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CLINICS / *CLINIQUE*

Family tetrodotoxin poisoning in Reunion Island (Southwest Indian Ocean) following the consumption of *Lagocephalus sceleratus* (Pufferfish)

Intoxication familiale à La Réunion (Sud-ouest de l'océan Indien), par ingestion de *Lagocephalus sceleratus* (Tétrodon)

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Abstract Pufferfish poisoning has rarely been reported in the southwestern Indian Ocean and in the French overseas territories. In Reunion Island, the last notified documented case occurred in 1989 and people are no longer aware of the potential toxicity of pufferfish. We report a family hospitalized for a tetrodotoxin poisoning following the consumption of *Lagocephalus sceleratus* caught on the coast of Reunion Island in September 2013. Two patients presenting acute vital functions failures were admitted in an ICU. Ten people were admitted simultaneously to the emergency department after consuming *L. sceleratus* with signs of toxicity appearing within 2 hours. Treatment was supportive, but included the need for mechanical ventilation for two patients. All those affected had complete and uneventful recoveries within a few days. The fish consumed was identified as *L. sceleratus*, a species known to contain tetrodotoxin. The diagnosis of tetrodotoxin poisoning was suggested by typical clinical manifestations together with the history of very recent consumption of tetrodotoxin-containing fish. Tetrodotoxin was later detected at high levels in food remnants. To the best of our knowledge, there has been no documented case series of tetrodotoxin poisoning reported from Reunion Island for the last 25 years and from the entire Indian Ocean area since 1998. Pufferfish intoxication is one of the most common causes of poisoning among people in coastal regions of Asia but it has also recently been reported in areas where it was previously unknown, particularly along the Mediterranean shores and in Spain. Public health education in French overseas territories and along the Mediterranean shores should be adapted to include increased awareness of the danger of consuming pufferfish. Health teams must be aware of such clinical presentations.

Keywords Tetrodotoxin · Pufferfish · *Lagocephalus sceleratus* · Intensive care · Fish poisoning · Indian Ocean · Reunion Island

Résumé Les intoxications par les tétrodons ont été rarement rapportées dans le sud-ouest de l'océan Indien et les territoires français d'outremer. À La Réunion, le dernier cas documenté remonte à 1989 et beaucoup de Réunionnais ne connaissent plus la toxicité des tétrodons. Nous rapportons le cas d'une intoxication familiale à la suite de la consommation d'un tétrodon (*Lagocephalus sceleratus*) pêché dans les eaux réunionnaises en septembre 2013. Dix personnes ont été hospitalisées en même temps deux heures après avoir consommé du *L. sceleratus*. Deux personnes âgées présentant des défaillances d'organes ont dû être admises en réanimation pour ventilation mécanique. Tous les patients ont parfaitement récupéré en quelques jours. L'intoxication par *L. sceleratus* a été suspectée d'après les signes cliniques, puis confirmée par la photographie du poisson prise par le pêcheur. La tétrodotoxine a été isolée à d'importantes

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concentrations dans les restes alimentaires. À notre connaissance, il n'y avait pas eu de cas documenté d'intoxication à la tétrodotoxine depuis 25 ans à La Réunion et depuis 1998 dans l'océan Indien. Ce type d'intoxication est fréquent en Asie, mais a également été récemment rapporté en Méditerranée (migration par le canal de Suez) et en Espagne, sur la côte Atlantique. L'information des populations et des personnels soignants de l'outremer français doit être renforcée, ainsi que dans les pays méditerranéens.

Mots clés Tétrodotoxine · Tétrodon · *Lagocephalus sceleratus* · Réanimation · Intoxication par les poisons · Océan Indien · La Réunion

Context

Pufferfish poisoning has been rarely documented in the southwest Indian Ocean. In Reunion Island, a French overseas territory, the last case was reported in 1989 and the population is no longer aware of the toxicity of the pufferfish, locally known as "*bouftangue*" or "*poisson ballon"* (Fig. 1). Alternative names are: Giant Toadfish, Giant Toado, North-west Blowfish, Silver Pufferfish, Silver-checked Toadfish, Silver-stripe Pufferfish. The species can be recognised by the silver stripe along the sides of the body and the greenish coloured back with dark spots. The pufferfish are consumed in Japan [11] as well as their cousins Takifugu that are known in Europe under the general term of fugu.

Objectives

We report a tetrodotoxin poisoning affecting an entire family following consumption of *Lagocephalus sceleratus* (Gmelin, 1789) caught along Reunion island coast, including two cases of acute respiratory failure.

