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Case diagnosis and characterization of suspected paralytic shellfish poisoning in Alaska

Jennifer S. Knaack [a,1,2], Kimberly A. Porter [b,1], Justin T. Jacob [c], Kate Sullivan [d], Matthew Forester [e], Richard Y. Wang [a], Vera L. Trainer [f], Steve Morton [g], Ginny Eckert [h], Ernest McGahee [a], Jerry Thomas [a], Joseph McLaughlin [i], Rudolph C. Johnson [a,*]

[a] Emergency Response Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway, MS F44, Atlanta, GA 30341, USA
[b] Epidemic Intelligence Service, (Alaska Section of Epidemiology), Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30333, USA
[c] Oak Ridge Institute for Science and Education Fellow at the Centers for Disease Control and Prevention, MC-100-44, P.O. Box 117, Oak Ridge, TN, 37831-0117, USA
[d] Fisheries Technology, University of Alaska Southeast, 2600 7th Ave, Ketchikan, AK 99901, USA
[e] Alaska Environmental Health Laboratory, Alaska Department of Environmental Conservation, 5251 Dr. Martin Luther King Jr. Ave., Anchorage, AK 99507, USA
[f] Marine Biotoxins Program, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, WA 98112 USA
[g] National Centers for Coastal Ocean Science, National Oceanic and Atmospheric Administration, Marine Biotoxins Program, 219 Fort Johnson Road, Charleston, SC 29412, USA
[h] School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 17101 Point Lena Loop Rd., Juneau, AK 99801, USA
[i] Section of Epidemiology, Alaska Department of Health and Social Services, 3601 C Street, Suite 540, Anchorage, AK 99503, USA

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ABSTRACT

Clinical cases of paralytic shellfish poisoning (PSP) are common in Alaska, and result from human consumption of shellfish contaminated with saxitoxin (STX) and its analogues. Diagnosis of PSP is presumptive and based on recent ingestion of shellfish and presence of manifestations consistent with symptoms of PSP; diagnosis is confirmed by detection of paralytic shellfish toxins in a clinical specimen or food sample. A clinical diagnostic analytical method using high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) was used to evaluate the diagnosis of saxitoxin-induced PSP (STX-PSP) in 11 Alaskan patients using urine specimens collected between June 2010 and November 2011. Concentrations of urinary STX were corrected for creatinine concentrations to account for dilution or concentration of urine from water intake or restriction, respectively. Of the 11 patients with suspected PSP, four patients were confirmed to have STX-PSP by urine testing (24–364 ng STX/g creatinine). Five patients had clinical manifestations of PSP though no STX was detected in their urine. Two patients were ruled out for STX-PSP based on non-detected urinary STX and the absence of clinical findings. Results revealed that dysphagia and dysarthria may be stronger indicators of PSP than paresthesia and nausea, which are commonly used to clinically diagnose patients with PSP. PSP can also occur from exposure to a number of STX congeners, such as gonyautoxins, however their presence in urine was not assessed in this investigation. In addition, meal remnants obtained from six presumptive PSP cases were analyzed using the Association of Official Analytical Chemists' mouse bioassay. All six samples tested positive for PSP toxins. In the future, the clinical diagnostic method can be used in conjunction with the mouse bioassay or HPLC–MS/MS to assess the extent of STX-PSP in Alaska where it has been suggested that PSP is underreported.

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Abbreviations: Cr, creatinine; HPLC–MS/MS, high performance liquid chromatography tandem mass spectrometry; LRL, lowest reportable limit; STX-PSP, saxitoxin-induced paralytic shellfish poisoning.

* Corresponding author.
E-mail addresses: Knaack_JS@Mercer.edu (J.S. Knaack), RJohnson6@cdc.gov (R.C. Johnson).

[1] These authors contributed equally to this investigation.
[2] Present address: Department of Pharmaceutical Sciences, Mercer University, 3001 Mercer University Dr., DV-114, Atlanta, GA 30341, USA.

