Public Health Response to Puffer Fish (Tetrodotoxin) Poisoning from Mislabeled Product

NICOLE J. COHEN,† JONATHAN R. DEEDS,‡ EUGENE S. WONG, ROBERT H. HANNER, HAILE F. YANCY, KEVIN D. WHITE, TREVONNE M. THOMPSON,‡‡ MICHAEL WAHL, TU D. PHAM, FRANCES M. GUICHARD, IN HUH, CONNIE AUSTIN, GEORGE DIZIKES, AND SUSAN I. GERBER

†Chicago Department of Public Health, Chicago, Illinois 60612, USA; ‡U.S. Food and Drug Administration Center for Food Safety and Applied Nutrition, College Park, Maryland 20740, USA; §Biodiversity Institute of Ontario, University of Guelph, Guelph, Ontario, Canada N1G 2W1; ‡U.S. Food and Drug Administration Center for Veterinary Medicine, Laurel, Maryland 20855, USA; ‡Illinois Poison Center, Chicago, Illinois 60606, USA; ‡Swedish Covenant Hospital, Chicago, Illinois 60625, USA; ‡Illinois Department of Public Health, Springfield, Illinois 62761, USA; and §Illinois Department of Public Health, Chicago, Illinois 60612, USA

MS 08-353: Received 15 July 2008/Accepted 16 November 2008

ABSTRACT

Tetrodotoxin is a neurotoxin that occurs in select species of the family Tetraodontidae (puffer fish). It causes paralysis and potentially death if ingested in sufficient quantities. In 2007, two individuals developed symptoms consistent with tetrodotoxin poisoning after ingesting home-cooked puffer fish purchased in Chicago. Both the Chicago retailer and the California supplier denied having sold or imported puffer fish but claimed the product was monkfish. However, genetic analysis and visual inspection determined that the ingested fish and others from the implicated lot retrieved from the supplier belonged to the family Tetraodontidae. Tetrodotoxin was detected at high levels in both remnants of the ingested meal and fish retrieved from the implicated lot. The investigation led to a voluntary recall of monkfish distributed by the supplier in three states and placement of the supplier on the U.S. Food and Drug Administration’s Import Alert for species misbranding. This case of tetrodotoxin poisoning highlights the need for continued stringent regulation of puffer fish importation by the U.S. Food and Drug Administration, education of the public regarding the dangers of puffer fish consumption, and raising awareness among medical providers of the diagnosis and management of foodborne toxin ingestions and the need for reporting to public health agencies.

Tetrodotoxin is a heat-stable neurotoxin that blocks sodium conductance and neuronal transmission in skeletal muscle, leading to weakness or paralysis and potentially death if ingested in sufficient quantities (17). The minimum lethal dose in humans for tetrodotoxin is estimated to be 2 mg (24), but this number can vary based on age, health, and sensitivity to the toxin. In fish, tetrodotoxin occurs mainly in members of the family Tetraodontidae (puffer fish) and is typically concentrated in the fish’s liver, followed by the ovaries, intestines, and skin (17). In most species, tetrodotoxin concentrations in the muscle are low, which is why, when properly processed, this fish is commercially exploited and safely consumed in countries such as Japan (24). Importation of puffer fish into the United States is strictly regulated by the U.S. Food and Drug Administration (FDA). In 1989, after 4 years of negotiations, an agreement was reached between the Japanese Ministry of Health and Welfare and the FDA that allowed the importation of Japanese puffer fish under a specific set of conditions. The intention of the parties was never to allow unrestricted importation for general distribution but to allow limited importation only on the basis of “special occasions.” The conditions were as follows: only meat, skin, and testicles of tiger puffer fish (Takifugu rubripes or torafugu) prepared in an authorized facility and certified as safe for consumption would be allowed entry by a single import organization through JFK International Airport, New York, NY. The product could be sold only to restaurants belonging to an organization known as the Torafugu Buyers Association, who were not allowed to transfer or resell the product to another establishment (2). Any puffer fish imported into the United States by countries other than Japan or by Japanese importers but outside the specific terms of this agreement are subject to FDA Import Alert #16–20, which allows for the detention without physical examination of puffer fish and products containing puffer fish (28).

