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SEA BIRD MORTALITY AT CABO SAN LUCAS, MEXICO: EVIDENCE THAT TOXIC DIATOM BLOOMS ARE SPREADING

A. SIERRA BELTRÁN,[1] M. PALAFOX-URIBE,[2] J. GRAJALES-MONTIEL,[3] A. CRUZ-VILLACORTA[1] and J. L. OCHOA[1]*

[1]Center for Biological Research NW, Box 128, La Paz, BCS 23000, Mexico; [2]Environmental Protection Agency (Procuraduria del Medio Ambiente en BCS), Delegación La Paz, BCS, Mexico and [3]Health Institute of BCS (Instituto Estatal de Salud en BCS), La Paz, BCS, Mexico

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A. Sierra Beltrán, M. Palafox-Uribe, J. Grajales-Montiel, A. Cruz-Villacorta and J. L. Ochoa. Sea bird mortality at Cabo San Lucas, Mexico: evidence that toxic diatom blooms are spreading. *Toxicon* **35**, 447–453, 1997.—Domoic acid was found to be responsible for an isolated event involving the massive death of brown pelicans (*Pelecanus occidentalis*) in January 1996, at the tip of the Baja California peninsula. The death of these sea birds was the result of feeding on mackerel (*Scomber japonicus*) contaminated by domoic acid-producing diatoms (*Pseudonitzschia* sp.). The number of dead birds (150 animals) found during a period of 5 days caused alarm and called for a govermental task force that would help to implement emergency measurements to protect other species of bird. Also, local canneries were inspected to verify the safety of their recent production and prevent the toxin entering the human market. Fortunately, the timing, response and coordination of this task force enabled identification of the origin and nature of the toxin that provoked such a phenomenon. Future monitoring is recommended to avoid a larger impact of domoic acid spreading and the occurrence of similar toxic events.

INTRODUCTION

The first reports on the massive, seemingly unnatural, death of sea birds, such as brown pelicans (*Pelecanus occidentalis*) and cormorants (*Phalacrocorax penicillatus*) in the American continent, refer to events that occurred in Monterey Bay in 1991 (Work *et al.*, 1991, 1993) and 1 year later in Santa Cruz, California, U.S.A. (Work *et al.*, 1993). The phenomenon now appears to have extended southwards, and took place for a second time at the Baja California peninsula early in January 1996 (the first event in the Baja California

*Author to whom correspondence should be addressed.

northern state was reported by Morales Chávez *et al.*, 1994, describing a case recorded in 1992 in which 110 pelicans were found dead at Ensenada BC, Mexico. The source, or cause of death, was not elucidated). In all these cases, affected pelicans showed evidence of central nervous disorders not known in other common causes of bird mortality; and hence a foreign toxic compound was suspected. As reported earlier by Work *et al.* (1993), the cause of death of brown pelicans at Monterey Bay and Santa Cruz was shown to be related to domoic acid (DA) produced by pennate diatoms, namely *Pseudonitzschia pungens* f. multiseries and *P. australis*, contaminating northern anchovies (*Engraulis mordax*) that were eaten by the birds. In our case, as shown later, we were able to explain the death of the sea birds by determining the presence of such toxic microorganisms and/or DA in the stomach content of both mackerel and pelicans.

MATERIALS AND METHODS

On 5 January 1996, The Marine Pathology Unit of CIB received four specimens of pelicans collected at Cabo San Lucas (BCS, Mexico), designated CSL-1–4, and one specimen from La Paz (BCS,Mexico), designated LAP-1. Only CSL-1 and CSL-2 were alive, and showed serious symptoms of illness. They were all dissected and smears prepared to observe their stomach content by light microscopy (× 400 and × 1000) and to obtain digestive tract (DT) and liver (L) extracts. The viscera of the dead animals were already in an advanced state of decomposition and were discarded, while those from the sick animals were extracted separately with a volume (w/v) of 0.1 N HCl, boiled for 5 min and centrifuged at 2800 ***g*** for 15 min. From each supernatant, 1 ml was injected i.p. into each mouse of a group of three albino Swiss strain mice (18–23 g, about 5 weeks old). As a standard, purified DA (Sigma Chemical Co., St Louis, MO, U.S.A.) was used at a concentration of 40 μg/ml, and from this solution 1.0, 0.7 and 0.5 ml were injected into each mouse of a set of three animals as above. Dose thus represented 40, 30 and 20 μg/animal, respectively. Surviving mice were all observed for 48 hr before being killed by cervical dislocation. In addition, fresh mackerel, obtained from a local bait retailer, and sardines, both fresh (eviscerated and headless) and in cans (from recent collection), were analysed as above, except that in these cases only the stomach content (SC) and whole fish ground meal (GM) were included as fractions.