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1. Introduction

The increasing concentration, duration, and frequency of HABs over the past few decades have been documented in many coastal regions of the world (Anderson, 1995; Burke et al., 2000). HABs cause significant ecosystem, human health, and economic impacts (Anderson, 2000; Hoagland and Scatasta, 2006; McFarren et al., 1960) and have become a national and international research focus within the past decade. Paralytic shellfish poisoning (PSP) currently is the primary health concern caused by harmful algal blooms (HABs) in Alaska. Around the world, PSP is caused by ingestion of seafood containing bioaccumulated toxins produced by certain species of marine dinoflagellates in the genera *Alexandrium, Gymnodinium*, and *Pyrodinium* (McFarren et al., 1960; Riegel et al., 1948; Schantz, 1961; Schantz et al., 1966; Wiese et al., 2010). Though commercially harvested shellfish are tested and considered safe, shellfish harvested for subsistence and recreational consumption are not routinely tested in Alaska, resulting in this state having one of the highest incidences of reported PSP in the world (Gessner and Schloss, 1996). Between 1973 and 1996, more than 200 incidences of PSP were reported in Alaska from 70 outbreaks, with the majority occurring in southeast Alaska (Gessner and Schloss, 1996). These cases likely represent a fraction of actual PSP cases because not all affected people seek medical treatment and there is no rapid and readily accessible diagnostic test or biomarker for assessing human exposure.

The congeners of saxitoxin (STX) have a common heterocyclic guanidine structure and they include STX, neosaxitoxin, gonyautoxins, and other derivatives that vary in toxicity based on structural determinants with different side chains and functional groups that affect their binding affinity to sodium channels (Henderson et al., 1974; Hille, 1975; Strichartz, 1984). Specifically, these toxins bind to the sodium channel pore, thereby blocking the influx of sodium into these cells (Hille, 1975). STX and neosaxitoxin are the most potent of the PSP toxins (Shimizu, 2000), however, biotransformation of toxins from low to high affinity isoforms (or vice versa) can occur in algae (reviewed in Wiese et al., 2010), shellfish (review in Bricelj and Shumway, 1998) or humans (Garcia et al., 2010; see discussion below). Clinical diagnosis of PSP is based on recent ingestion of shellfish and the presence of clinical manifestations consistent with toxicity, including nausea, vomiting, paresthesia (skin sensations such as tingling), dysarthria (abnormal speech), dysphagia (difficulty swallowing), and weakness that can lead to paralysis and respiratory failure (McFarren et al., 1960; Schantz, 1960; Tennant et al., 1955). The detection of a paralytic shellfish toxin in a clinical specimen, such as blood or urine, or an uneaten food sample also can be used to confirm the diagnosis of PSP in a patient (AOAC, 2005a,b) .

Saxitoxin has been detected in urine specimens collected from victims of PSP (Garcia et al., 2004; Gessner et al., 1997; Llewellyn et al., 2002; Rodrigues et al., 2012), although other congeners can be present at concentrations higher than STX or at the exclusion of STX. In one report, STX was detected at a higher concentration than neosaxitoxin in urine specimens from two patients with PSP from Alaska (Gessner et al., 1997). However, gonyautoxins (GTX2 and GTX3), and C1 toxins comprised a larger portion of the total measured toxin in these patients. Although these toxins can be detected in clinical specimens using high performance liquid chromatography with fluorescent detection (AOAC, 2005b; Garcia et al., 2004), this method is not commonly used for clinical investigations. Recently, a mass spectrometry-based clinical method was developed for diagnosing PSP by detecting STX in urine (Johnson et al., 2009). This is the only fully characterized diagnostic method available for clinical use and directly detects STX without the need to oxidize or conjugate the analyte.

The purpose of this investigation was to apply this diagnostic test for STX (Johnson et al., 2009) to urine samples from patients with suspected PSP in Alaska to confirm STX-induced PSP (STX-PSP). This method was previously used to evaluate two patients from Alaska in 2011 with presumed PSP (Centers for Disease Control and Prevention, 2011). In this investigation, these two patients are described in more detail with respect to their clinical manifestations. Results are documented using this diagnostic test in seven other Alaskan patients with presumptive PSP and two Alaskan patients who consumed contaminated shellfish but did not report symptoms. The clinical findings in the context of the urinary STX concentrations are presented, when available, in conjunction with the total paralytic shellfish toxin concentration in shellfish, assessed by mouse bioassay. Other STX congeners causing PSP were not assayed in this investigation.