Case details

On the evening of September 10th, 2013, 6 adults and 4 children of the same family were admitted to the emergency room with various neurological, gastrointestinal, cardiac symptoms: peri-oral paraesthesia, weakness of both lower limbs, paraesthesia all over the body, headache, dyspnea, nausea and vomiting, blurring of vision, and vertigo. The medical history highlighted the ingestion of *L. sceleratus* as a curry – a typical cooked meal in Reunion Island – two hours prior to hospital admission. The prompt identification of the fish by attending doctors from photographs taken by the fisherman who caught the 4 kg fish at a depth of 70-80 meters (Fig. 2), off the city of Saint-Paul, enabled an immediate diagnosis and prompted symptomatic treatment.

Elderly people (the fisherman's father and mother) who had eaten liver, flesh and eggs of the fish required immediate ventilatory support for an ascending paralysis involving the limbs and the respiratory muscles and were transferred to our ICU.

Patient 1 (74-year-old man suffering from chronic obstructive bronchitis) presented with dysarthria, motor disturbances, myoclonus and a motor deficit that expanded rapidly requiring intubation and mechanical ventilation. Upon arrival in the ICU the patient had bradycardia (41 bpm), hypotension (60/38 mm Hg), nonreactive mydriasis, absent deep tendon reflexes and hypothermia (33.8°C). Hypotension was controlled with IV norepinephrin bitartrate (0.5 mg/hour over 20 hours). Bradychardia was controlled with small doses of atropine (1/2 mg IV, twice). Motor disturbances, mydriasis

Fig. 1 *Lagocephalus sceleratus* (Picture by Jean-Lou Justine, Muséum National d'Histoire Naturelle, France, http://www.fishbase.org). See more at: http://australianmuseum.net.au/Silver-Toadfish-Lagocephalus-sceleratus#sthash.4qT6Jqju.dpuf / *Lagocephalus sceleratus* (Photographie de Jean-Lou Justine, Muséum National d'Histoire Naturelle, France, http://www.fishbase.org). En voir plus sur : http://australianmuseum.net.au/Silver-Toadfish-Lagocephalus-sceleratus#sthash.4qT6Jqju.dpuf

Fig. 2 The fisherman and the yet unidentified poisonous pufferfish / *Le pêcheur et le tétrodon toxique encore méconnu*

and bradychardia subsided within 24 hours, but hyporeflexia lasted 48 hours. The patient was weaned from mechanical ventilation 36 hours after admission and discharged from the ICU.

Patient 2 (68-year-old obese woman suffering hypertension and type 2 diabetes) rapidly developed tetraparesis and deep coma with a GCS of 3, requiring ventilatory support. Upon arrival at the ICU, blood pressure was 114/72 mmHg, pulse rate 57 bpm, body temperature 35,2°C. She had generalised hyporeflexia and bilateral nonreactive mydriasis that subsided within 24 hours. She was weaned from the mechanical ventilation within 24 hours and discharged from the ICU.

Both patients exhibited unexplained severe thrombocytopenia (patient 1: 56 G/L, patient 2: 75 G/L) with normal bone marrow examination results.

The other eight members of the family did not require any life support measures. Their specifications, clinical signs and outcomes are summarized in the table 1.

Biochemical studies

The remains of the cooked meal of the causative food poisoning were recovered and frozen until the assay carried out. Extractions and analyzes were performed on muscle and liver samples separately.

Muscle (30 g) and liver (20 g) were separately homogenized (UltraTurax) for 5 min with 3 volumes of 1% acetic acid in methanol and centrifuged (1.000 g, 20 min). The operation was repeated twice. The supernatants were combined, concentrated under reduced pressure at 45°C. Residues were dissolved in 1 volume of 1% acetic acid in water and kept at −20 ◦C until use. We have examined toxicity by the mouse assay for TTX. Lethal potency was expressed in mouse units (MU). One MU is equal to 0.178 g TTX, defined here as the amount of toxin required to kill 20 g of OF1 strain mice in 30 minutes after intraperitoneal injection.

Muscle and liver aliquots (50 μL) were diluted with 950 μL of 5mM ammonium formiate in methanol (50/50) and homogenised for 1 min using a vortex. Homogenised aliquots were directly infused in the electrospray source at a 10 μL/min flow rate with a syringe pump. A mass spectrometry was then performed using the 3200Q TRAP mass spectrometer (ABI-Sciex, Toronto, Canada) equipped with an electrospray ion (ESI) source on the positive-ion acquisition data mode. Ion source setting parameters were optimized as follows: temperature at 500°C, ion spray voltage at 5500V, curtain gas at 10 psi and ion source gas at 20 psi.

In order to get the best signal to noise, data acquisition was operated in positive enhanced product ion (EPI) in targeted ion mass at *m/z* 320.0 Da, which corresponded to TTX (MH^+). Declustering potential (DP) was at 65 V, Entrance potential (EP) and collision cell exit potential (CXP) were optimised at 8 V and 4 V respectively. Collision energy (CE) was performed at 45 eV with collision energy spray (CES) at 15 eV.

