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Third- and Fourth-Generation Cephalosporin Resistant *Morganella morganii* Associated to an Abscess on the Perineum of a Male Bottlenose Dolphin (*Tursiops truncatus*)

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Abstract

A male bottlenose dolphin (*Tursiops truncatus*) of approximately 16 years of age was found to have a swollen lesion on the skin of the perineum. This lesion first appeared as a regular cutaneous wound that had problems healing due to the swelling, extension, and depth. It was treated using systemic antibiotics, NSAIDs, and wound care. Three years later, the animal presented new superficial skin lesions in the same area. Although they resolved with basic wound care, a 5-cm circular and erythematous swelling developed. Leukocytosis with neutrophilia and increased γ-globulins resulted from the blood exams. However, no abnormalities were found in the serum biochemistry. Ultrasound examination of the swelling together with a cytological sample and its culture confirmed the diagnosis of a septic abscess. Due to the nature and location of the abscess, a medical approach was preferred. Systemic antibiotherapy, analgesics, periodic drainage and irrigation of the lesion with an antibiotic solution, and inoculation of plasma rich in growing factors resulted in the successful control of the infection. *Morganella morganii* was isolated in pure culture. Initial antibiogram showed sensitivity of the bacterium to third-generation cephalosporins. However, successive control demonstrated resistance not only to third-generation cephalosporins but also to fourth-generation cephalosporins.

Key Words: bottlenose dolphin, *Tursiops truncatus*, ultrasound, medical training, abscess, *Morganella morganii*, third-generation cephalosporin, fourth-generation cephalosporin, antibiotic resistance

Introduction

Bottlenose dolphins (*Tursiops truncatus*) can be found in temperate and tropical coastal waters worldwide. They can be as long as 3.8 m and weigh up to 635 kg. Males tend to be somewhat larger than females (Wang et al., 2014). *T. truncatus* is catalogued as Least Concern by the IUCN *Red List*, and the population is estimated to be more than 750,000 individuals. Human-related activities such as fishing and harvesting, maritime transportation, and pollution are considered the biggest threat for free-ranging bottlenose dolphins (Wells et al., 2019).

Abscesses have been reported in marine mammals historically. Cockrill (1960a) described them as a collection of pus localized in an encapsulated cavity forming a round or ovoid body. They have been frequently observed affecting different organs, tissues, and body cavities in both free-ranging and managed pinnipeds and cetaceans (Cockrill, 1960a, 1960b; Dunn et al., 2001; Tryland et al., 2018). Multiple bacteria and fungi have been isolated from abscesses in marine mammals (Tryland et al., 2018).

Methods & Results

A 16-y-old male bottlenose dolphin (*Tursiops truncatus*) hosted in a marine park in Malta was presented with two superficial scratches in the ano-genital space (perineum) that merged into a bigger and swollen skin lesion with granulation tissue that was painful at touch. Bloodwork showed marked leukocytosis with neutrophilia, an increase in the eritrocyte sedimentation rate (ESR), and high levels of fibrinogen. Systemic oral antibiotherapy (amoxicillin-clavulanic acid [Augmentin™], 875 mg/125 mg film-coated capsules; GlaxoSmithKline [Ireland] Limited, Dublin, Ireland; 10 mg/kg PO q12h for 14 d; and enrofloxacin [Baytril®], 150 mg tablets; KVP Pharma + Veterinär Produkte GmbH, Kiel, Germany; 5 mg/kg PO q12h for 14 d) and anti-inflammatory and pain relief (meloxicam [Novacam®], 1.5 mg/ml; AST Beheer B.V., Oudewater, The Netherlands; 0.1 mg/kg PO once) therapies were implemented together with daily topical wound care and frequent addition of platelet rich plasma to promote healing. Ultrasonographic examination (Aloka SSD-900;

Aloka Co. Ltd, Mitakashi, Tokyo) of the affected tissue showed an underlying area of 4.5 × 4.5 cm with mixed echogenicity that could indicate the presence of an inflammatory process, hematoma, or an abscess. No further diagnostics were performed at the time to determine the nature of the soft tissue variations as the lesion resolved with the treatment plan.

