examination she was found to have bilateral ptosis and was admitted with her husband.

Laboratory evaluations for both patients, including complete blood cell counts, and serum level of sodium, potassium, blood glucose, blood urea nitrogen and creatinine were normal. Cranial computed tomography and magnetic resonance imaging were normal. Both patients underwent lumbar punctures; cerebrospinal fluid analyses were unremarkable. Both patients underwent electromyography which showed low-voltage compound motor-units, consistent with axonal neuropathy. The possibility of Guillain-Barré syndrome was suggested in the differential diagnosis, however, it is not plausible in outbreaks. Therefore, Guillain-Barré syndrome was excluded from the diagnosis. Three days following hospital admission the condition of both cases had deteriorated, with descending quadriparesis, and worsening respiratory function. Foodborne botulism was suspected and the dietary history re-explored from their relatives. This revealed that the couple had eaten the same brand of jarred vegetarian mushroom pâté produced in Viet Nam approximately 20 to 36 hours before the first symptoms occurred.

Cases 3, 4 and 5 were three Vietnamese women aged 20, 24 and 26, respectively, again referred to our department from their local hospitals at the end of July 2020. The three were friends and worked as office staff for a company in Dong Nai province. The cases had no social link to cases 1 and 2 and lived

approximately 400 kilometers from them. They gave a history of gastrointestinal symptoms (nausea, vomiting and abdominal pain) followed by the development over the next 2 days of neurological deficits including dysarthria, bilateral ptosis, difficulty in breathing and limb weakness (strength 2-3/5 Medical Research Council grade). One patient had reported a mild fever on the first day of illness. All 3 patients underwent lumbar puncture, routine hematological and biochemical investigations, and brain computed tomography scan in our hospital, and all these were unremarkable. Because of the similarity of their presentations with cases 1 and 2, a detailed history of food consumption was taken. They had eaten the same brand of vegetarian pâté as the previous couple between 24 and 48 hours before the appearance of their symptoms. Clinical specimens obtained from all 5 patients (serum and stool), and samples of suspected food (the remainder of the canned pâté at their house), were sent to the Institute of Hygiene and Public Health, Ho Chi Minh city. The presence of *Clostridium botulinum* in the food samples of all cases was confirmed by bacterial culture method, mouse bioassay method of Association of Official Analytical Chemists (AOAC) 977.26¹ and the diagnosis of botulism was established. The mouse bioassay is a functional assay that detects biologically active toxin. The assay requires a three - part approach including detection of viable *C. botulinum*, selection of typical *C. botulinum* colonies, and finally detection and identification of botulin toxin. However, at the last step of this process, we could not identify the typing of *C. botulinum* toxin due to a lack of monovalent antitoxin for toxin neutralization. *C. botulinum* was not isolated from patient specimens.

The last case was a 54-year-old Vietnamese salesman who presented to our department in August approximately 3 weeks

after the first 5 cases. He gave a history of dizziness, nausea, vomiting and abdominal pain which occurred 24 hours after eating the same brand of vegetarian pâté. Over the next 24 hours, he developed double and blurred vision, bilateral ptosis, dysarthria, dysphagia and a descending paralysis. Laboratory investigations including complete blood count, urea and electrolytes were normal. Cerebrospinal fluid examination was normal. Now with a high index of suspicion, the diagnosis of botulism was made promptly. As with the previous 5 cases we were unable to isolate *C. botulinum* from blood and stool. The serological testing of BoNTs is not available at Cho Ray hospital, therefore all patients were not performed with this test. The clinical and laboratory findings of all cases are summarized in [Table 1](#) and [Table 2](#).

The vegetarian pâté consumed by all cases was made by a small-scale family-run business using locally-sourced ingredients and handmade methods. Pâté bought by the first 5 cases and the sixth case were prepared separately in July 2020 and August 2020 respectively. All consumption of pate occurred within the use-by dates (December 2020 and January 2021 respectively). The manufacturer recommended the product be stored frozen and the expiration date was 6 months following production. All cases told us they kept once-opened pâté at room or fridge temperature (5–10 °C). The exact source of contamination during the production process is not clear.