2. Materials and methods

A human subject research determination was conducted at Centers for Disease Control and Prevention (CDC) in accordance with the Code of Federal Regulations, Title 45, Part 46. As a result, this dataset was determined not human subject research.

2.1. Study design and selection of participants

PSP is a mandatorily reportable condition in Alaska. Clinicians reporting a patient with suspected PSP to the Alaska Section of Epidemiology (SOE) provide clinical data and, if known, the patient's recent history of shellfish consumption. A case of presumptive PSP is one that is assessed by a physician to demonstrate clinical manifestations consistent with PSP (Gessner et al., 1997). Clinical specimens were obtained from 11 patients with presumptive PSP from June 2010 to November 2011 and were analyzed for STX in the National Center for Environmental Health, Division of Laboratory Sciences at the Centers for Disease Control and Prevention (CDC). Specimens from other patients with suspected PSP during this time were not collected by clinicians or were not sent for urinalysis. Urine specimens were stored and shipped frozen to the Alaska State Public Health Laboratory, repackaged, and shipped frozen to CDC for testing. When possible, unconsumed portions of shellfish were obtained from patient meals and analyzed by the Alaska Department of Environmental Conservation's environmental health laboratory for the presence of paralytic shellfish toxins.

2.2. Methods and measurements

Clinical data were obtained from the following sources: patient interview with a structured questionnaire, consultation with the patient's healthcare provider, and the patient's medical record. Data from medical records were summarized, including: time to onset of signs and symptoms since shellfish consumption; clinical manifestations of PSP including paresthesia, nausea, light-headedness, dysarthria, dyspnea, and dysphagia, and clinical course and outcome (Table 1). A case of confirmed STX-PSP was the detection of STX in the urine of a case of presumed PSP.

At CDC, urine specimens were analyzed for STX using a method compliant with the Clinical Laboratory Improvement Amendments Act of 1988 as previously described (Johnson et al., 2009). Saxitoxin was extracted from urine by solid phase extraction using a weak cation exchange sorbent and detected by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS). The lowest reportable limit (LRL) for the analytical method, which was defined as the lowest standard in the assay, is 4.8 ng/mL (Johnson et al., 2009). Creatinine in urine (Cr) was

Table 1
Clinical data, manifestations, urinary saxitoxin (STX) concentrations, and shellfish toxin concentrations, for 11 patients with suspected paralytic shellfish poisoning (PSP).

Patient identifier	Approximate amount and type of shellfish consumed (μg STX eq/100 g)	Time to develop clinical manifestations after consumption	Nausea or vomiting	Paresthesia and light-headedness, dizziness, or weakness	Dysarthria or dysphagia	Blood pressure (mmHg)	Approximate urine sampling time post consumption (h)	Creatinine corrected urinary STX (μg STX/g Cr)	Disposition
A	2 pounds (weight of shells included) clams (1096) and cockles (32)	2 h	Yes	Yes	Yes	146/90	48	24.3	Discharged
B	Mussels (not tested)	28 h[a]	Yes	Yes	No	121/71	48	<LRL[b]	Discharged
C	1 butter clam (not tested)	15 min	Yes	Yes	No	170/80	>120	<LRL	Discharged
D[c]	80–90 mussels (5037)	1 h	Yes	Yes	Yes	175/104	24	47.8	Discharged
E	4 mussels (5037)	–	No	No	No	NA[d]	24	<LRL	Discharged
F	3 mussels (5037)	–	No	No	No	NA	24	<LRL	Discharged
G	20–40 mussels (5037)	1 h	Yes	Yes	Yes	182/121	24	364	Discharged
H	12 butter clams (not tested)	9.5 h	Yes	Yes	Yes	160/180	36	24	Discharged
I	crab (not tested)	8 h	Yes	Yes	No	118/76	<24	<LRL	Discharged
J	crab (not tested)	<5 min	Unknown	Yes	No	133/85	38	<LRL	Deceased
K	4 cockles (2044)	3 h	Yes	Yes	Yes	124/60	48	<LRL	Deceased