Three years later, the same animal was presented with a small skin lesion on the area previously affected (Figure 1). Initially, the lesion seemed to be healing without medical treatment. However, 13 d after the lesion appeared, the area swelled, became congested, and the skin reopened, including a new smaller skin lesion. Even though the lesions were closed after 24 h, blood samples were collected by voluntary training, and examination showed a clear leukocytosis with an increase of the γ-globulins, with all other inflammatory parameters (ESR, fibrinogen, and iron) within the normal limits. In a differential diagnosis, a clear increase of γ-globulins is normally associated with a suppurative disease or abscessation. Oral systemic antibiotics (amoxicillin-clavulanic acid [Augmentin™], 875 mg/125 mg film-coated capsules, GlaxoSmithKline [Ireland] Limited; 10 mg/kg PO q12h for 12 d; and enrofloxacin [Baytril®], 150 mg tablets, KVP Pharma + Veterinär Produkte GmbH; 5 mg/kg PO q12h for 12 d) and nonsteroidal anti-inflammatory drugs (NSAIDs; meloxicam [Novacam®], 1.5 mg/ml, AST Beheer B.V.; 0.1 mg/kg PO once) were administered empirically. The skin wounds healed and white blood cell counts (WBC) lowered, but the affected area remained swollen 12 d after starting the oral treatment. Ultrasonographic exam of the area (LogiqTMV2; General Electric Healthcare, Chicago, IL, USA) revealed a well-demarcated rounded hypoechoic and superficial area of approximately 4.2 × 5.3 cm compatible with an encapsulated chamber containing fluid that was also compressing the retractor muscle, the m. ischocavernosus, and the m. bulbourethralis (Figure 2). No foreign objects were observed inside the chamber. A fine needle aspirate was performed by applying a local anesthetic cream with lidocaine/prilocaine (EMLATM cream 5%; Aspen Pharma Trading Limited, Dublin, Ireland) 10 min prior to cleaning and disinfecting with iodine solution and alcohol alternatively to aseptically prepare the area. A 21G and 19-mm-long butterfly needle was used to puncture percutaneously. Approximately 15 ml of a pale brown purulent fluid was retrieved, leaving the chamber almost empty. No physical restriction was needed at any time during the process due to advanced voluntary training behavior achieved by operant conditioning with positive reinforcement.

Samples of the fluid were prepared for microbiological analysis as well as cytological mounts. Microscopically, the sample contained a large amount of degenerated white blood cells (pus). However, no bacteria were observed, even with Gram staining. A couple of hours after the drainage, the abscess filled up again. A total of almost 80 ml of pus was drained in the following days, while the abscess was internally rinsed with sterile saline solution until arrival of the microbiological