Treatment and progress

All six cases required intubation and mechanical ventilation due to weakness of respiratory musculature. The median time to intubation and mechanical ventilation following consumption of the pâté was 6.5 days (range 4 to 9 days). Botulinum antitoxin was not available in Vietnam at the time. Because of the severe

Table 1. The summary of clinical findings of six cases.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age, gender	36, male	36, female	20, female	24, female	26, female	54, male
Time interval from ingestion to the symptom onset	21 hours	36 hours	24 hours	48 hours	48 hours	24 hours
Nausea, vomiting	yes	yes	yes	yes	yes	yes
Abdominal pain	no	no	yes	yes	yes	yes
Blurred, double vision	yes	yes	no	no	no	yes
Dysarthria	yes	yes	yes	yes	yes	yes
Dysphagia	yes	yes	yes	yes	yes	yes
Ptosis	yes	yes	yes	yes	yes	yes
Dyspnea	yes	yes	yes	yes	yes	yes
Respiratory failure	yes	yes	yes	yes	yes	yes
Limb weakness	yes	yes	yes	yes	yes	yes
Fever	no	no	no	no	no	no
Impaired consciousness	no	no	no	no	no	no

Table 2. The summary of laboratory investigations.

Lab tests (unit, normal range)	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Hemoglobin (g/dL, 12–17)	14.8	11.4	13.0	12.8	13.6	15.5
White blood cell (/mm ³ , 4000–11000)	8060	9880	2920	14000	6250	11500
Neutrophil (%)	64	83	73	86	75	82
Platelet (/mm ³ , 200000–400000)	239000	268000	167000	230000	227000	249000
Aspartate transaminase (U/L, 5–49)	22	23	19	19	47	22
Alanine transaminase (U/L, 9–48)	14	27	14	29	38	21
Blood urea nitrogen (mg/dl, 7–20)	23	7	8	13	12	28
Creatinine (mg/dl, 0.7–1.5)	2	0.37	0.58	0.6	0.68	0.82
Lactate dehydrogenase (U/L, 200–400)	168	143	235	197	161	432
Creatin phosphokinase (U/L, 34–171)	205	Not done	37	Not done	Not done	81
Urine myoglobin (ng/ml, <5)	>1000	8.37	8.87	8.9	19.8	7.7
CSF ¹ Cell count (/mm ³ , <5)	0	1	1	1	1	1
CSF ¹ Protein (mg/dl, 15–45)	38	6.5	15	21	37.5	29.5
CSF ¹ /blood glucose (mg/dl, >0.5)	57 / 116	58 / 98	89 / 130	90 / 152	67 / 112	74/117
PCR CMV and EBV ²	Negative	Negative	Negative	Negative	Negative	Not done
Electromyography	The motor axonal neuropathy;	The motor axonal neuropathy; and test for myasthenia gravis negative	The motor axonal neuropathy	The motor axonal neuropathy; and test for myasthenia gravis negative	The motor axonal neuropathy	The motor axonal neuropathy; and test for myasthenia gravis negative
Electroencephalography	Normal	Normal	Normal	Not done	Not done	Not done
Cranial MRI ³ and CT ⁴ scan	Normal	Normal	Normal	Normal	Not done	Normal
Food samplings	Isolation of <i>Clostridium botulinum</i>					

Note: ¹: Cerebrospinal fluid, ²: Polymerase chain reaction of Cytomegalovirus and Epstein-Barr virus, ³: Magnetic resonance imaging, ⁴: Computed tomography.

deteriorating status of the patients, and the lack of antitoxin, we administered therapeutic plasma exchange (TPE) in addition to standard supportive therapy. TPE was administered on alternate days on 3 occasions during the third week of hospitalization for each of the first 5 cases, and during the first week of hospitalization for the sixth case. Amongst the first 5 patients, immediately following TPE four showed recovery of ptosis and some improved limb strength sufficient to warrant attempts at weaning from ventilation. However, there did not appear to be any long-lasting/permanent benefit of TPE in any patient. We could not detect any benefit of TPE in the 6th patient.

All patients underwent tracheotomies after 14 days of intubation. The first five cases remained in our hospital for 4 to 5 weeks to referral back to their local hospitals for on-going intensive care unit care. All patients required on-going invasive mechanical ventilation when discharged. The sixth patient remains in intensive care in our department. Table 3 details the conditions of all the cases at the time of this report.

In addition to these six cases, seven further cases of botulism were identified during this time. These patients had similar

clinical manifestations to the six reported here, and also had eaten the same brand of vegetarian pâté as our patients. In two of these seven cases, *Clostridium botulinum* was detected in both clinical samples (stool) and pâté. None of these additional cases received botulinum antitoxin until the beginning of September when this was kindly provided by the World Health Organization. The antitoxin was administered after at least 4 weeks of illness for 10 of 13 patients (by this time, cases 2, 4 and 5 described above had been successfully weaned from mechanical ventilation; hence, they did not receive antitoxin). The recovery of the remaining 13 patients is ongoing.