DA, ([2S-[2α,3β,4β (1Z, 3E, 5R)]]-2-carboxy-4-(5-carboxy-1-methyl-1,3-hexadienyl)-3-pyrrolidineacetic acid), has a mol. wt of 311.34 and the formula $C_{15}H_{21}NO_6$. It is a white powder with m.p. 223–224°C, is soluble in water and resembles kainic acid (IOC, 1995). DA analysis was carried out by high-performance liquid chromatography (HPLC) using the method described by Quilliam *et al.* (1995) and IOC (1994). Samples from the supernatants of liver and digestive tract extracts were first cleared through 0.20 μm Gelman Acrodisc 13CR PTFE filters. The DA (1 ng/μl) standard was prepared in acetonitrile HPLC grade. A 4.6 mm × 25 cm of Ultrasphere ODS-C18 column was adapted to a Beckman HPLC System Gold using 20 μl Rheodyne as injector and the UV detector at 242 nm. The mobile phase consisted of 3 ml of 85% w/v H_3PO_4, 873 ml of HPLC grade water, and 125 HPLC grade acetonitrile; the flow rate was set at 1.5 ml/min.

RESULTS

All pelicans showed an empty stomach (which suggested earlier vomiting episodes) while the digestive tract was still occupied by the remains of recent feedings from which smears and extracts were prepared. Under the light microscope, the samples of Cabo San Lucas Pelicans and mackerel showed some structures resembling empty frustules corresponding to the diatom *Pseudonitzschia* sp. Unfortunately, it was not possible to establish a culture or confirm their identity by electronic microscopy. The samples analysed by HPLC analysis are indicated in Fig. 1 and a summary of mouse bioassay results is shown in Table 1. The chromatographic pattern of the DT extract from pelican CSL-1 is shown in Fig. 1(a); Fig. 1(b) illustrates the corresponding pattern of the DT extract from pelican LAP-1, which presumably died of natural causes. The DT extract pattern of mackerel M-SB is shown in Fig. 1(c) and that of sardine M-SB in Fig. 1(d). The DA standard pattern is shown in Fig. 1(e). From these data we may conclude that the peak that corresponds to DA appears only in the samples from pelican CSL-1 and mackerel. The HPLC analysis showed no

indication of the presence of the toxin in the dead pelican LAP-1, the fresh sardine samples or the canned fish.

Of the bird extracts tested, none but the stomach content of pelican CSL-1 elicited visible symptoms of DA intoxication as detected by the mouse bioassay (Table 1). Most of the seabird extracts injected into mice indicated very low amounts of DA and, as previously mentioned, we were unable to detect them quantitatively (see Fig. 1). This test confirmed the presence of DA in mackerel sample M-SB, which showed appreciable amounts of the