Figure 1. First appearance of the lesion on the perineal area

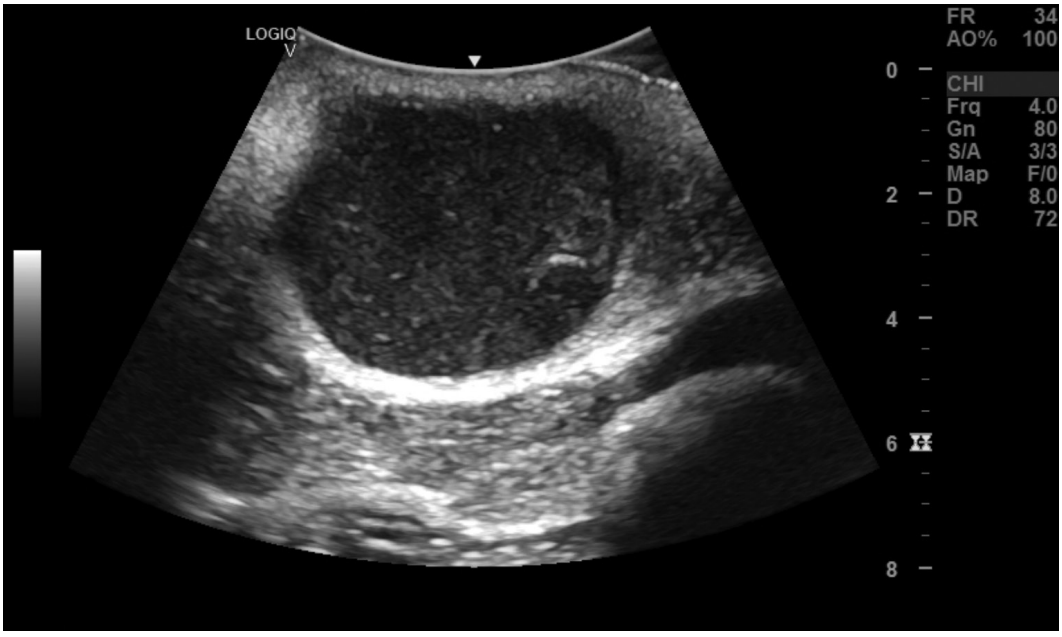

Figure 2. Ultrasound examination performed with a GE 4C convex probe. The animal was positioned in dorsal recumbency, and the probe was placed longitudinally over the median line. The image shows a spheroidal-shaped hypoechoic structure (size: 44.8 × 56.2 mm), characterized by a well-defined wall (thickness: 2.8 mm), and hypoechoic fluid (to the surrounding tissues), containing coarse fluctuating echoic particles (hyperechoic "sparkles").

analysis results. Anaerobic and fungal cultures yielded no growth, but *Escherichia coli* and *Morganella morganii* grew in the aerobic culture. Both bacteria were sensitive to third-generation cephalosporins and aminoglocosydes (Table 1). Cefovecin as a long lasting systemic antibiotic and gentamicin as an irrigation solution were chosen for the treatment of the septic abscess following the antibiogram results. Eight milligrams/kilograms of cefovecin (Convenia©; Haupt Pharma Latina S.R.L, Borgo San Michele, Italy) was then injected intramuscularly in the left flank in the longissimus muscle in a single dosage. A 1.1% solution of gentamicin and saline was used to irrigate the interior of the abscess after draining the pus completely and flushing it with a sterile physiological solution. To promote the healing, 1 ml of homologue growing factors rich plasma (GFRP) was prepared, following the technique described by Griffeth et al. (2014), and was injected in the cavity.

During the process, and once the abscess was drained, the animal managed to pass urine, probably triggered by the clear reduction of pressure produced by the abscess on the structures of his genitals. Together with urine, the animal excreted white fluids that were presumed to be either pyuria or sperm, although ultrasonographic examination did not show abnormalities in the urinary bladder. After this, the animal was observed to be uncomfortable, refused interaction and food, and released what was described as "white urine" several times in the following 24 h. Tramadol (Altadol®, 50 mg tablets, Labiana Pharmaceuticals, S.L.U., C/Casanova 27-31, 08757 Corbera de Llobregat, Spain; 0.1 mg/kg PO q12h for 5 d) was added to the therapy to control pain, and the animal responded to it positively. It was then possible to measure his body temperature (36.4ºC) and perform an ultrasound exam of the abscess. Since it was not clear if the animal was having a major problem, blood for hemoculture and a general check was collected, as well as a sample of the serous-like liquid content of the abscess in a bottle for blood culture. Again, once the abscess was drained, the dolphin passively released a whitish fluid that was also collected and which cytology confirmed to be sperm. No passive ejaculation was observed again—just normal urination. The blood culture result was sterile. A pure culture of *M. morganii* was obtained from the aerobic culture of the pus collected in the hemoculture bottle, while *Enterococcus* sp. grew from the anaerobic culture of the same sample. Antibiogram did not suggest changes in the therapy regarding gentamicin. However, *M. morganii* was resistant—not only to first-generation cephalosporins but also to third- (cefovecin and ceftiofur) and fourth-generation cephalosporins (cefquinome) as shown

