Successful rehabilitation of an oiled sea turtle (*Lepidochelys olivacea*) affected by the biggest oil spill disaster in Brazil

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Citation: Oliveira REM, Attademo FLN, Galvincio JS, Freire ACB, Silva AS, Pires JML, Lima LRP, Aguiar JMF, Moreira AB, Melo LIS, Gavilan SA, Lima SA, Lima MA, Silva FJL, Oliveira MF (2021): Successful rehabilitation of an oiled sea turtle (*Lepidochelys olivacea*) affected by the biggest oil spill disaster in Brazil. Vet Med-Czech 66, 313–319.

Abstract: The standardisation of protocols and discussion of therapeutic procedures in the rehabilitation of turtles affected by oil spills are necessary to optimise the recovery time and increase the chances of survival of these animals. This study aimed at reporting the processes adopted for the stabilisation, decontamination, rehabilitation and release of an oiled olive ridley sea turtle (*Lepidochelys olivacea*), rescued alive on September 23, 2019, at Santa Rita Beach, Extremoz municipality, Rio Grande do Norte, Brazil. Its entire body was covered by oil. At first, the animal was mechanically dry cleaned using a gauze soaked in mineral oil in the keratinised regions (carapace and plastron) and a gauze soaked in vegetable oil was used on the oral, nasal, ocular, and cloacal mucous membranes. The second stage of the oil removal consisted of washing the animal with heated pressurised water (39 °C) and a neutral detergent using a soft foam sponge. The animal received treatment with antitoxins, antibiotics, analgesics, gastrointestinal protectors, and fluid therapy. After 7 days of treatment, the blood count showed that all the parameters were within the normal range. The oil cleaning process and the therapeutic protocol used in the rehabilitation of the olive ridley sea turtle were efficient.

Keywords: Cheloniidae; contamination; crude oil; intoxication; Testudines

Financed by Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES, Financial Code 001).

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The olive ridley sea turtle is classified as "vulnerable" according to the International Union for Conservation of Nature (IUCN) red list of threatened species (Abreu-Grobois and Plotkin 2008). Interacting with fishing represents one of the main causes of mortality of this species, mainly due to the interaction with gillnets or trawls used on spawning beaches (Silva et al. 2010).

Oil spills affect animals directly through contact with the oil or indirectly by reaching all levels of the food chain, thus enduring for many years in the environment on a large scale (Lauritsen et al. 2017). This occurs because the effects of an oil spill on animals are not always clearly observed immediately after the accident (Soares et al. 2020). Sea turtles are vulnerable to oil at all stages of life, including the embryonic stage, in the nest, in the hatchling and juvenile stages, in the ocean gyres, in the sub-adult stage, in habitats close to the coast, and the adult stage when they migrate between nests and food areas (Lutcavage et al. 1995).

The effects of oil spills on sea turtles are rarely reported, despite the prevalence of this type of accident occurring in areas where these animals inhabit (Wallace et al. 2020). In this sense, the standardisation of techniques and discussion of the treatments in the rehabilitation of sea turtles affected by oil spills are necessary to optimise the recovery time and increase the chances of survival of these animals. Thus, this study aimed at reporting the processes adopted for the stabilisation, decontamination, rehabilitation and release of an oiled olive ridley sea turtle (Lepidochelys olivacea) with 100% of the body affected by oil, in order to guide the clinical measures for providing greater success in the rehabilitation processes of oiled turtles and, consequently, to provide relevant knowledge for conservation planning for these animals.

Case description

In September 2019, Northeast Brazil was affected by an oil spill that reached nine states of the region and that later extended to Southeast Brazil (Espírito Santo and Rio de Janeiro). The origin of the oil spill remains unknown to date. It caused the death of hundreds of marine animals and placed the population living in the country's coastal region at risk. Five months after the first record, oil spills have been recorded on 1 009 beaches, directly af-

fecting 159 marine and estuarine animals, including sea turtles, sea mammals, sea birds, and other aquatic organisms, resulting in the death of 112 animals. Of the animals affected, 105 were sea turtles and, of these, 83 died, making this oil spill the most extensive and severe environmental disaster ever recorded in the history of Brazil. Of the affected sea turtles, fourteen were recorded across the Rio Grande do Norte (RN) state, thirteen of which were already found dead while one stranded animal was found alive (IBAMA 2020).