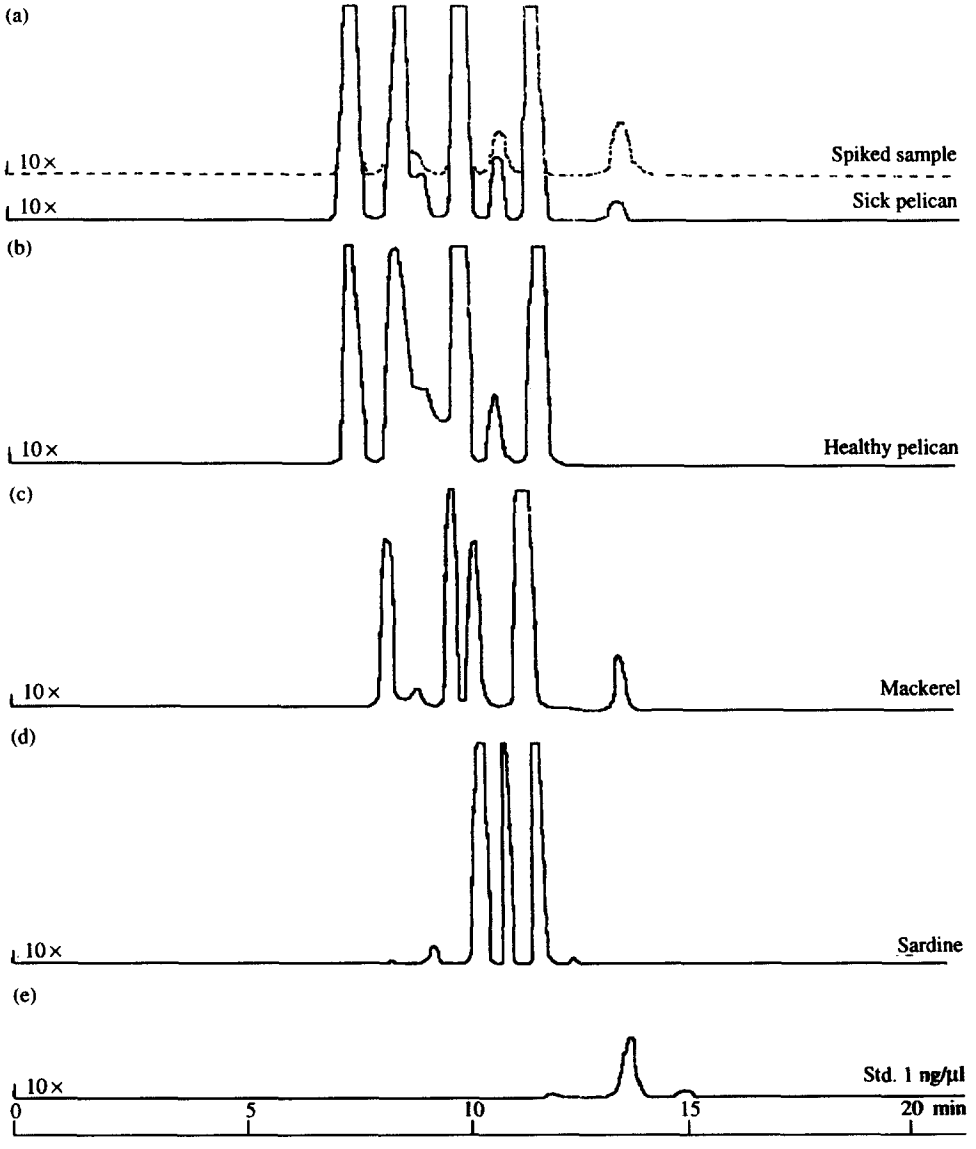

Fig. 1. HPLC analysis by Quilliam's method (1994).
To confirm the authenticity of the peak, pure domoic acid was added to the sick pelican sample.
(a) CSL-1 DT; (b) LAP-1 DT; (c) M-SB SC; (d) M-SS SC; (e) pure domoic acid. Gain: 1 × and 10 × . Retention time in minutes.

Table 1. Mouse bioassay and HPLC analysis of pelicans and fish

Pelican/fish	Organ/tissue	Weight (g)	Mouse bioassay	DA concentration by HPLC (μg/g)
CSL-1	L	38	+	Traces
CSL-1	DT	5	+ + +	37.17
CSL-2	L	45	+	Traces
CSL-2	DT	11	+	Traces
LAP-1	DT	2	−	Negative
M-SB	SC	3	+ + +	142.85
M-SS	SC	15	−	Negative
FSLM	DT	8	−	Negative
CSLM (0816)	GM	25	−	Negative
CSLM (09M5)	GM	25	−	Negative
CSLM (1016)	GM	25	−	Negative
FSC	GM	25	−	Negative
SSC (M1-202)	GM	25	−	Negative
SSC (M2-185)	GM	25	−	Negative
SSC (M3-197)	GM	25	−	Negative
SSC (M4-001)	GM	25	−	Negative

CSL, Pelican specimens obtained from Cabo San Lucas (BCS, Mexico); LAP, pelican from La Paz (BCS, Mexico); M-SB and M-SS, mackerel from Cabo San Lucas (BCS, Mexico); FSLM, sardine from López-Mateos (BCS, Mexico); CSLM (lot no.), canned sardine from López-Mateos (BCS, Mexico); FSC, sardine from San Carlos (BCS, Mexico); SSC (lot no.), canned sardine from San Carlos (BCS, Mexico); L, liver extract; DT, digestive extract; SC, stomach content; GM, ground meal extract.

toxin in the HPLC analysis (Fig. 1). The sardine extracts were all negative in the mouse bioasssay and can be considered safe for consumptiom.