Table 1. Antibiogram results of the bacteria yielded from the cultures performed. *M. morganii* presented resistance to third- and fourth-generation cephalosporins during the second culture. Although not included in the table, *Escherichia coli* was sensitive to Polymyxins (Polymyxim B and E); and *Enterococcus* sp. was sensitive to Glycopeptide antibiotics (Vancomycin and Teicoplanin) but resistant to Macrolides (Azithromycin, Clarithromycin, and Erythromycin) and Lincosamides (Clindamycin and Lincomycin). S = susceptible, I = intermediate, and R = resistant.

		Results 1st culture		Results 2nd culture	
		E. coli	*M. morganii*	*M. morganii*	*Enterococcus* sp.
Penicillins					
	Penicillin	--	--	--	S
	Ampicillin	S	R	R	S
β-lactam/β-lactamase inhibitor combination					
	Amoxicillin	S	R	R	S
	Amoxicillin-clavulanic acid	S	R	R	S
Cephalosporins					
1st-generation	Cephalexin	S	R	R	R
	Cefazolin	--	R	R	R
3rd-generation	Cefovecin	S	S	R	R
	Ceftiofur	S	S	R	R
4th-generation	Cefquinome	S	S	R	R
Carbapenems					
	Imipenem	S	R	S	S
	Meropenem	S	R	S	--
Aminoglycosides					
	Gentamicin	S	S	S	--
	Amikacin	S	S	S	R
	Tobramycin	S	S	I	R
Tetracyclines					
	Tetracycline	S	R	R	S
	Doxycycline	S	R	R	S
Fluorquinolones					
	Enrofloxacin	S	R	R	I
	Marbofloxacin	S	I	R	I
	Pradofloxacin	S	R	R	I
Folate pathway inhibitors					
	Sulfamethoxazole-trimethoprim	S	S	S	I
Phenicols					
	Chloramphenicol	S	S	S	S
Nitrofuran derivates					
	Nitrofurantoin	S	R	R	S

in Table 1. Although the molecular identification of the bacterium was not performed, for sensitivity testing, the laboratory used a fully automated Minimum Inhibitory Concentration (MIC) determination method by using the VITEK® 2 platform by BioMerieux (IDEXX Laboratories, 2019).

Follow-up checks by ultrasounds demonstrated a continuous reduction in size of the abscess (Figures 3 & 4). Still, aspirations retrieved considerable amounts of serosanguinous fluid that were decreasing with time (from 42 to 15 ml) while irrigations with gentamicin continued regularly every 3 d. Inoculations of GFRP were discontinued. At this stage, 1 mo after the beginning of the problem, the dolphin had lost 7 kg of body weight, his blood analyses were unremarkable, and he was displaying normal behavior and excellent attitude. Externally, it was difficult to notice the area of the abscess, and it was very soft to the touch. The walls of the abscess became more hyperechoic, suggesting they were fibrotic, and the cavity had reduced by almost half of the initial size. Drainage and irrigation of the abscess was then carried out every 5 d over the following 3 wks. For the irrigation, less volume of saline solution was used and, as a result, the concentration of gentamicin increased (3.33, 5, and 10%, respectively). Five milliliters of fluid were retrieved during the last drainage. Fibrin would make it more complicated as it would block the needle. A follow-up ultrasonographic exam performed slightly over 2 mo after the first lesion appeared demonstrated the presence of granulation tissue filling the cavity of the abscess (Figure 5). This was also noticeable externally, at touch, since the area had a homogenous consistency. It took four more months for the lesion to resolve completely (Figure 6).