Discussion

This is the first case report of an outbreak of botulism in Viet Nam. It demonstrates the need for a high index of suspicion in order to make the diagnosis in a timely manner, the severe associated morbidity, and the need to have rapid access to antitoxin.

Botulism is caused by *C. botulinum* through the action of BoNTs. BoNTs are divided into several toxinotypes (A, B, C, D, E, F, G, H, and X) and each toxinotype is further

Table 3. Assessment of the recovery of six cases at discharged.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Time interval from onset to assessment (days)	33	33	30	28	27	14
Blurred vision	no	no	no	no	no	yes
Dysarthria, dysphagia	Assessment was not obtained					
Ptosis	no	yes	yes	mild	mild	yes
Spontaneously breathing	yes	yes	no	yes	yes	no
Weaning from mechanical ventilation	Periodically	Periodically	Totally depending on mechanical ventilation	Periodically	Periodically	Totally depending on mechanical ventilation
Limb weakness	5/5	5/5	2/5	3-4/5	4-5/5	2-3/5
Days of hospitalization	34	34	31	29	27	Has not been discharged yet
Days of ventilation	31	31	31	31	26	Has not been discharged yet
Ventilation at discharged	yes	yes	yes	yes	yes	Has not been discharged yet
General assessment by attending doctors	Moderate recovery	Moderate recovery	Re-paralysis after 2 days of improvement	Mild recovery	Mild recovery	Not changed

divided into subtypes. Until now, 41 such subtypes have been described⁸. The sophisticated understanding of the complexity of the toxin subtypes is at odds with our ability to diagnose foodborne botulism, which depends in the first instance upon clinical suspicion based upon the history and clinical signs. There are no rapid tests available to aid diagnosis at the time of presentation. Confirmation of diagnosis comes through epidemiological investigation to identify potential exposure, with microbiological confirmation of the presence of the organism or toxins in the source patient samples. As seen in our cases, identifying the organism in human clinical samples has low sensitivity. Epidemiological confirmation of the diagnosis takes considerable time; given that a potentially effective antitoxin is available, the development of more sensitive and more rapid diagnostics would be welcomed, particularly in settings such as the tropics where other foodborne neurotoxins are prevalent.

The incubation period of botulism can range from several hours to a week⁹. The presentation of our cases was typical and consistent with previous studies, but we saw some variability in the time to development of life-threatening neurological compromise. The first symptoms involved the digestive system and included nausea, vomiting and abdominal pain. These usually appear within 12 to 36 hours of ingestion of the food source⁹. However, both the gastrointestinal and neurological symptoms may be delayed by as much as eight days after

exposure¹⁰. The variability in presentation may represent a dose effect of the botulinum toxin. Our experience offers circumstantial evidence supporting this. In our series, case 2, who had eaten smaller amounts of pate compared with her husband, had a relatively delayed presentation, with neurological signs occurring on day 5, in contrast to after 24 hours as seen in her husband. A recent report of two cases from Germany, describes a similar finding. Here, the patient who had ingested a smaller amount of contaminated food developed descending paralysis later than the other¹¹. However, while variability in the time to onset of symptoms appears to depend on how much contaminated food has been eaten, the huge potency of the toxin resulted in all patients in our series ultimately requiring intubation and mechanical ventilation¹².

The presentation of botulism can be subtle. The earliest neurological symptoms tend to involve the eyes, with blurred and double vision, and ptosis¹³. These maybe followed by dysarthria, in turn, followed by progressive weakness of limb muscles and respiratory insufficiency. Around 68% of cases of foodborne botulism present with simultaneous neurological and gastrointestinal symptoms¹³. Autonomic dysfunction can also be an important clue to botulism. Symptoms and signs may include resting tachycardia, supine hypertension, and orthostatic hypotension, explained by inhibition of the parasympathetic nervous system. Such autonomic dysfunction is thought to be particularly associated with botulism type B, the absence of

such symptoms/signs in our patients suggest an alternative toxin subtype was responsible¹⁴.