The turtle that was found alive, which belonged to the species Lepidochelys olivacea, was a subadult. It was rescued on September 23, 2019, at the Santa Rita Beach (5°43'54.37"S, 35°12'15.46"W), Extremoz municipality, Rio Grande do Norte, Brazil (Figure 1). The stranding was notified and the team of the Costa Branca Cetacean Project -State University of Rio Grande do Norte (PCCB-UERN) carried out the rescue. The animal was forwarded to the Marine Life Stabilization Center (PCCB-UERN), Natal, Rio Grande do Norte (RN), Brazil. The performance of activities with marine animals is authorised by SISBIO-ICMBio (License No. 13694-9) and the Ethics Committee on the Use of Animals of the Federal Rural University of the Semi-arid Region (License No. 01/2019).

The animal had 100% of its body surface affected by the crude oil, directly affecting the oral, ocular and cloacal mucous membranes. The animal was active, responsive to stimuli, was tachypnoeic with noisy breathing, had a good body score and was moderately hydrated. The specimen had a 50.5 cm curved carapace length (CCL), 54.5 cm curved carapace width (CCW) and 13.8 kg of body mass. Starting with the stabilisation procedure, the animal was first manually dry cleaned using a gauze pad soaked in mineral oil in the keratinised regions (carapace and plastron) and a gauze pad soaked in vegetable oil was used on the oral, nasal, ocular, and cloacal mucous membranes (Figure 2A-C). After removing the crude oil from the animal, congestion of the oral, ocular and cloacal mucosa was observed.

The external jugular veins (right and left) and the dorsal cervical sinus were the routes for the blood collection, fluid therapy and drugs administration routes. The animal was maintained out of water on a mattress during the venous assessment either for the fluid therapy or drug administration. Containment was performed holding the animal's carapace. This containment was sufficient to main-

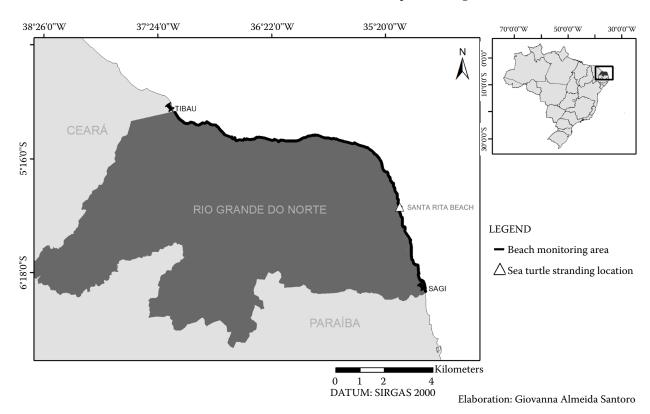


Figure 1. Beach monitoring area of the Cetáceos da Costa Branca Project and sea turtle stranding location

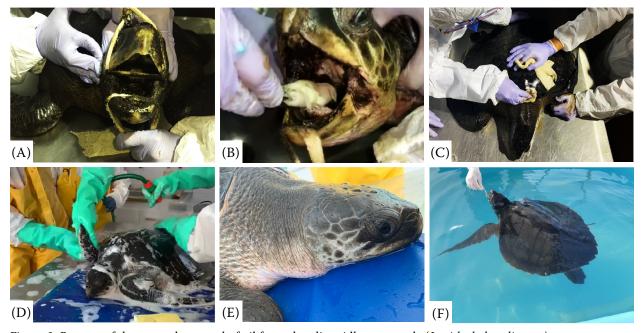


Figure 2. Process of the manual removal of oil from the olive ridley sea turtle (*Lepidochelys olivacea*)
(A) Presence of oil on the entire body surface and mucous membranes. (B) Manual removal of the oil from the oral cavity using a gauze pad soaked with vegetable oil. (C) Removal of the oil from the body surface using a gauze pad soaked with mineral oil. (D) Removal of the oil with heated water (39 °C) and neutral detergent. (E) Clean animal, without any traces of oil. (F) Animal in a pool feeding at will

tain the animal in a satisfactory condition to perform the necessary procedures. After removing the

oil for the first time, the animal's blood samples were collected sequentially for the complemen-