Discussion

Marine mammals are susceptible to skin disorders of different etiology. Trauma, viruses, and parasites are the primary cause of most of the bacterial skin problems (Dunn et al., 2001). Several authors have reported septic abscesses in dolphins (Ketterer & Rosenfeld, 1974; Palmer et al., 1991; Dagleish et al., 2008; Cassle et al., 2013; Traversi et al., 2013; Lee et al., 2019). *Staphylococcus aureus* has been frequently reported to be involved in subcutaneous abscesses (Ketterer & Rosenfeld, 1974) and purulent skin lesions (Palmer et al., 1991), and has been isolated from mixed bacterial infections together with *E. coli* and *Enterococcus* spp. (Tryland et al., 2018). In the present case, *M. morganii* was isolated from the abscess.

M. morganii is a commensal Gram-negative rod, facultatively anaerobic, known to cause opportunistic infections of the respiratory and urinary tract, cellulitis, abscessation, sepsis, diarrhea, and bacteremia in humans and animals, including

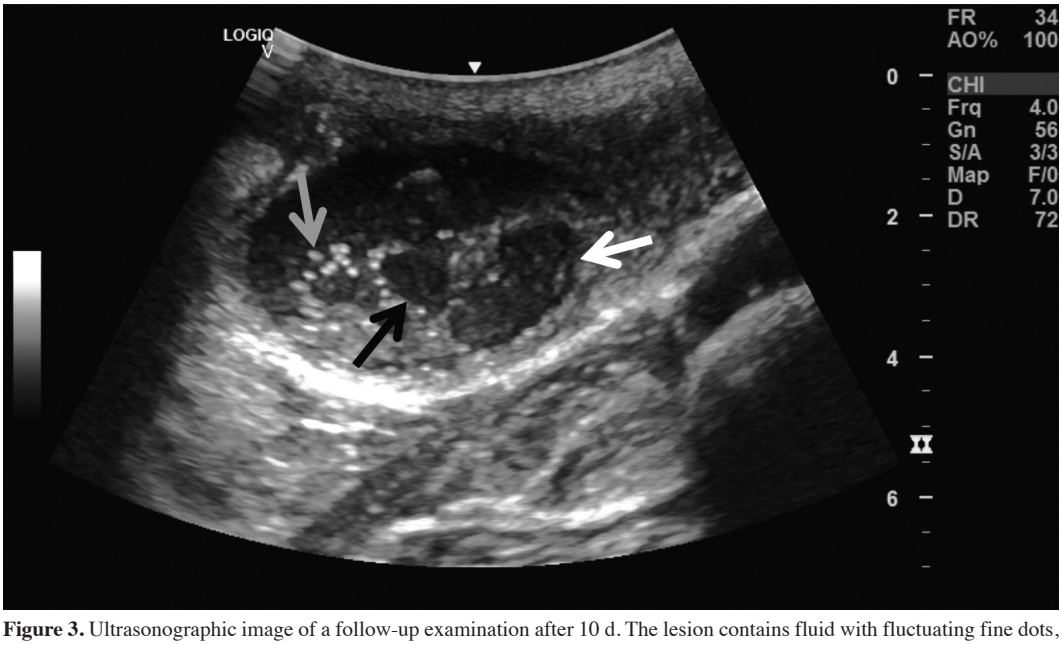

Figure 3. Ultrasonographic image of a follow-up examination after 10 d. The lesion contains fluid with fluctuating fine dots, anechoic cavitations of different sizes (white arrow: 22.1 × 15.1 mm; black arrow: 10.9 × 6.9 mm), and hyperechoic foci echoes representing gas bubbles (grey arrow).

Figure 4. Ultrasonographic image of the follow-up exam 22 d after the first evaluation. The abscess presents a reduction in volume (22.2 × 32.1 mm), a well-defined wall (1.7 mm), and homogeneous content with fine fluctuating dots.