Botulism should be considered within a broad differential diagnosis, including seafood poisoning (brevetoxin, saxitoxin, ciguatera toxin), heavy metal intoxication, myasthenia gravis, tick paralysis, Guillain Barre, Lambert–Eaton syndrome, poliomyelitis/Japanese encephalitis, and stroke¹⁵. A detailed history investigating potential exposures, the health of contacts, and the disease progress, are crucial in obtaining the correct diagnosis. Lambert–Eaton and myasthenic syndrome can be excluded by electromyography and antibody studies. Guillain–Barre syndrome usually involves an ascending rather than descending paralysis, associated sensory findings, and an elevated cerebrospinal fluid protein.

Identifying the food source of botulism is crucial in confirming the diagnosis and managing the risk to public health. Improper food storage and preservation can provide specific conditions such as the anaerobic, low salt, low acid environments which facilitate the growth and development of the toxin producing *C. botulinum*. Identification of the food source should lead to an examination of food handling practices with education and remediation as needed. The vegetarian pâté consumed by our patients contained nuts (almond, walnuts, cashew), and mushroom, was produced in metal containers which were able to provide the anaerobic conditions needed for bacterium growth and toxin production.

Key to the diagnosis of botulism in our case series was the presentation of multiple patients with consistent syndromes. However, we were unable to isolate *C. botulinum* from any clinical specimen. Identifying sporadic cases affecting only single individuals remains extremely challenging, requiring a high index of suspicion; developing more rapid, sensitive, and affordable tests would enable a better understanding of the epidemiology of this disease and the more timely intervention of treatment.

While the rarity of disease means no randomized controlled trials have been performed, it is believed that administration of antitoxin can shorten hospital stay and decrease the duration of mechanical ventilation¹⁶. The benefit of antitoxin depends on neutralization of that toxin which is unbound to neuromuscular junctions, and this requires administration within the first 24 hours of presentation^{17,18}. However, it must be noted

that there is no constraint for the latest time of effective antitoxin administration (see WHO botulism factsheet), with benefit having been reported in patients treated with antitoxin up to 8 days after the onset of symptoms¹⁹.

Unfortunately antitoxin was not available in Vietnam when we received the cases reported here, and given their severe condition this led us to try TPE, which has been used to treat myasthenic-type crises following therapeutic Botox injections²⁰. The first five of our cases underwent TPE in the third week of illness and we observed clinical improvement in 4 of 5 cases. However, it is impossible to tell whether this was the normal disease course or due to the intervention, and contrasts with the sixth case who received TPE on three occasions in the first week of disease. Early intervention might be expected to be more effective but we could discern no clinical improvement in muscle strength following the treatment. Other treatments suggested for botulism have included dalfampridine or 4-aminopyridine, prescribed to control symptoms in multiple sclerosis. This drug has been used in some cases of severe botulism, and offered some signs of enhancement in peripheral muscle strength, but it needs further study²¹.

Conclusion

We report the first recognized outbreak of botulism in Vietnam. All patients were severely unwell and ultimately required mechanical ventilation. Diagnosis requires a high index of suspicion, and has to be distinguished from other intoxications that are more common in tropical climates, such as those associated with seafood. The syndrome should be considered in patients presenting with absence of fever, a normal conscious level, and an acute descending paralysis. Detailed exposure history is essential to identify sources that may be continuing to put the wider community at risk. The logistics of maintaining stocks of costly antitoxin for what are rare diseases is a challenge; cross-border cooperatives with rapid dissemination of stocks as needed may be one solution.

Data availability

Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

Consent

Written informed consent for publication of their clinical details was obtained from the patients.

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Reviewer Report 21 June 2021

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Michel Popoff

¹ Bacteries Anaerobies et Toxines, Institut Pasteur, Paris, France

² Veterinary Academy of France, Paris, France

The authors have improved the manuscript. However, the identification of *C. botulinum* from food is too imprecise. The authors mention the general methodology of *C. botulinum* identification, but without any result regarding the *C. botulinum* strain(s) which are supposed to have been isolated. If the authors are unable to provide bacteriological characteristics of the isolated strains, they can send the strains to a more specialized laboratory to perform full identification including typing by PCR and DNA sequencing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clostridium toxins

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 18 June 2021

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John W Austin

Botulism Reference Service for Canada, Microbiology Research Division, Bureau of Microbial

Hazards, Food Directorate, Health Products and Food Branch, Ottawa, ON, K1A 0K9, Canada

The changes to this article – especially the change to the title to indicate “Suspected botulism outbreak” – have improved the article. I am willing to change my opinion to “Approved with reservations”.