[a] Time to presentation at the hospital after shellfish consumption. Time of onset for manifestations was unknown.
[b] Lowest reportable limit for the laboratory diagnostic method was 4.8 ng/mL.
[c] Some details were previously reported for these patients (Centers for Disease Control and Prevention, 2011).
[d] Not available.

measured using the Roche Creatinine Plus™ assay and a Roche Hitachi analyzer. Urinary STX concentrations were reported in volumetric and creatinine-corrected units.

During PSP outbreaks, shellfish samples were collected by SOE staff, a local public health nurse or an environmental health officer, and shipped frozen to the Alaska Department of Environmental Conservation's environmental health laboratory. Seafood samples were analyzed for paralytic shellfish toxins by the standard mouse bioassay using the protocol described in the Association of Official Analytical Chemists method 959.08 for paralytic shellfish poison (AOAC, 2005a). The assay assesses a biological response in mice, resulting from cumulative toxicity of both STX and STX congeners. Paralytic shellfish toxin concentrations were reported in μg STX equivalents per 100 g shellfish tissue.

3. Results

3.1. Characteristics of study subjects

From June 2010 to November 2011, urine specimens from 11 Alaskan patients were sent to CDC for laboratory diagnostic testing for STX (Table 1). These patients were all seen at the hospital for suspected PSP and ranged in age from 22 years to 61 years. Nine patients (A, B, C, D, G, H, I, J, and K) presented with presumptive PSP (Fig. 1). The remaining two patients, E and F, were not presumed to have PSP because they lacked clinical signs and symptoms despite consuming noncommercially harvested shellfish that caused symptoms in other patients (Table 1).

Seven of the urine specimens analyzed in this report were collected from seven independent exposure events (patients A, B, C, H, I, J, and K). Patient A developed paresthesia approximately 2 h after consuming clams and cockles. The symptoms had largely abated approximately 10 h after consumption. Patient B arrived at the hospital with paresthesia, dizziness, and chest pain at approximately 28 h after consumption of noncommercially harvested mussels. Patients C, H, and I reported the onset of paresthesia at 15 min, 9.5 h, and 8 h following shellfish consumption, respectively.

Two patients, J and K, died after consumption of shellfish; therefore the cause of death was initially attributed to PSP. Patient J was admitted to the hospital after consuming a large amount of noncommercially harvested crab meat and viscera. The patient had dizziness, weakness, disorientation, and ataxia. The patient improved, left the hospital, and died one day later. Death was attributed to coronary artery disease by the state medical examiner's office. Patient K developed paresthesia approximately 3 h after consuming 4 cockles. The patient described difficulty swallowing and was initially monitored in the critical care unit and then on the medical floor after symptoms improved. The following day, the patient was found unresponsive and later died. The pathologist attributed death to complications from hepatic steatosis (fatty liver disease).

Patients D, E, F, and G reported consumption of noncommercially harvested mussels at the same event as previously reported (Centers for Disease Control and Prevention, 2011). Patients D and G reportedly consumed between 80–90 and 20–40 mussels, respectively. Both patients developed significant manifestations of PSP within one h after shellfish consumption, including paresthesia, generalized weakness, and dysphagia. Patient G required mechanical ventilation because of severe respiratory distress. They were accompanied to the emergency department by patients E and F who consumed only 3–4 mussels each and did not develop any symptoms.