Mouse bioassay is used to identify an unknown toxin extract in comparison with a TTX-specific dose–death time relationship curve [9]. A series of test solutions are prepared by diluting the unknown toxin extract with 1% acetic acid. One ml of each test solution is intraperitoneally preliminary injected into a mouse. The solution that causes the death of the mice within 15 minutes was selected and injected a lot of 3 mice to determine the toxicity. After injection with extracts, the mice show the characteristic signs and symptoms, like uncoordinated movement, rapid breathing difficulties, convulsion, and jumping, followed by respiratory failure.

Toxicity of the liver and flesh were 95 MU/g (equivalent to 17 μg/g of TTX) and 5 MU/g (equivalent to 5 μg/g of TTX) respectively. The analysis (Figs. 3, 4) confirmed the presence of TTX.

Accordingly to intensities obtained for the two samples using EPI acquisition, TTX concentration is greater in the liver approximately 5 times more than in the muscle.

Tableau 1 Specifications, clinical signs and outcomes of the 8 patients not admitted in the intensive ward / *Caractéristiques, signes cliniques et devenir des 8 patients non admis en service de réanimation*.

Patient	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Sex	M	M	M	M	M	M	F	M
Age (year)	49	46	36	23	13	10	9	10
Initial clinical signs	Nausea, mouth and distal paresthesia	Mouth paresthesia, limbs gait disturbance	Mouth paresthesia,	Right thigh paresthesia,	None	Nausea, generalised pruritus without skin rash	None	None
Pulse (beats/mn)	63	72	73	84	75	65	65	86
Blood pressure (mmHg)	130/81	145/100	145/106	131/85	138/95	132/71	100/75	99/74
Secondary clinical signs	H8: kinetic and static cerebellar syndrome, vertigo	H8: kinetic and static cerebellar syndrome, vertigo	H8: no signs	None	None	None	None	None
Treatment	None	None	None	None	None	None	None	None
Outcome	Medical ward	Medical ward	Emergency room	Home at H 10	Paediatric ward	Paediatric ward	Paedia-tric ward	Paedia-tric ward
Duration of hospitalization	5 days	5 days	10 hours	10 hours	18 hours	36 hours	18 hours	18 hours
Eaten part of the fish	Flesh, liver++, eggs+	Flesh, liver++, eggs+	Flesh: 2 mouth-fuls	Flesh: 2 mouthfuls	Flesh: 1 mouth-ful	Flesh: 4 mouthfuls	Flesh: 1 mouth-ful	Flesh: 2 mouth-fuls

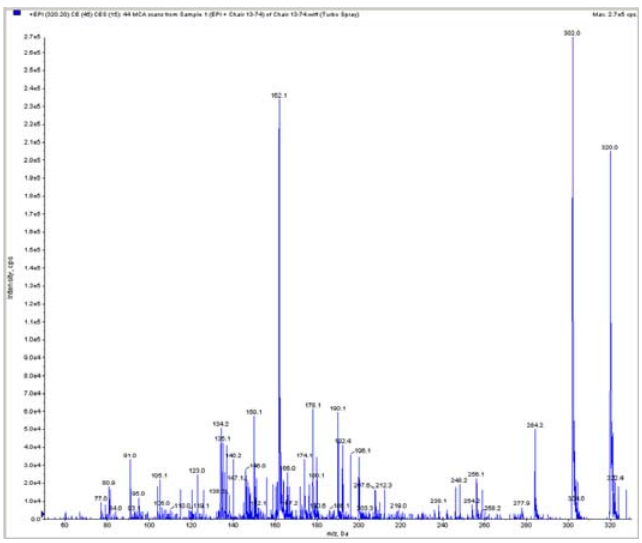

Fig. 3 Fragmentation ion profile in LC–MS/MS system on EPI of (A) muscle and (B) liver of *L. sceleratus* cooked / *Profil de la fragmentation ionique dans le système LC–MS/MS sur EPI du (A) muscle et (B) du foie des restes cuisinés de L. sceleratus*