Neurologic illness associated with ingestion of puffer fish is rare in the United States, particularly when associated with imported product (6, 7). This report describes the public health investigation and regulatory response to an unusual episode of human tetrodotoxin poisoning following illegal importation and sale of puffer fish. The objectives of this investigation were to (i) determine the cause of neurologic illness, (ii) determine the identity of the suspect product, and (iii) ensure that any remaining suspect product was removed from wholesale and retail commerce.
CASE REPORT

A 47-year-old woman purchased two frozen fish at a Chicago Asian market on 4 May 2007 and prepared them as a soup for herself and her family on 9 May 2007. The head and viscera of the fish had been removed prior to purchase. Preparation included removing the skin and soaking the fish in water at room temperature for 7 to 8 h prior to cooking. Through interviews with the couple, it is estimated that the woman consumed approximately 3 cups (700 ml) of the soup. Her 55-year-old husband ate approximately 1 cup (230 ml), while their 10-year-old daughter tasted only one spoonful of the soup.

Within 30 min of consuming the soup, the woman experienced nausea and vomiting, followed by numbness and tingling of the perioral region and extremities, lower extremity weakness, headache, and chest pain. She was taken to a local hospital emergency department, where a physical examination revealed profound extremity weakness, more pronounced on the left side; gross truncal ataxia; and generalized paresthesias. She was admitted to the hospital for supportive care but had no progression of symptoms and did not require ventilatory support. Her sensory deficits resolved within 24 h; however, the motor weakness persisted, requiring inpatient therapy for 3 weeks, after which time she was discharged to a long-term care facility for additional rehabilitative care.

Her husband experienced perioral and extremity paresthesias and extremity weakness of approximately 1 week’s duration. He was evaluated as an outpatient but did not require hospital admission. Their daughter remained asymptomatic.

MATERIALS AND METHODS

Field investigation. The admitting hospital reported the case to the Illinois Poison Center, who subsequently notified the Chicago Department of Public Health on 11 May 2007. The retail establishment where the fish was purchased and the local wholesaler were investigated by the Chicago Department of Public Health Food Protection Division, who also notified the local FDA field office (Chicago District Office). Leftover soup was retrieved by the Illinois Poison Center and submitted to the Illinois Department of Public Health Laboratory, where it was packaged and shipped to the FDA Center for Food Safety and Applied Nutrition (CFSAN), located in College Park, MD, for laboratory evaluation. FDA field officers additionally retrieved the uncooked skins of the two implicated fish from the patients’ home and submitted them to CFSAN for visual identification. Although the investigation was conducted immediately following receipt of the case report by the Chicago Department of Public Health, the Chicago retailer’s remaining lot of suspect fish had already been returned to a supplier in California. The local FDA field office in California (Los Angeles District Office) inspected the facility and collected one unopened 22-lb box of individually wrapped fish, marked as “monkfish, gutted and head-off, product of China,” from the returned lot. Two additional unopened boxes, also marked as monkfish but imported at different times, were also collected by the Los Angeles District Office. All samples were sent to CFSAN for toxin analysis and species identification.

On 14 May 2007, health alert notices were sent out electronically by the Chicago Department of Public Health and the Illinois Department of Public Health to Chicago and Illinois hospitals, respectively, as well as to local health departments, informing them of the reported case and exposure risk. The health alerts requested enhanced surveillance for, and prompt reporting of, any patients with symptoms and exposure history consistent with tetrodotoxin poisoning.

Sample extraction. Two samples of cooked white muscle and one sample of gelatinized broth obtained from the leftover implicated soup, as well as five individual fish from the suspect box and one fish from each of the additional “monkfish” boxes were analyzed for tetrodotoxin as well as saxitoxin. The fish was screened for saxitoxin due to a recent outbreak in which saxitoxin was found in meal remnants implicated in several cases of domestically imported puffer fish poisoning that occurred on the U.S. East Coast between 2002 and 2004 (7, 11, 20). Procedures for the extraction of tetrodotoxin and saxitoxin were adapted from the method of Chen and Chou (8). Briefly, 5-g subsamples of homogenized tissues or gelatinized broth were extracted twice with 10 ml of 1% acetic acid in methanol. Extracts were centrifuged, and combined supernatants were concentrated to <1 ml under vacuum. Samples were redissolved in 5 ml of 1% (vol/vol) acetic acid in high-performance liquid chromatography (HPLC)-grade water and then defatted with chloroform. Defatting was performed by adding 5 ml of chloroform to each sample, vortexing to mix, and then separating by centrifugation. The top aqueous layer was saved; 5 ml of additional acidified water was added to the lower chloroform layer and mixed by vortexing, and the process was repeated. Supernatants were combined, and a subsample was filtered with a 0.22-μm-pore-size cellulose acetate syringe filter, adjusted to 25% acetonitrile, and 10 μl was analyzed for tetrodotoxin and saxitoxin content by liquid chromatography electrospray-ionization multiple-reactions monitoring mass spectrometer (LC/ESI/MRM/MS).