Paresthesia, nausea, and light-headedness or dizziness was experienced by the four patients with a confirmed diagnosis of STX-PSP (Table 2). Three of these four patients also had dysphagia, three had weakness, and two reported headaches. Blood pressure was elevated in three patients upon arrival at the hospital and it was treated in two patients because of systolic and diastolic

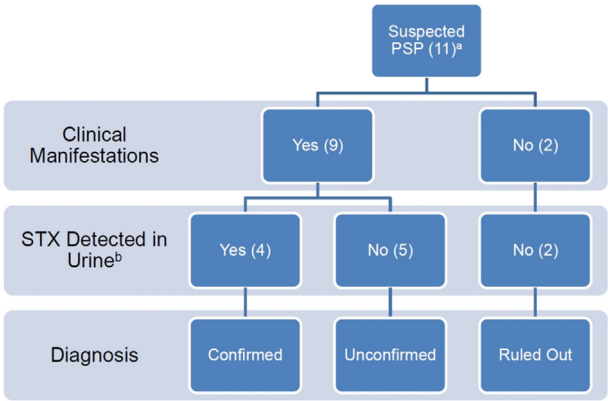

Fig. 1. Summary of diagnosis of Paralytic Shellfish Poisoning (PSP) in 11 patients with suspected PSP. [a] Number of patients. [b] The analytical limit of detection for STX in urine was 4.8 ng/mL.

Table 2
Clinical manifestations of patients presenting with suspected PSP and their final diagnosis.

	Total number of patients	Number of patients experiencing selected manifestations					
		Paresthesia	Nausea	Light-headed or dizzy	Weakness	Dyspnea	Dysphagia or dysarthria
Confirmed PSP[a]	4	4	4	4	3	1	4
Unconfirmed PSP[b]	5	5	4	3	5	4	1
Ruled Out for PSP[c]	2	0	0	0	0	0	0

[a] STX-PSP was confirmed by urine testing.
[b] Based on presence of clinical findings and no STX detected in the urine.
[c] Based on absence of clinical findings and no STX detected in the urine.

hypertension. These patients did not require further treatment for blood pressure control at the time of discharge from the hospital.

3.2. Laboratory diagnostic test

All patients provided urine specimens for detection of STX within 48 h of shellfish consumption, except for patient C who provided a specimen six days after consumption. Urine specimens were collected within 4 h after shellfish consumption for patients D, E, F, and G. These specimens were shipped to CDC and analyzed within 36 h of their receipt. Specimens from patients A, D, G, and H contained STX at concentrations of 24.3, 47.8, 364, and 24 μg STX/g Cr, respectively. STX concentrations in urine specimens from other patients were below the LRL for the analytical method (Table 1). Among the 11 urine specimens tested, seven were below the LRL for STX (Table 1, Fig. 1). Two of these patients, E and F, lacked any clinical findings. The other five patients had paresthesia and four patients reported dyspnea. None of these patients had dysarthria and only one had dysphagia (patient K). Dysarthria or dysphagia was present among those with confirmed cases of STX-PSP (Table 2). Patient G complained of dysphagia and dyspnea and had the highest urinary STX concentration among these patients. A HPLC–MS/MS chromatogram of STX in this specimen is shown in Fig. 2. In contrast, patients A and H, who had the lowest concentrations of STX, experienced only dysphagia or dysarthria, but no dyspnea.