As mentioned, the mouse bioassay yielded positive results for the stomach content extract of pelican CSL-1. The injected mice showed symptoms corresponding to DA intoxication such as akinesia, postration and scratching during the first 40 min. At the end of 139 min, all the animals had diarrhea. The first animal died after 18 hr, while the other two showed convulsions, loss of lateral movement and motor incoordination. They died 20 hr after injection. The mice that survived the 48 hr period of observation also showed symptoms of slight intoxication, e.g. akinesia, scratching and lack of motor coordination. In these cases none showed diarrhea. The positive control group at a dose of 20–40 μg showed first akinesia and postration, then tremors, gating, motor incoordination, hindlimb stroll and convulsions, and died at 95–200 min. All symptoms were dose related, being maximum at higher doses. A pelican (LAP-1) found dead in La Paz Bay (Mexico) was used as a negative control. This animal, as the ones collected dead from Cabo San Lucas, was handled to obtain only the digestive tract extract. In contrast to the specimens from Cabo San Lucas which showed at least traces of DA, the extract of pelican LAP-1 had no toxic effect in mice. This excludes the possibility that its death was caused by a similar intoxication, and no effort was made to clarify the reason for death.

DISCUSSION

DA was first isolated from the red algae *Chondria armata* and later also found in diatoms such as *P. pungens*, *P. pseudodelicatissima*, *P. australis*, *Nitzschia actydrophila*, *P. seriata* and *Amphora coffaeiformis* Cl. Only in cultures of the latter, but not in the case of *P. pungens f. pungens*, has the production of DA been observed (IOC, 1995).

DA acts as an agonist to glutamate receptors (IOC, 1995) which open Na^+ ion chanels in the postsynaptic membrane, inducing depolarization. This in turn increases the Ca^{2+}

permeability which ultimately leads to cell death. After consumption of DA-contaminated seafood, the following gastrointestinal symptoms have been observed within the first 24 hr: nausea, vomiting, headache, abodominal cramps, or at least one of the following neurological symptoms within 48 hr: confusion, lost of memory, disorientation, or other serious neurological signs such as seizures, coma and death. Symptoms such as hypoactivity, sedation-akinesia, rigidity, stereotypy, loss of postural control, tremors and convulsion have been observed in mice. The level of action for DA in shellfish is 40 μg/g wet weight. The upper limit allowed for human consumption of shellfish is 20 μg/g.

In late December 1995 and the first week of January 1996, a singular event related to sea bird mortality was observed at Cabo San Lucas (Mexico). Only a few seagulls but over 100 pelicans were found dead in a place considered as their shelter. Many more live pelicans showed symptoms of intoxication such as disorientation and agitation, and when in the water they submerged and had difficulty in swimming; if they turned upside-down, they could not right themselves and drowned. Similar behavior was observed in the cases of massive pelican deaths reported in Monterey Bay and Santa Cruz 4–5 years earlier (Work *et al.*, 1993), where DA was found to be the cause.

DA was isolated for the first time after hundreds of reports of illness in humans related to consumption of mussels harvested in Prince Edward Island, Canada (Bates *et al.*, 1989). The mouse bioassay indicated that these animals showed unusual neurotoxic clinical signs before dying of suffocation. DA is an agonist of glutamate receptors, producing in rodents effects such as inactivity, followed by seizures, as with many other toxins, with a characteristic scratching response (Work *et al.*, 1993). Neurons from the hippocampus area are seriously damaged or lost (Peng *et al.*, 1994). When administered orally, large amounts of DA are recovered in the feces, indicating that absorption is difficult (Iverson and Truelove, 1994) and that some human preexisting medical conditions, such as diabetes, hypertension and chronic renal disease, may exacerbate its effects (IOC, 1995).

The lowest level of DA detected by the mouse bioassay, which uses behavioral effects rather than death endpoint, is 23 μg when injected i.p., which is equivalent to 46 ppm in mussels. No symptoms are observed at doses of 20 ppm or below, therefore for monitoring purposes this level has been adopted as the maximum tolerated dose in humans (IOC, 1995).