Toxin analysis. Initial toxin separations were performed by LC/ESI/MRM/MS according to Negri et al. (22) by using an Agilent 1100 HPLC system equipped with a 250-mm column with inner diameter of 2 mm packed with 5-μm-diameter Tosohaas TSK-GER Amide-80 material. Toxins were eluted with 0.3 ml of acetonitrile:HPLC-grade water (70:30, vol/vol) per min with 5 mM ammonium formate and 26.5 mM formic acid. Mass spectrometry was performed with an API5000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Framingham, MA) equipped with a turbo spray ionization source and operated in positive-ion mode. The following instrument parameters were used: source temperature, 300°C; curtain gas, 45 liters of N2/h; nebulizer gas (GS1), 40 liters of N2/h; turbo heater gas (GS2), 35 liters of N2/h; spray voltage, 3.200 V. Multiple-reaction monitoring (MRM), in which the parent ion for each toxin is fragmented and monitored for the appearance of specific fragments characteristic of that compound, was used for measurement of the toxins. The MRM data acquisition method was separated into two periods, monitoring three fragmentation channels (MRMs) each. The three reactions monitored for tetrodotoxin were from the decomposition of the protonated tetrodotoxin molecule [M + H]+ at m/z 320 fragmenting to ions at m/z 302, 256, and 162. The three reactions monitored for saxitoxin were from the protonated saxitoxin molecule [M + H]+ at m/z 300 fragmenting to ions at m/z 282, 204, and 179. Dwell time for each reaction was 200 ms, and the entrance potential was 10 V for each. Declustering potential, collision energy, and exit potential were independently optimized for each reaction. Saxitoxin and tetrodotoxin were quantified by linear regression of the sum of the three fragment ions for each toxin, using mixed standards of the following concentrations: 1, 10, 100, 1,000, and 10,000 ng of saxitoxin/tetrodotoxin per ml. Tetrodotoxin standard was purchased from Sankyo Co. Ltd., Tokyo, Japan, while saxitoxin standard was from FDA in-house stocks (FDA reference standard saxitoxin). Standards were diluted in 1% aqueous acetic acid with 25% acetonitrile. Linear regressions were performed using GraphPad Prism (v. 4.03) software (GraphPad Software, Inc., San Diego, CA).
**Fish identification.** Recovered skins from two fish utilized to make the implicated soup were visually compared with samples of headless fish from the returned lot. These samples were also compared with whole monkfish and monkfish tails also obtained from the California supplier and to a whole puffer fish (†Lagocephalus sp.) from North American waters. After removal of a small tissue sample for genetic analysis, three whole monkfish were transferred to the Smithsonian National Museum of Natural History Museum Support Facility located in Suitland, MD, for identification and vouchering. Due to a lack of appropriate Asian puffer standards, the currently accepted regulatory method for fish identification of protein isoelectric focusing (3) was not available. In addition, isoelectric focusing is not possible with cooked samples due to thermal degradation of proteins. Therefore, an alternative genetic approach was attempted both to determine the identity of the cooked fish flesh in the implicated soup and to confirm the visual finding that the suspect fish from the returned lot were not monkfish as the supplier claimed.

Genetic analyses of the two cooked fish flesh samples obtained from the implicated soup, subsamples of five fish from the suspect box, and subsamples of monkfish (one each) from the two additional boxes obtained from the California supplier were performed at the Biodiversity Institute of Ontario (University of Guelph, Ontario, Canada). The analysis was conducted using a molecular diagnostic technique known as DNA barcoding (12). This technique uses the mitochondrial marker cytochrome c oxidase I (COI), which has been shown to work well for the species identification of fish (27, 33) and has been validated for use in forensic analysis (10). DNA was extracted from approximately 2 to 3 mm³ of tissue by using the protocol detailed by Ivanova et al. (18). The DNA barcode fragment of the COI mitochondrial gene was PCR amplified and sequenced using the protocols and primers described by Ivanova et al. (19).