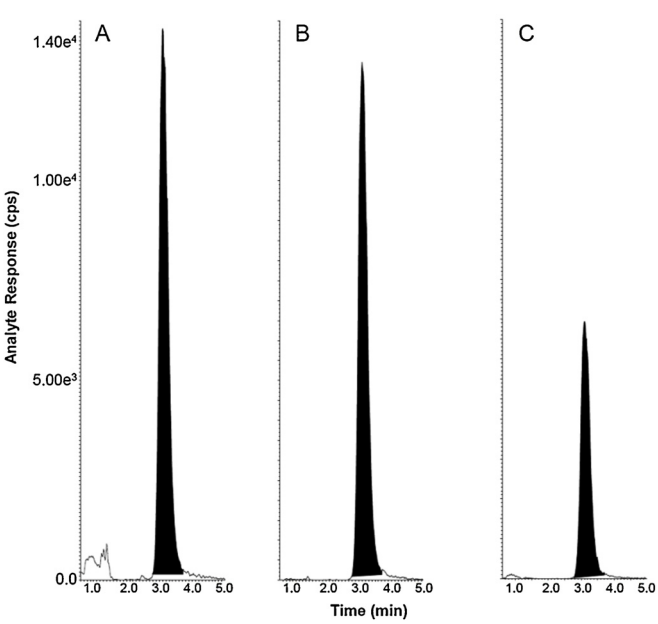

Fig. 2. HPLC–MS/MS chromatogram of STX in the urine specimen collected from Patient G. Ion peaks used to quantitate STX (A) and confirm STX identity (B) in the specimen are shown. The ion peak corresponding to the internal standard (C) is also shown and was used to correct for loss of STX during sample preparation and analysis.

3.3. Shellfish analysis

Paralytic shellfish toxin concentrations were determined by mouse bioassay for shellfish consumed by six patients from three independent exposure events. Patient A reported consumption of two pounds of shellfish including clams (1096 μg STX equivalents/100 g) and cockles (32 μg STX equivalents/100 g). Patients D, E, F, and G consumed mussels containing 5037 μg STX equivalents/100 g (Centers for Disease Control and Prevention, 2011). Patients E and F reported consuming four and three mussels, respectively, and patients D and G reported consuming 80–90 and 24–40 mussels, respectively. Patient K reported eating four cockles that were found to contain 2044 μg STX equivalents/100 g. No shellfish testing was conducted for patients B, C, H, I, and J. Although the exact locations where shellfish were collected cannot be divulged to protect the identity of the patients, a description of the shellfish toxicity and the environmental factors associated with this PSP event near Ketchikan, Alaska, are described in a recent publication (Trainer et al., 2014).

3.4. STX-PSP diagnosis

The diagnosis of STX-PSP was based on the recent consumption of shellfish, the presence of clinical findings, and the detection of STX in the urine, which confirmed the diagnosis. The diagnosis of STX-PSP in four patients (A, D, G, and H) (Fig. 1) was confirmed and ruled out STX-PSP in two patients (E and F) based on no STX detected in the urine and the lack of clinical manifestations. PSP could not be confirmed in five patients (B, C, I, J, and K), because STX was not detected in the urine and these patients remained as presumed PSP. Paralytic shellfish toxins were detected in shellfish consumed by all patients with confirmed STX-PSP and in two patients who were ruled out for STX-PSP. This testing was not performed for shellfish consumed by patients with unconfirmed PSP, except for patient K.

3.5. Limitations

STX is the primary and most potent toxin (Shimizu, 2000) associated with PSP, but many congeners of the toxin exist including decarbamoyl saxitoxins and gonyautoxins 1-4 (Etheridge, 2010; Henderson et al., 1974; Hille, 1975; Wiese et al., 2010). Currently, no validated diagnostic methods for measuring exposure to these congeners exist and so exposure to these toxins is not discussed here. However, the presence of these toxins in urine specimens cannot be ruled out. Additionally, the diagnostic limit of detection for STX in urine is the lowest reportable limit, 4.8 ng/mL. The presence of toxin concentrations below this limit also cannot be ruled out.

4. Discussion

Findings indicate that the detection of STX in urine can confirm the diagnosis of STX-PSP in patients with suspected PSP. The diagnosis of PSP based only on the recent consumption of shellfish

and the development of clinical manifestations is difficult and can be inaccurate because these findings are nonspecific and similar to other diseases such as stroke and acute coronary syndrome. In 11 patients, the diagnostic test for STX allowed us to confirm or rule out the diagnosis of STX-PSP in six patients. PSP could not be confirmed for five patients whose diagnosis remained as presumed PSP. In addition, it is suggest that dysphagia and dysarthria are significant manifestations of toxicity based on the urinary STX concentration in patients with confirmed STX-PSP. Thus, laboratory testing for the presence of STX in urine enhances the accuracy of the clinical diagnosis of STX-PSP.