The most worrying of the events described above is the ease with which the disease can spread into the human food chain, thus it is absolutely necessary to implement strategies for an effective monitoring program on edible species known to accumulate the toxin, e.g. sardine, mackerel, razor clam, Dungeness crab, and shellfish in general (Wekell *et al.*, 1994). Canada and the U.S.A. have introduced monitoring programs for DA which have identified the relationship between different species of diatoms that provoked toxic episodes. For example, in 1992 sequenced blooms of *P. pungens* and *P. australis* were detected on the west coast of Vancouver Island (Taylor, 1993). First, *P. pungens* was shown not to be responsible for producing the toxin. Only when its blooming was followed by a bloom of *P. australis* did the DA assays become positive. Such observations indicate that, in general, *P. australis* and not *P. pungens* was the primary source of DA. It is important to mention that the taxonomy of these toxic diatoms is complex and its distribution in the American continent usually corresponds to cold environments (Argentina and Alaska). Nevertheless, the events of Monterey, Santa Cruz and Cabo San Lucas may indicate that different, but closely related species may be involved. Since such diatoms are usually found in temperate waters, the hypothesis that the ballast water of the ships traveling from Alaska and north-west Canada to Cabo San Lucas, Mexico, serves as a carrier could

explain the dissemination of these organisms along the coast. However, in order to bloom, special conditions have to be met to produce noticeable cell quantities in the sea.

The tip of the Baja California peninsula is in a unique setting where two of the main oceanic currents meet: the temperate California stream that travels down the west coast of the U.S.A. until it reaches the Californian peninsula of Mexico; and the Costa Rica dome stream (Wyrtki, 1966) that originates in the warm ecuatorial waters with an upward direction until it meets the California stream. As a result, a local subclimate is formed that creates an impressive abundance and variety of sea and land species, many of them not found elsewhere, as would be expected for a typical transition zone. Global climate variation has also made its impact in this part of the world, and phenomena never observed before appear to be a warning signal about changing natural conditions. This is the case with fluctuations in fisheries that were normally abundant and have suffered serious declines in recent years, e.g. with shrimp, shellfish and tuna catches. 'El niño', an oceanic phenomenon that appears as an abnormal increase in sea surface temperature at Christmas, has been frequently linked to such undesirable effects. The same assumption can be made with regard to the recent appearance of harmful algal blooms (HAB) along the coasts of the Californian peninsula.

The HAB produced by toxic diatoms and dinoflagellates are considered unpredictable; however, coastal upwellings may be involved in such phenomena. For example, diatom blooms have been observed mostly during active upwelling, while during relaxation or reversals, dinoflagellates replace diatoms (Fraga, 1993; Work *et al.*, 1993). Thus, it is not only a matter of nutrients and turbulence, but also of river plumes that flow along the coast during relaxation. Typical coastal upwellings are usually found associated with deserts, and the peninsula of California is one of them. Hence, it may be assumed that diatom and dinoflagellate blooms in the area should be a common event. The effect of winds on water surface produces an Eckman layer, when the coast is on their left, it moves offshore owing to Coriolis effect (the opposite is true for the southern hemisphere). The water of this upper layer is then replaced by subsurface water, which is richer in nutrients. Since turbulence is inherent to this phenomenon, the diatoms are the phytoplankton organisms best equipped to overcome the situation in which turbulence and nutrients are associated (Margalef, 1978).

In summary, in spite of the small number of samples analysed, and considering the symptoms shown by the sick pelicans, it is concluded that the event observed at Cabo San Lucas (BCS, Mexico) in late December 1995 and early January 1996 was a case of seabird intoxication by DA. The suspected source was identified as *Pseudonitzschia* sp., which could be observed in smears of the stomach content of pelicans and mackerel under the light microscope. Thus, the pelicans presumably ate contaminated fish in the open sea, felt sick and returned to their refuge.

From a colony previously estimated at 300 animals, 50% died as a result of the intoxication herein described, and the surviving birds still showed symptoms of weakness and disorientation 2 months after the event. At this time we cannot anticipate whether the phenomenon may occur again but, as discussed above, a monitoring program is recommended, especially as the area is a source of sardine and mackerel for local canneries, and important aquaculture programs for mussel and shellfish are in progress.

DA is difficult to detect unless a mouse bioassay is conducted, and chromatographic analysis is too expensive to be used as the routine method of detection. New tools should be developed to make economically feasible a permanent monitoring program in countries such as Mexico that lack sufficient resources for this purpose.

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