Barcode sequences derived from each test sample were queried against the identification engine of the Barcode of Life Data System (BOLD) (25). The BOLD identification engine displays the 99 closest matches to the query sequence found in the barcode database in the form of a neighbor-joining tree. For comparison, frozen tissue samples of monkfish, salmon, and snapper were processed for DNA barcode analysis along with the suspect fish soup samples. Salmon and snapper controls were from the FDA regulatory fish encyclopedia authenticated set of reference fish tissues (34). Tissue samples from whole monkfish used in the analysis were from specimens sent to the Smithsonian National Museum of Natural History for species identification and vouchering.

Since the full BOLD database contains sequences from both authenticated and nonauthenticated fish, an additional Bayesian inference analysis was conducted for the suspect puffer fish samples to provide a quantitative assessment of nodal confidence for the relationship between the suspect samples and other Tetraodontidae species in the BOLD database. A Bayesian inference of a phylogeny is based on the posterior probability of the tree, which can be interpreted as the probability that the tree is correct (15). The analysis was conducted with MrBayes v3.1.2. under the following parameters: a GTR+I+G model, 5,000,000 generations, 4 chains, and 2 runs (14). Sequences from two of the unknown test samples, an authenticated monkfish sample, and a total of 51 sequences from authenticated, vouchered tetraodontiform specimens were used in the Bayesian inference analysis of tree nodal confidence.

Many of the sequences used in the additional Bayesian inference analysis were from new specimens, not yet in the public portion of the BOLD database, that were collected as part of this study. These included 25 samples, representing 21 distinct species, from the Tetraodontidae (puffer) family and 26 additional non-Tetraodontidae, tetraodontiform species representing five closely related families. These reference sequences were obtained from several sources, including (i) vouched specimens collected by Professor Keiichi Matsuura of the National Museum of Nature and Science, Tokyo, Japan, now housed as part of the FDA Regulatory Fish Collection at the Smithsonian National Museum of Natural History (Washington, DC); (ii) vouched specimens collected as part of the Moorea Biocode Project (http://moorea.berkeley.edu/biocode/), provided by Dr. Serge Planes (CRIOBE, UMS 2978 EPHE-CNRS, Moorea, Polynesia) and Dr. Chris Meyer (Smithsonian National Museum of Natural History, Washington, DC); and (iii) sequences from the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/) containing the reserved keyword “BARCODE,” which includes by definition that each sequence be linked to a museum voucher specimen with all associated metadata available through a public database from the sample voucher’s repository.

**RESULTS**

**Field investigation.** The retail package label of the implicated fish identified it as “frozen bok fish” in both English and Korean. Bok is the Korean name for puffer fish (translation by I.H.). Both the Chicago retailer and the California supplier denied having sold or imported puffer fish and claimed the product was monkfish. The California supplier imported this lot of product into the United States in September 2006, in 22-lb boxes (282 boxes in total) labeled and invoiced as “monk fish, gutted and head-off, product of China.” This product was distributed to three states (California, Illinois, and Hawaii). The subsequent FDA investigation led to a voluntary nationwide recall of monkfish sold by the California supplier due to concerns of product safety and possible mislabeling (29).

No additional cases consistent with tetrodotoxin poisoning were reported in Illinois. Three additional suspect cases of puffer fish poisoning were eventually traced back to fish purchased from the same California supplier: two in California in November 2006 and one in New Jersey in July 2007. All three cases were associated with the restaurant consumption of, as listed on the menu, puffer soup/ stew. Investigations by the California Department of Health and the FDA found that in all cases the soup was made with product originally invoiced by the California supplier as monkfish. No meal remnants were available from these incidents, so toxin presence and species identification could not be confirmed.