There is still insufficient information regarding the microbiology laboratory work, and how *Clostridium botulinum* isolated from the food was identified as *C. botulinum*.

“The mouse bioassay is a functional assay that detects biologically active toxin. The assay requires a three - part approach including detection of viable *C. botulinum*, selection of typical *C. botulinum* colonies, and finally detection and identification of botulinal toxin. However, at the last step of this process, we could not identify the typing of *C. botulinum* toxin due to a lack of monovalent antitoxin for toxin neutralization. *C. botulinum* was not isolated from patient specimens.”

It is clear that monovalent antitoxin was not available. Was polyvalent typing sera available? Were lipase and lecithinase reactions considered? How was this isolate identified as *C. botulinum*?

Also, while isolation of *C. botulinum* from food is important to note, as the other reviewers have indicated, *C. botulinum* can be found in the environment and its presence in a food does not necessarily implicate the food in an outbreak of botulism. The organism must grow and produce toxin in the food.

In Table 2, it is indicated that *C. botulinum* was isolated from the food. Even though they were negative, the results from the clinical samples should also be indicated (ie. that *C. botulinum* was not isolated from the stool of any of the patients, and botulinum toxin was not detected in the serum or stool of any of the patients).

Also, as indicated by Dr. Anniballi, *C. botulinum* cannot be isolated from blood specimens. The authors should correct the following sentence:

“As with the previous 5 cases we were unable to isolate *C. botulinum* from blood and stool.”

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 3

Reviewer Report 19 May 2021

<https://doi.org/10.21956/wellcomeopenres.18238.r43724>

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Fabrizio Anniballi

Department of Food Safety, Nutrition and Veterinary Public Health, Higher Institute of Health, Rome, Italy

The manuscript entitled “Botulism outbreak after the consumption of vegetarian pâté in the south of Viet Nam” submitted by Dr. Hung describes the first botulism outbreak in Viet Nam.

I read carefully also the comments provided by John Austin and Michel Popoff that reviewed the manuscript before me. I agree with the comments provided by Dr. Austin; however, I think that the manuscript could be helpful for all countries in which botulism is not reported yet or is not known. Here my comments for the manuscript.

Title:

- I would like to suggest modifying the title as “Suspected botulism outbreak after the consumption of vegetarian pâté in the south of Viet Nam”. This title is more detailed and overcomes the drawbacks related to the lack of botulinum toxin detection in clinical specimens and leftover food.

Abstract:

- Please include “and other BoNT-producing clostridia” in the first sentence... botulism is a potentially life-threatening disease caused by toxins produced by *Clostridium botulinum* and other BoNT-producing clostridia.

Introduction:

- Please modify the first sentence as aforementioned for the abstract.
- Describing foods involved in botulism cases and outbreaks, the authors lack to mention meat products. I would like to remember that the word botulism derives from the Latin term “botulus”, which means sausage. Please also include meat products.
- Botulism diagnosis is mainly a clinical diagnosis; however, laboratory confirmation is essential to exclude all diseases included in the differential diagnosis for the definitive diagnosis. I would like to suggest to the authors to form a sentence in this respect. This statement could make more robust the activities carried out by the authors.
- Definitive diagnosis is based on laboratory confirmation that first consists of detecting botulinum toxins in clinical specimens and leftover foods. The detection and isolation of BoNT-producing clostridia (and not only *C. botulinum*) from clinical samples and leftover food is informative and can be used as criteria for laboratory confirmation combined with the clinical picture by the patients. Please kindly note that BoNT-producing clostridia are environmental microorganisms, and so foods could be contaminated with spores.

Case presentation:

- Guillain-Barré syndrome is included in the differential diagnosis of botulism; however, it is

not plausible in outbreaks. The authors should comment on this.

- Botulism is not a bacteriemia, and so the microorganism cannot be isolated into the blood. Blood may contain only toxins. The recovery of BoNTs in serum is considered the gold standard for botulism laboratory confirmation.
- AOAC method includes a step of isolation of *C. botulinum*; however, isolated strains have to be tested for their capability of producing botulinum toxins. Since the authors stated that they had not accessed the detection of BoNTs, they should detail the criteria adopted to identify *C. botulinum*, the isolated strains.

Table 1:

- Please include in the table a new row including “respiratory failure”.