Table 1. Blood count tests performed on the oiled turtle (*Lepidochelys olivacea*)

Complete blood count							
Erythrogram							
Parameters	test 1	test 2	range	reference			
Red cells	0.37	0.75	$0.12 - 0.40 \times 10^6 / \mu l$	Zhang et al. (2011)			
Haemoglobin	102	93	73.7–97.6 g/l	Ramakrisham et al. (2018)			
Haematocrit	35	29	13.6-20.8%	Pinto et al. (2015)			
MCV	945	385	255.5-341.5 fl	Ramakrisham et al. (2018)			
MCHC	290	320	260-359 g/l	Santoro and Meneses (2007)			

Leukogram						
Parameters	test 1		test 2		reference Zhang et al. (2011)	
	relative (%)	absolute (μl)	relative (%)	absolute (µl)	range (μl)	
Leukocytes	100	7 700	100	10 395	3 750-13 000	
Heterophiles	79	6 083	75	7 875	2 170-8 840	
Eosinophils	0	0	1	105	90-650	
Basophils	0	0	0	0	0-70	
Lymphocytes	10	770	19	1 995	920-2 540	
Monocytes	11	847	4	420	400-2 310	
Thrombocyte count	3 500		30 000		6 750-19 750	
Erythrocyte series	hemoglobinemia		no visible change			

MCHC = mean corpuscular haemoglobin concentration; MCV = mean corpuscular volume

Test 1 = September 25, 2019; test 2 = October 2, 2019

tary analyses (blood counts and biochemicals; Tables 1 and 2). The treatment administered during the first 24 h was composed of: fluid therapy with

Table 2. Biochemical tests performed on the oiled turtle (*Lepidochelys olivacea*)

Biochemical						
Parameters	test 1	test 2	reference Espinoza-Romo et al. (2018)			
ALT	30	31	$12-40 \text{ g/l} \times 10^{-6}$			
AST	275	11	$28-402 \text{ g/l} \times 10^{-6}$			
ALP	46	17	$15-94 \text{ g/l} \times 10^{-6}$			
Albumin	14	15	6–15 g/l			
Globulin	46	15	21-37 g/l			
Phosphor	0.074	0.075	0.065-0.147 g/l			
Calcium	0.087	0.090	0.001-0.098 g/l			
Urea	3.82	1.78	0.19-0.78 g/l			
Total proteins	60	30	27-52 g/l			

ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase

Test 1 = September 25, 2019; test 2 = October 2, 2019

a 0.9% NaCl solution (KabiPac®, dose: 20 ml/kg/i.v., velocity: 30 drops/min) associated with a multivitamin (Hertavita®, dose: 1.4 ml/kg/i.v., once a day for 15 days), and antitoxin (Mercepton®, dose: 0.2 ml/kg/i.v., once a day for 15 days) in addition to an activated charcoal adsorbent (Enterex®, dose: 8 g/animal/orally, once a day for 3 days).

On the second day of treatment, other drugs were included in the protocol, an antibiotic therapy with ceftiofur hydrochloride (CEF 50®, dose: 4 mg/kg/i.m., once a day for 30 days), an analgesia with tramadol hydrochloride (Tramal®, dose: 5 mg/kg/i.m., once a day for 5 days), meloxicam (Maxicam®, dose: 0.1 mg/kg/i.m., once a day for 5 days) and dipyrone (D-500®, dose: 25 mg/kg/i.m., twice a day for 5 days) in addition to a sucralfate gastric protector (Sucrafilm®, dose: 0.4 ml/kg/orally, twice a day for 10 days) and a liver protector (Ornitil®, dose: 0.2 ml/kg/i.v., once a day for 10 days).