**Toxin analysis.** The three suspect soup samples (two cooked flesh samples and one broth sample) were confirmed by LC/ESI/MS/MS analysis to contain tetrodotoxin (463 ± 167 μg/100 g) (Table 1). Based on the estimated amount of soup consumed, the female patient consumed as much as 3 mg of tetrodotoxin, while her husband consumed as much as 1 mg of tetrodotoxin. Subsamples of white muscle from five fish from the box of returned suspect product all contained tetrodotoxin (range, 10 to 961 μg/100 g) (Fig. 1 and Table 1). All of these samples were found to be free of detectable levels of saxitoxin. There are currently no regulatory action levels for tetrodotoxin in the United States because no product legally sold in the United States should contain detectable
TABLE 1. Summary of quantitative toxin analysis and molecular fish identification for soup samples and controls

<table>
<thead>
<tr>
<th>Sample</th>
<th>BOLD nearest-neighbor result</th>
<th>Amt of tetrodotoxin (µg/100 g)</th>
<th>Amt of saxitoxin (µg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soup with fish flesh 1</td>
<td>Tetraodontidae&lt;sup&gt;a,b&lt;/sup&gt; (puffer fish)</td>
<td>374</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soup with fish flesh 2</td>
<td>Tetraodontidae&lt;sup&gt;a,b&lt;/sup&gt; (puffer fish)</td>
<td>655</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soup broth</td>
<td></td>
<td>361</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Returned lot, fish 1</td>
<td>Tetraodontidae&lt;sup&gt;a,b&lt;/sup&gt; (puffer fish)</td>
<td>961</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Returned lot, fish 2</td>
<td>Tetraodontidae&lt;sup&gt;a,b&lt;/sup&gt; (puffer fish)</td>
<td>876</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Returned lot, fish 3</td>
<td>Tetraodontidae&lt;sup&gt;a,b&lt;/sup&gt; (puffer fish)</td>
<td>214</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Returned lot, fish 4</td>
<td>Tetraodontidae&lt;sup&gt;a,b&lt;/sup&gt; (puffer fish)</td>
<td>10</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Returned lot, fish 5</td>
<td>Tetraodontidae&lt;sup&gt;a,b&lt;/sup&gt; (puffer fish)</td>
<td>10</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Monkfish 1</td>
<td>Lophiidae (goosefish/monkfish)</td>
<td>ND</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Monkfish 2</td>
<td>Lophiidae (goosefish/monkfish)</td>
<td>ND</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salmon control</td>
<td>Salmonidae (salmon/trout)</td>
<td>ND</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Snapper control</td>
<td>Lutjanidae (snapper)</td>
<td>ND</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> An additional Bayesian inference analysis of nodal confidence produced a value of 1.00 for the proposed nearest-neighbor relationship.
<sup>b</sup> Cytochrome c oxidase I sequences diverged by <2%.
<sup>c</sup> ND, not detected.

levels of this toxin. For comparison, the action level for saxitoxin, responsible for paralytic shellfish poisoning, which has a similar potency and pharmacology, is 80 µg/100 g. Both monkfish samples were found to be free of detectable levels of tetrodotoxin or saxitoxin.

**Fish identification.** Whole fish skin recovered from the patients’ home was consistent with the fish from the returned suspect lot and was not consistent with authentic monkfish (Figs. 2 and 3). Due to incompleteness of the specimens, visual species authentication of the suspect puffer samples was not possible. However, a presumptive identification of *Lagocephalus lunaris* (whose common names are “doku-sabafugu” or green rough-backed puffer) could be made based on coloration, shape of the tail, and the pattern of dorsal prickles (21). In addition, fish skins and headless fish from the suspect lot were more consistent with authentic puffer fish (a North American *Lagocephalus* sp.) than with whole monkfish collected from the California supplier, identified by expert fish taxonomists at the Smithsonian National Museum of Natural History as *Lophius litudon*, a known Asian commercial species (Figs. 2 through 4). At the time of analysis (24 July 2007) the validated subset of the reference sequences in the public BOLD database, defined as species represented by three or more specimens with sequences of at least 500 bp in length that diverge by <2%, included 7,413 barcode records representing 1,235 fish species. Of these, 41 barcode records represented the family Tetraodontidae (puffer fish), including nine distinct species. A comparison of sequences derived from the two fish flesh samples from the suspect soup and the five fish from the suspect lot did not result in an exact species level match within BOLD due to incomplete reference coverage at that time. However, a qualitative nearest-neighbor relationship with the sequences representing the Tetraodontidae family was obtained from the tree-based identification output of BOLD. Monkfish, salmon, and snapper samples showed correct nearest-neighbor relationships to the Lophiidae (goosefish/monkfish), Salmonidae (salmon), and Lutjanidae (snapper) families, respectively.
The additional Bayesian inference analysis utilizing 51 authentic tetraodontiform sequences, most of them acquired from sources outside BOLD, resulted in a posterior probability value of 1.00 for the nearest-neighbor relationship between the suspect soup samples and several specimens from the genus *Lagocephalus*, which fall within the Tetraodontidae (puffer fish) family (Fig. 5). This finding confirmed both the initial BOLD nearest-neighbor identification as Tetraodontidae and the presumptive visual identification of the suspect fish as *Lagocephalus* sp. The COI sequences from the implicated soup and the five fish from the returned suspect lot all diverged by <2%, which strongly suggests that they belong to the same species (13).