In rare cases, shellfish can be collected from unconsumed meals or from areas where consumed shellfish were harvested to be tested for paralytic shellfish toxins. Levels at or above the regulatory guidance level, 80 μg STX equivalents/100 g shellfish (Wekell et al., 2004), could substantiate a diagnosis of PSP. However, the presence of toxins in these shellfish does not necessarily indicate a significant human exposure to these toxins (Gessner and Middaugh, 1995) as was observed with patients E and F. Relevant dose of the toxin must be considered when diagnosing PSP, however, insufficient information was available to determine a relevant dose in this investigation. Between June 2010 and November 2011, 11 urine specimens from Alaskan patients with a suspected PSP were sent to CDC. Of these specimens, only four had detectable levels of STX, which ranged from 8.32 to 118 ng/mL. These concentrations represented a nearly 1000-fold lower urinary STX concentration than reported in a post-mortem urine specimen (Garcia et al., 2004) and are in agreement with the urinary STX concentration reported for a patient who survived PSP in Portugal (Rodrigues et al., 2012). All four patients with confirmed PSP reported nausea, paresthesia, and dysphagia or dysarthria. One patient (G) also reported dyspnea and had the highest concentration of urinary STX among the confirmed cases and required mechanical ventilation indicating that the combination of dysphagia and dyspnea might represent substantial exposure to STX. In these patients, STX-PSP appears to progress from paresthesia and nausea, to near syncope and generalized weakness, to dysphagia or dysarthria, and then to dyspnea. There was no observation of dysarthria in patients with unconfirmed PSP and dysphagia was only observed in one unconfirmed case, suggesting that these findings are significant manifestations of PSP and specific to saxitoxin toxicity. The latter awaits confirmation by future investigations evaluating additional paralytic shellfish toxins, such as GTXs, which are less toxic than STX (Usleber et al., 1997).

Laboratory diagnostic testing for STX could not confirm STX-PSP in five patients with presumed PSP. The delayed collection of urine for one patient at 6 days after ingestion of shellfish might have contributed to this finding because the half-life of saxitoxin in urine is between 16 and 20 h (Poli, 2011). An alternative explanation for the inability to confirm PSP in these patients is that they were exposed to shellfish not containing STX as the major isoform but comprised mostly GTX or other paralytic shellfish toxins, which were measured in Alaska shellfish during the same time as the PSP events (Trainer et al., 2014) and described in a patient with PSP off the coast of Chile (Garcia et al., 2005), or because the analytical sensitivity was not sufficient to detect low concentrations of STX in urine. The decreased frequency of patients with dysphagia or dysarthria with presumed PSP compared to those with confirmed STX-PSP (1/5 vs. 4/4, Table 2) suggests the presence of a paralytic shellfish toxin with less toxicity than STX. Thus, the ideal method used to detect toxins in urine must be sensitive to all toxin congeners to avoid underestimation of the actual human exposure to the suite of saxitoxin congeners.

During typical progression of PSP, clinical manifestations develop within the first several hours after consumption of shellfish and resolve as the toxin is excreted (Gessner et al., 1997). Substantial improvement typically occurs within 24 h of consumption of contaminated shellfish and there have been no reports of delayed recrudescence to paralytic shellfish toxicity (Garcia et al., 2004; Llewellyn et al., 2002). In the cases of patients J and K, symptoms improved before death, which is not consistent for a PSP diagnosis.

In conclusion, this study demonstrated the ability of a new laboratory test to confirm the diagnosis of STX-PSP. This test can help lead to the identification of outbreaks and appropriate public health interventions in affected communities. Additionally, confirming the diagnosis of STX-PSP in the patients in this study allowed us to identify dysarthria and dysphagia as significant manifestations of STX-PSP. In Alaska, the consumption of shellfish harvested from unmonitored beaches is a common practice, therefore PSP likely is underreported. Thus, a biomonitoring study of the most affected regions and communities is recommended to reveal the actual extent of PSP in these populations. Future studies should incorporate the analysis of other paralytic shellfish toxins, including the gonyautoxins and neosaxitoxin, to determine their contribution to PSP and to better understand toxin metabolism in humans.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of Centers for Disease Control and Prevention.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.hal.2016.03.006.

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