Discussion:

- Toxins H and FA are the same. Please also consider the Toxin type X, as reported by Zhang S and colleagues – Nat Commun 2017;8:14130.¹ Please also consider the manuscripts published by Michel Popoff - Toxins 2020;12(11):716. Toxins 2020;12(9):570.²
- Page 6 of 13. Fourth line. Please check for +/- it appears as a typo.

References

1. Zhang S, Masuyer G, Zhang J, Shen Y, et al.: Identification and characterization of a novel botulinum neurotoxin. *Nature Communications*. 2017; **8** (1). [Publisher Full Text](#)
2. Popoff M: Bacterial Toxins, Current Perspectives. *Toxins*. 2020; **12** (9). [Publisher Full Text](#)

Is the background of the cases' history and progression described in sufficient detail?

Yes

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?

Partly

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?

Partly

Is the conclusion balanced and justified on the basis of the findings?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Botulism; Detection of Foodborne pathogens; Foodborne diseases epidemiology surveillance system.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 01 Jun 2021

Ngan Thi Thuy Nguyen, Cho Ray hospital, Ho Chi Minh city, Vietnam

Thank you very much for your comprehensive review. I really appreciate your consideration. I also modified my article based on what you suggested.

Competing Interests: No competing interests were disclosed.

Reviewer Report 12 May 2021

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John W Austin

Botulism Reference Service for Canada, Microbiology Research Division, Bureau of Microbial Hazards, Food Directorate, Health Products and Food Branch, Ottawa, ON, K1A 0K9, Canada

The six cases described in this report all displayed symptoms typical of botulism and were all linked epidemiologically by having consumed the same brand of vegetarian pâté. A further seven clinical cases were reported as having consumed the same food product, making 13 clinical cases.

Case definitions differ depending upon the country. Since *C. botulinum* may be isolated at high incidence from some foods, several countries including the United States and Canada, do not consider the isolation of *Clostridium botulinum* from food as laboratory confirmation of a case. Confirmation of botulism involves detection of botulinum toxin in serum, stool, stomach contents or patient's food; or isolation of *C. botulinum* from stool or gastric aspirate. None of these criteria were met in this investigation.

The only laboratory result presented was the detection of *C. botulinum* from the commercial vegetable pate using cultural methods. The authors indicate that the presence of *C. botulinum* in food samples was confirmed using the bioassay method, but no information is presented on the serotype (ie. toxinotype, but not subtype) of the organism. *C. botulinum* was not isolated (or detected?) from stool specimens, nor was botulinum toxin detected in extracts of stool, gastric liquid, serum or food. Neutralization of toxicity in extracts of any of the clinical specimens or in the food would have been sufficient to confirm this outbreak as botulism, however, this was not done. The authors also cite a CDC webpage for the AOAC method for the mouse bioassay.

If typing sera were not available, it is not clear how the food isolate could have been identified as

C. botulinum. The mouse bioassay method involves detection of botulinum toxin in an enrichment culture, or a pure culture, by neutralization of toxicity using sero-specific antitoxins. Since it appears that typing antisera (which are not the same as therapeutic antisera) were not available for this, it was impossible to confirm the presence of *C. botulinum*, or other botulinum toxin-producing clostridia, in the food.

There is some additional confusion in this manuscript regarding laboratory testing. It is mentioned that *C. botulinum* was not isolated from blood and stool. Since *C. botulinum* does not pass into blood, it would not be expected to isolate the organism from blood specimens. However, serum could have been tested for botulinum toxin.

Is the background of the cases' history and progression described in sufficient detail?

Yes

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?

No

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?

Partly

Is the conclusion balanced and justified on the basis of the findings?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clostridium botulinum and botulism.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 27 January 2021

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Michel Popoff

¹ Bacteries Anaerobies et Toxines, Institut Pasteur, Paris, France

² Veterinary Academy of France, Paris, France

The authors have improved the manuscript. However, they said that they have performed the

identification of *C. botulinum* in food by mouse bioassay, but they don't have the facilities to perform botulinum toxin identification. This is contradictory. Please, provide further details about the identification of the *C. botulinum* strain.

Is the background of the cases' history and progression described in sufficient detail?

Partly

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?

Partly

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?