**DISCUSSION**

Despite the potential dangers associated with accidental ingestion of tetrodotoxin, puffer fish remains a sought-after delicacy among many cultures, particularly those of Southeast Asia (1, 6). In the United States, legal importation of puffer fish is limited to a single Japanese importer certified by the Japanese Ministry for Health and Welfare to ensure that the fish have been properly processed to be safe for
human consumption; the fish may be served only in Japanese restaurants by certified fugu (puffer fish) chefs on special occasions (2). However, previous cases of tetrodotoxin poisoning (6), as well as the case described in this report, demonstrate that illegal importation of puffer fish into the United States continues in response to consumer demand. Subsequent sale of the fish for home or commercial preparation puts consumers, who may not be aware of the illegality of the sale, at risk for tetrodotoxin poisoning. Although the hospitalized female patient described in this report survived the event, it is estimated that she consumed as much as 3 mg of tetrodotoxin, a potentially lethal dose. The severity of her illness highlights the importance of puffer fish toxicity as a public health and safety issue in the United States.

The multiagency response to this case of tetrodotoxin poisoning allowed the rapid identification of this dangerous food product and its removal from wholesale and retail markets. The subsequent investigation resulted in a voluntary nationwide recall of monkfish imported and sold by the implicated supplier on suspicion of product safety and possible...
incorrect labeling (29), potentially preventing additional illnesses and saving lives. Collaboration between poison control centers, local and state health departments, and federal agencies, including the FDA and Centers for Disease Control and Prevention, is essential to rapidly detect and respond to cases of foodborne toxin ingestion as well as other threats to the food supply. Local and state health departments provide a valuable coordinating link between healthcare providers, local response agencies, and federal partners. In this instance, the Chicago and Illinois Departments of Public Health worked together to ensure the collection and submission of fish specimens to the FDA CFSAN Laboratory, the investigation of the local retail and wholesale markets together with the FDA field offices, and the notification of the local medical community to enhance surveillance for additional cases of tetrodotoxin poisoning. Also, collaboration between federal agencies and academic institutions allowed for access to the latest testing techniques with transfer of these methods for use in regulatory compliance.

The FDA’s case for illegal importation and improper labeling (i.e., that the fish purchased by the patient was puffer fish, and not monkfish as claimed by the retailer) was strengthened by several pieces of evidence, including (i) visual indication that both the fish skins recovered from the patients’ home and fish from the suspect lot recovered from the California supplier were not consistent with monkfish; (ii) detection of significant quantities of tetrodotoxin, a toxin not previously reported in monkfish; and (iii) COI sequences consistent with reference sequences from the family Tetraodontidae in both the leftover soup and the fish from the returned suspect lot. As a result of this investigation, in October 2007 the California supplier was placed on FDA Import Alert for species misbranding (30). This case underscores the importance of retrieval and analysis of fish specimens and meal remnants following a clinical diagnosis. Testing at the FDA CFSAN laboratory is not available commercially and should be coordinated through local and/or state public health authorities and the Laboratory Response Network to ensure appropriate request for testing and interpretation of results. Lack of laboratory confirmation may result in misdiagnosis or missing of cases which could have far-reaching consequences, such as additional illnesses or even deaths, if the source of the toxin is not identified. Laboratory confirmation is also an important epidemiologic tool in determining the geographic and species range of marine toxins, as was demonstrated in 2002, when the presence of saxitoxin (a marine toxin with symptoms indistinguishable from those of tetrodotoxin) was confirmed in puffer fish caught off the coast of Florida, an area where puffer fish were previously believed to be free of neurotoxins (7, 11, 20). While additional work is needed to establish regulatory action levels for tetrodotoxin, the novel laboratory techniques used in this investigation will likely improve the FDA’s capability to prevent illegal toxic fish importation. Fish identification through the use of short standardized COI mitochondrial sequences is currently in the process of being validated at FDA CFSAN as a new tool for regulatory compliance.