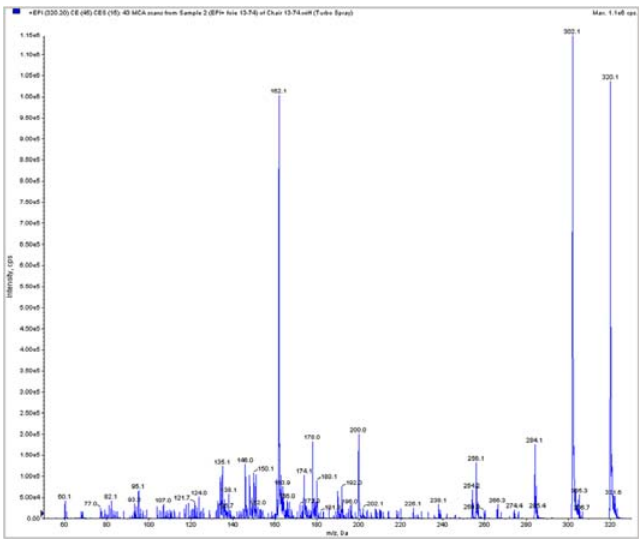

Fig. 4 Presence of TTX (MH^+, *m/z* 320 Da) in the two aliquots (tissue and liver) with specific selected ions *m/z* 162, 256 and 302 corresponding to the ions of TTX fragmentation / *Présence de TTX (MH^+, m/z 320 Da) dans deux aliquots (chair et foie) avec des ions spécifiques m/z 162, 256 et 302 correspondant aux ions de la fragmentation de TTX*

Discussion

TTX and its derivatives are known to be produced not by the fish themselves but by bacteria in which they are detected by HPLC analysis [18].

The scarcity of reported local cases of puffer toxicity led to this collective fish poisoning. Moreover, the type of local food preparation "carri" eased the dissemination of the toxin by mixing different parts of the fish: normally the fish is peeled, cut into steaks, keeping the head and tail, fried in a little oil. Onion, garlic, ginger and tomatoes are added and a glass of water is poured on the mixture that is brought to the boil. In the case presented, the fish was cooked with the skin and liver.

Local people do not seem to be aware of the current European Union legislative requirements that ban poisonous fish of the family Tetraodontidae and products derived from them from the European markets, including Reunion Island.

Questioning of relatives revealed that the severity of the clinical presentation was linked to the quantity of fish ingested and to the parts of the fish eaten. With respect due to their position within the family as grandparents, the two most severe cases had enjoyed the liver, flesh and eggs.

In humans, tetrodotoxin (TTX) inhibits voltage-gated sodium channels in a highly potent and selective manner without effects on any other receptor or ion channel systems. Recently, TTX-resistant sodium channels have been discovered in the nervous system and received much attention because of their role in pain sensation [14].

In fatal cases, the death results from respiratory arrest. Patients should survive if they arrive at a well-equipped hospital, conscious and prior to the respiratory arrest. A high mortality is reported in countries were a rapid admission in an ICU is not warranted, like in Bangladesh [12]. To our knowledge, thrombocytopenia has never been reported.

There is currently no effective antidote to tetrodotoxin poisoning. Treatment is entirely supportive and symptomatic. That is including mechanical ventilation or inotropic support in most severe cases. Some advocated treatments [9] : cholinesterase inhibitors, naloxone, activated charcoal, gastric lavage with sodium bicarbonate, antihistamines and steroids are nowadays losing favour in the treatment of most acute poisoning because of their side effects or because of the lack of proven benefit.

Our patients were not treated with cholinesterase inhibitors [7] for rapid reversal of TTX toxicity, as mechanical ventilation was immediately available.

Pufferfish intoxication is one of the most common causes of poisoning among people in coastal regions of Asia. However, several cases of poisoning have been recently reported in areas where it was previously unknown, particularly along the Mediterranean shores: Lebanon [2], Israel [5], Greece [13], Turkey [4], Marocco [1]. This appears to be the result of migration of the Lessepsian migrant pufferfish *L. sceleratus* through the Suez Channel. Furthermore, Europe is not anymore spared, since smooth puffer *Lagocephalus laevigatus* was reported from Galician waters (Northwest Spain) with three considered mechanisms of introduction: natural displacement, the aquarist trade and transport in ballast water [3]. The first European case was recently reported in Spain in a patient who ingested a trumpet shellfish (*Charonia lampas lampa*) caught along the Portuguese coast (Atlantic ocean), that also contains tetrodotoxin [8].

In French Guiana, another French overseas territories, TTX poisoning cases have been reported in 1992 and in 2010 [10,17].

In the Indian Ocean, a fatal case was reported in a Zanzibari fisherman in 1967 [6]. Four cases with one death were reported in Nosy Bé (Madagascar) in 1998 [16]. In Reunion Island, pufferfish poisoning cases have been reported in the past [15]: in 1959, one person died and twelve survived following the ingestion of *L. sceleratus*; in 1972, two children died and five persons survived following the ingestion of *L. sceleratus*; in 1980 two survived and in 1989, a woman survived. Since then, no case was reported.

Conclusion

Health information campaigns should make the public more aware of the potential risk of consuming pufferfish, in the French overseas territories, as well as along the Mediterranean countries. Emergency and ICU practioners must be aware of such clinical presentations that require a prompt supportive treatment.

Ethical statement

All human and animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All study participants gave verbal informed consent for participation in this investigation and publication of the photograph of the fisherman.

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Declaration of interest : On behalf of all authors, the corresponding author states that there is no conflict of interest.

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