After the first 24 h, the animal was sent to the Oiled Marine Live Decontamination Centre of the Cetáceos Costa Branca Project, located at the State University of Rio Grande do Norte (UERN),

Mossoró Municipality, Rio Grande do Norte, Brazil. The second stage of the oil removal consisted of washing the animal with heated pressurised water (39 °C) and a neutral detergent using a soft foam sponge (Figure 2D). This procedure was performed within a closed system capable of containing all the oil residue and water contaminated by it. Then, they were sent to a specific reservoir prepared to separate the oil from the water and efficiently dispose of the by-products generated in the washing process.

After 4 days of washing and complete oil removal (Figure 2E), the animal was transferred to the Wild Animal Rehabilitation Centre of the Cetáceos Costa Branca Project – UERN, Areia Branca municipality to continue the rehabilitation. The animal was put in a pool (1 m³ = 200 cm in diameter \times 75 cm in depth), with a water temperature between 25 °C and 28 °C and with natural lighting from 5:30 am to 5:30 pm approximately. The specimen, which was fed with a small fresh fish right after the washing process, immediately showed interest in the food (Figure 2F). The animal received ad libitum feeding corresponding to an average of 500 g to 600 g per meal. One meal was offered in the morning and the other in the afternoon. In addition to fish, the diet included shrimps and crabs.

During treatment, other multivitamins were administered through an oesophageal tube (Hemolitan®, dose: 0.1 ml/kg, once a day for 10 days; Glicopan®, dose: 0.1 ml/kg, once a day for 10 days; Emulsion Scott®, dose: 0.4 ml/kg, once a day for 10 days) in addition to 150 g of fish porridge, diluted in 300 ml of the 0.9% NaCl solution associated with the sucralfate gastric protector (Sucrafilm®) previously mentioned.

On the ninth day of treatment, another gastric protector was included in the protocol, omeprazole (Oprazon®, 4 mg/kg/i.v., for 16 days). After 16 days of using this drug, gastroparesis occurred triggering intestinal constipation. Thus, we opted to stop the administration of the drug. Following the clinical signs, metoclopramide hydrochloride (Plasil®, 0.5 mg/kg/i.v., for 10 days) was included to increase the gastrointestinal motility, and the animal spontaneously defecated.

After 67 days of rehabilitation, the animal was re-evaluated by the veterinary medical team considering the physiological, clinical, and behavioural parameters for the species, and was considered ready to be released.

Before releasing it, two metallic washers with an individual identification were inserted in both anterior fins (left: BRA17527, right: BRA17528). The animal was transported to the open sea (4°54′20.16″S, 37°7′51.60″W) in a motor vessel and reintroduced there.

DISCUSSION AND CONCLUSIONS

The oil ingested by a sea turtle does not pass quickly through the digestive tract. It can be retained for several days, increasing the internal contact and the likelihood of the absorption of toxic compounds. The internal effects of oil exposure include significant changes in the blood haematological and biochemical profile (Milton et al. 2010). Prolonged exposure to oil can worsen the body condition of the animals, interrupting the feeding and increasing the susceptibility to diseases, and the subsequent mortality (Mitchelmore et al. 2017).

The oil spill disaster that occurred in the Gulf of Mexico in 2010 was one of the largest in the world, affecting not only the environment, but the animals living there. All five species of sea turtles that are present in the Gulf of Mexico were affected by this incident. The changes found in the 319 turtles that were analysed were non-specific, thus, it was not possible to observe the toxicological effects (Stacy et al. 2017).

However, evidence shows that approximately 30% of all ocean turtles in the region affected by the Gulf of Mexico spill that were not heavily oiled would have died from oil ingestion, while that 100% of the heavily oiled turtles would have died from the physical effects of the heavy oiling (Mitchelmore et al. 2017). Due to the dispersive, migratory, and surface-dwelling behaviour of sea turtles, many populations can be negatively impacted by oil spills. Thus, the spill's impacts may extend far beyond the current focus on the northern Gulf of Mexico (Putman et al. 2015). Although direct toxicological changes could not be observed in our study, a supportive and antitoxin prophylaxis treatment was prescribed from the time that the animal arrived at the Rehabilitation Centre. On the first day, activated charcoal was used to reduce the absorption of oil toxins according to the recommendations of Walsh (1999), with an active function only in the first 24 hours. It was only used in the first three days, in a preventive way, being associated with antitoxins and liver protectors which were both administered intravenously. Considering

that the liver is the main site of the chemical detoxification, it is expected that the toxic effects cause changes in the serum levels of several liver enzymes (Milton et al. 2010).