A note of particular interest in this case is the high concentrations of tetrodotoxin found in the puffer muscle. In most commercial Asian puffer species, muscle is nontoxic or weakly toxic in properly prepared specimens. This has allowed the commercial exploitation of even highly toxic (in the viscera) Takifugu spp. In 1983, the Japanese Ministry of Health and Welfare enacted guidelines for edible puffer species and parts, which include only muscle, skin, and testicles (24). Most poisonings occur either from cross-contamination of the muscle due to improper preparation or from consuming nonapproved parts, for example, liver, a Japanese delicacy known as “kimo.” One exception appears to be the species Lagocephalus lunaris, which is one of the few species found to commonly contain high levels of tetrodotoxin in the muscle (4, 5, 23, 26). Consumption of products such as dried fish filets or fish balls made from L. lunaris or a mixture of L. lunaris with other nontoxic species has resulted in poisonings, even though the sale of this species is prohibited in many Asian countries (9, 16). Complicating matters is the fact that L. lunaris is similar in appearance to several other Lagocephalus spp., such as L. inermis, L. gloveri, L. wheeleri, and, in particular, L. spadiceus. The muscle in all of these look-alike species is considered safe for consumption, and harvesting of these species is allowed in several Asian countries (5, 16, 24). The unprecedented 28 cases of puffer fish poisoning that occurred on the East Coast of the United States between 2002 and 2004 due to the consumption of domestic southern puffer fish (Sphoeriodes nephelus) from the Northern Indian River Lagoon in Florida was similarly due to the fact that saxitoxin was found predominantly in muscle tissue, making safe preparation of this product impossible, even by individuals trained in the preparation of toxic Asian puffers (11, 20). In this investigation, we could not genetically confirm the species identity of the suspect fish product as L. lunaris, due to a lack of authenticated standard, but DNA barcode analysis clearly identified this product as Lagocephalus sp.; visual inspection of recovered skin and headless product was highly suggestive of L. lunaris, and genetic analysis ruled out two of the potential look-alike species, L. specideus and L. inermis. We are currently attempting to obtain an authentic specimen of L. lunaris to confirm this presumptive identification.

This case of tetrodotoxin poisoning demonstrates the need for continued stringent regulation of the importation of puffer fish by the FDA. However, the problem of inadvertent or deliberate mislabeling of fish imported for personal or commercial use is not easily addressed. Neither is the fact that individuals will continue to purchase illegally imported puffer fish if it is available, either knowingly or through ignorance of the import regulations. It is essential to educate the public of the dangers of puffer fish consumption, including information that tetrodotoxin is not destroyed by freezing or cooking; to recognize the signs and symptoms of tetrodotoxin poisoning; and to seek immediate medical attention if symptoms occur following puffer fish ingestion. Due to the above concerns, in October 2007 the FDA released separate consumer and industry advisories informing the public of the proper, legal, and safe sources of puffer fish in the United States (31, 32). It is also important to raise awareness among medical providers in the United States of the diagnosis and management of tetrodotoxin and other foodborne toxin ingestions, the resources
available at poison control centers, and the need to immediately notify local public health authorities if foodborne toxin exposure is suspected.

ACKNOWLEDGMENTS

The authors acknowledge the contributions of the FDA’s Chicago and Los Angeles District Office personnel for the collection of field samples, and Professor Keiichi Matsuura of the National Museum of Nature and Science, Tokyo, Japan; Drs. Jeffrey Williams and Chris Meyer of the Smithsonian National Museum of Natural History, Washington, DC; and Dr. Serge Planes (CRIOBÉ, UMS 2978 EPHE-CNRS, Morcea, Polynesia) for providing reference tissues and expert fish identifications used in this investigation. The Canadian Barcode of Life Network received funding for DNA barcoding from Genome Canada (through the Ontario Genomics Institute), NSERC and other sponsors listed at www.BOLNET.ca. The authors additionally acknowledge the California Department of Public Health, Los Angeles County Department of Public Health, New Jersey Department of Health and Senior Services, and Bergen County Department of Health Services for their roles in investigating cases in their jurisdictions.

REFERENCES