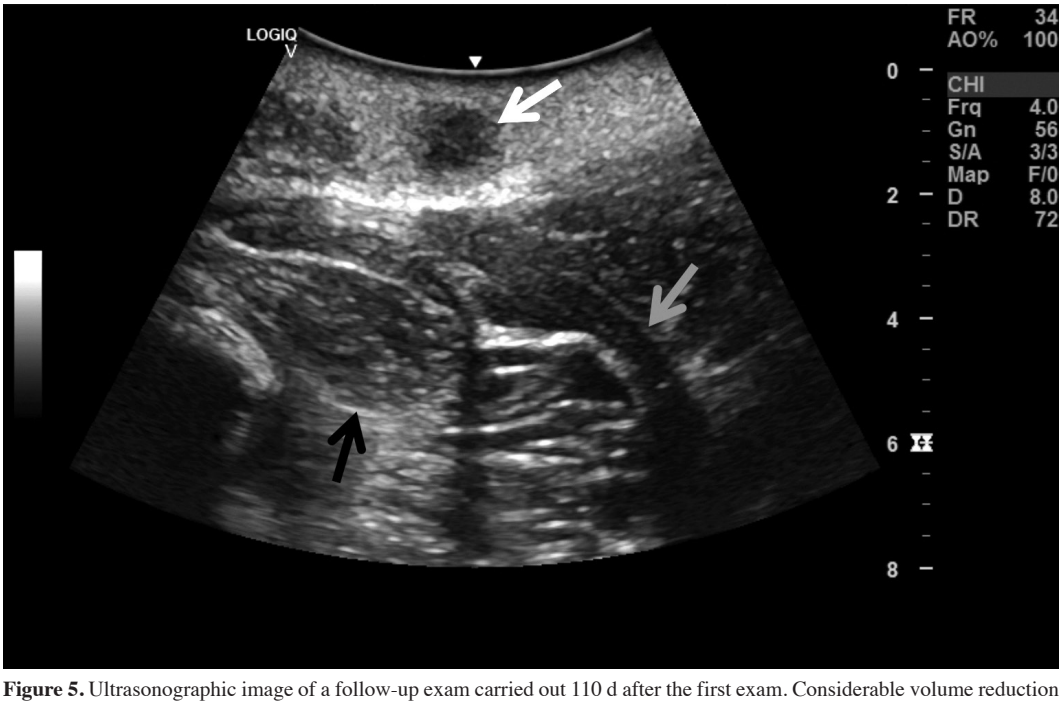

Figure 5. Ultrasonographic image of a follow-up exam carried out 110 d after the first exam. Considerable volume reduction is shown (14.3 × 18.3 mm; wall thickness: 1.6 mm), and slightly echoic fluid is present (white arrow). The image shows portions of the prostatic gland (black arrow) and retractor muscle (grey arrow).

Figure 6. Image of the perineal area after the lesion resolved

marine mammals (De Guise et al., 1995; Higgins, 2000; Bonar et al., 2007; Dove, 2009; Nielsen et al., 2013; Wallace et al., 2013; Sakaguchi et al., 2014; Elfadl et al., 2017; Nakajima et al., 2017; Li et al., 2018). This member of the Family *Morganellaceae* is also considered part of the normal microbiota of the respiratory and gastrointestinal tract in bottlenose dolphins (Reidarson et al., 1998; Chan et al., 2001; Buck et al., 2006; Machado, 2014; Fiorucci, 2016; Soverini et al., 2016; Suzuki et al., 2019). Elfadl et al. (2017) reported the first fatal case in a bottlenose dolphin suffering from a fibrino-hemorrhagic bronchopneumonia primarily produced by *M. morganii*. However, other authors have isolated this bacterium associated with other microorganisms from the lung and blood in a case of purulent pneumonia and hepatic failure (Ueda et al., 2013), in an abscess of a pre-anal gland (Traversi et al., 2013), potentially involved in a pulmonary aspergillosis (Reidarson et al., 1998), in a renal bilateral necrosis (Stephens et al., 2014), and in respiratory samples from dolphins suffering from bronchitis and bronchopneumonia or with undiagnosed diseases (Kleiva, 2013).