The regulation of the osmolarity in the blood of sea turtles is highly dependent on the availability of water for consumption, the reabsorption of water in the urinary bladder/cloaca, and the loss of salts by the salt glands, rather than the reabsorption of water by the kidneys. When a negative water balance occurs, all the body systems are affected, and a fluid therapy becomes an integral part of the restoration and maintenance of the cellular homeostasis, where adequate hydration provides support for cells and a means of transport for molecules (Rudloff 2005).

In an experiment conducted by Lutcavage et al. (1995), the physiological and clinical-pathological effects of exposure to crude oil on loggerhead turtles (Caretta caretta) in the southern US state of Louisiana were evaluated. Sea turtles ingested oil incidentally, and contamination was observed in the nostrils, eyes, upper oesophagus and faeces. The white blood cell counts of oiled turtles had a fourfold increase, a 50% reduction in the red blood cell counts, and changes in the colour of the red cells. Histological changes were observed on the skin, and mucosa surface of the oiled turtles, including acute inflammatory cell infiltrates, dysplasia of the epidermal epithelium and loss of cellular architecture of the skin layers. Cellular changes in the epidermis are of particular concern, as they may increase susceptibility to secondary infectious conditions (Milton et al. 2010). Thus, the administration of antibiotics in this report is justified as a preventive approach. Vitamin B12 (cyanocobalamin) and B9 (folic acid) are essential nutrients for normal haematopoiesis. Since macrocytic anaemia is related to the deficiency of these vitamins in cases of intoxication (Aslinia et al. 2006), supplementing vitamin B12 and B9 is necessary to reverse the conditions observed in the first blood count test (haemoglobinaemia and mean corpuscular volume). After 7 days of treatment, a second blood count showed that all the parameters were within the normal range.

Proton pump inhibitors (PPIs) are substances that selectively and completely inhibit the $H^+/-K^+$ -ATPase proton pump in the parietal cell membrane. Thus, the gastric acid secretion is suppressed, increasing the gastric pH (Strand et al. 2017). Typical doses of PPIs are incredibly useful in inducing the

remission of oesophagitis and gastritis symptoms (Khan et al. 2007; Fass et al. 2012). For this reason, using omeprazole was essential to reduce the damage caused by the ingested oil on the gastroesophageal mucosa and the occasional gastrointestinal adverse effects of meloxicam. Adverse effects related to omeprazole are rare, but can occur with prolonged use. Adverse effects in humans include headaches, diarrhoea, abdominal pain, nausea, skin rashes, constipation, and a vitamin B12 deficiency (Marchetti et al. 2003). After 16 days of using omeprazole, the turtle had constipation. Thus, we opted to stop the administration of the drug.

In conclusion, we found that the oil cleaning process and the therapeutic protocol used in the rehabilitation of the olive ridley sea turtle were efficient.

Moreover, the short time between the stranding of the animal and the first visit was essential to avoid the greater absorption of oil toxins. Although PPIs are considered safe drugs, caution should be exercised in their use in sea turtles for an extended period. It is worth mentioning that the therapeutic protocol can be tailored to suit individual cases.

Acknowledgement

We thank Coordination for the Improvement of Higher Education Personnel (CAPES, Financial Code 001) for the PhD scholarship granted to the Postgraduate Program in Animal Science, to the entire team of veterinarians and biologists of the Costa Branca Cetacean Project (PCCB-UERN/CEMAM) working in the Potiguar Basin Monitoring Program, who helped rescue the animal and Giovanna Almeida Santoro by making the map.

Conflict of interest

The authors declare no conflict of interest.

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Received: April 7, 2020 Accepted: December 30, 2020