Partly

Is the conclusion balanced and justified on the basis of the findings?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clostridium toxins

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

Reviewer Report 05 November 2020

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Michel Popoff

¹ Bacteries Anaerobies et Toxines, Institut Pasteur, Paris, France

² Veterinary Academy of France, Paris, France

The manuscript of Thi Tuy Ngan *et al.* reports the first outbreak of food borne botulism which has been confirmed in Vietnam. Food-borne botulism is rare but it is the most severe food intoxication which is often lethal without treatment. Here, the authors describe a botulism outbreak including six patients. The clinical symptoms, treatment, and outcomes are reported for each patient. All the patients were hospitalized in an intensive care unit with mechanical ventilation. Laboratory

investigations include regular hematology analysis, cerebrospinal fluid analysis, electromyography, cranial computed tomography, magnetic resonance imaging, as well as food analysis. The diagnosis of botulism was suspected based on the epidemiological investigations showing that the six patients and seven additional patients ate the same food. It was pointed out that anti-botulinum serum, which is the only specific treatment of botulism, was not available in Vietnam and that global access to antiserum is required.

It is indicated that the incriminated food is a vegetarian mushroom paté. More details are required about the preparation of this food: industrial, small scale, or home-made product? Date of preparation of the food and dates of consumption of the food by the patients? Recommended conditions of storage of this food? How this food was preserved, room temperature, low temperature? Which is the possible origin of food contamination?

Laboratory investigations. It is indicated that *Clostridium botulinum* was confirmed in food but not in patient's specimens. More details about the identification of *C. botulinum* are required: by which method *C. botulinum* has been identified in food? Which is the *C. botulinum* typing? Botulinum typing is important to adapt specific antiserum for eventual treatment by serotherapy. Has botulinum toxin been investigated in food? Which patient's specimens have been investigated for botulism? Usually, *C. botulinum* and botulinum toxin are investigated in stool samples and botulinum toxin in serum sample.

Is the background of the cases' history and progression described in sufficient detail?

Yes

Are enough details provided of any physical examination and diagnostic tests, treatment given and outcomes?

Partly

Is sufficient discussion included of the importance of the findings and their relevance to future understanding of disease processes, diagnosis or treatment?

Yes

Is the conclusion balanced and justified on the basis of the findings?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clostridial toxins including botulinum toxins

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 16 Dec 2020

Ngan Thi Thuy Nguyen, Oxford University Clinical Research Unit, Ho Chi Minh city, Vietnam

Questions: It is indicated that the incriminated food is a vegetarian mushroom pâté. More details are required about the preparation of this food: industrial, small scale, or home-made product? Date of preparation of the food and dates of consumption of the food by the patients? Recommended conditions of storage of this food? How this food was preserved, room temperature, low temperature? Which is the possible origin of food contamination?

Answer: The vegetarian pâté consumed by all cases was made by a small-scale family-run business using locally-sourced ingredients and handmade methods. Pâté bought by the first 5 cases and the sixth case were prepared separately in July 2020 and August 2020 respectively. All consumption of pate occurred within the use-by dates (December 2020 and January 2021 respectively). The manufacturer recommended the product be stored frozen and the expiration date was 6 months following production. All cases told us they kept once-opened pâté at room or fridge temperature (5-10 °C). The exact source of contamination during the production process is not clear.

We also added this information into the new version of manuscript.

Question: Laboratory investigations. It is indicated that *Clostridium botulinum* was confirmed in food but not in patient's specimens. More details about the identification of *C. botulinum* are required: by which method *C. botulinum* has been identified in food? Which is the *C. botulinum* typing? Botulinum typing is important to adapt specific antiserum for eventual treatment by serotherapy. Has botulinum toxin been investigated in food? Which patient's specimens have been investigated for botulism? Usually, *C. botulinum* and botulinum toxin are investigated in stool samples and botulinum toxin in serum sample.

Answer: *C. botulinum* was identified in the food product according to the mouse bioassay method of Association of Official Analytical Chemists (AOAC) 977.26. Unfortunately, we do not currently have the facility to detect and type *C. botulinum* toxin.

The diagnosis of botulism was made in our patient clusters because of the consistent clinical syndrome and the isolation of C. botulinum from pâté, consumed by all patients. We were unable to isolate C. botulinum from any patient specimen and lacked the resources to isolate the toxin. However, we think the clinical, epidemiological and microbiological evidence associated with our patients is sufficient to allow us to be confident in our diagnosis. We addressed the limitations of our diagnostic methods in the discussion. We agree there is a need for improved, sensitive and specific point of care and laboratory tests which would enable rapid diagnosis and treatment, also addressed in our discussion.

Competing Interests: No competing interests were disclosed.