Although bacteria of the Family *Morganellaceae* did not appear to be within the most represented families of the normal gut microbiome (Soverini et al., 2016), in the present case, fecal contamination of the initial skin lesion cannot be excluded, and this might have led to the development of the abscess from which *M. morganii* was isolated. The fast healing skin of cetaceans (Zasloff, 2011) might have helped to encapsulate the bacterium.

Septic abscesses caused by bacteria in dolphins have been described in several anatomical locations (Palmer et al., 1991; Ketterer & Rosenfeld, 1994; Cassle et al., 2013), including one report of a pre-anal gland abscess of unknown etiology (Traversi et al., 2013). The latter represents the closest anatomical area of affection compared to the present case. Traversi et al. (2013), from the only culture performed in their study, isolated different microorganisms from the exudate that included *M. morganii* and *Enterococcus* spp. In the present case study, the initial treatment with amoxicillin-clavulanic acid and enrofloxacin was administered empirically immediately after diagnosing the leukocytosis. Culture analysis received a few days later showed that *M. morganii* was resistant to both antibiotics. The antibiogram showed that the bacterium was sensitive to cefovecin and gentamicin instead, thus the change in the treatment. The second culture showed that *M. morganii* became resistant to cefovecin but was still sensitive to gentamicin, which was applied locally by irrigation. This correlates with the results reported by Park et al. (2020) on *M. morganii*'s antibiotic resistance pattern, especially to third-generation cephalosporins. *M. morganii* is

intrinsically resistant to a wide range of antibiotics such as first- and second-generation cephalosporins and amoxicillin-clavulanic acid (Clinical and Laboratory Studies Institute [CLSI], 2021). To our knowledge, fourth-generation cephalosporin resistance for this bacterium has not been previously reported. Since the molecular identification of the strain of *M. morganii* was not specifically performed, the possibility of the presence of two different strains involved in this case with different resistance profile to antimicrobials cannot be excluded.

Abscesses in cetaceans tend to dissect or migrate dorsally (McBain, 2001). The location of the abscess (perineum) and the compression it was producing over the retractor muscle, the m. ischocavernosus, and the m. bulbourethralis were of great concern. Due to the fully aquatic lifestyle of the dolphins and the difficulties to keep the area dry until complete wound healing, surgery was not performed to avoid potential secondary contamination of the abdominal cavity with the aquatic environment. The fact that the dolphin was trained for voluntary participation in any clinical activity made possible the collection of samples and the application of different diagnostic techniques. It also helped in deciding the medical approach and in implementing the treatment plan in a safe and unstressful way. Additionally, ultrasonography was a very useful non-invasive technique to diagnose and monitor the abscess, as also reported by Dennison & Saviano (2018).

Conclusions

Bacteria forming part of the normal microbiota of dolphins can opportunistically take part in infections, including in those animals under human care. *M. morganii* has the potential to cause an abscess and become resistant to third- and fourth-generation cephalosporins. Thus, antibiotic treatments should be considered and directed based on antibiogram results to avoid the worsening of antimicrobial resistances—a problem that has become a serious concern in human and veterinary medicine. Further studies should be carried out to characterize the multiresistant strains of *M. morganii* and its resistance mechanism and to identify the break point for these newer antibiotics. A medical treatment is a valid approach to resolve perineal abscesses in managed bottlenose dolphins, especially if the animal is trained for voluntary medical behaviors that allow such treatment. To our knowledge, this is the first case of a perineal abscess with the involvement of a multiresistant *M. morganii* reported in a bottlenose dolphin.

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