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Short Note

Histopathology, Immunohistochemical Diagnosis, and Management of Penicillin-Resistant *Staphylococcus delphini* Cutaneous Infection in a Bottlenose Dolphin

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Staphylococcus are widespread bacteria that cause superficial and invasive infections in several species. The species *Staphylococcus delphini* is a Gram-positive bacterium belonging to the *Staphylococcus intermedius* group, being a mucosa colonizer and an opportunistic pathogen in some animals, including delphinids (Canver et al., 2019; Vrbovská et al., 2020). The first isolation was from two aquarium dolphins suffering from multifocal purulent skin lesions (Varaldo et al., 1988). *S. delphini* has subsequently been isolated from other mammals (e.g., cows, horses, donkeys, camels, mustelids) and avian species (e.g., Adélie penguins, pigeons, cinereous vultures, red kites, ducks) (Sasaki et al., 2007; Guardabassi et al., 2012; Mama et al., 2019; Ruiz-Ripa et al., 2019; Vrbovská et al., 2020). Recently, the zoonotic potential of *S. delphini* has been recognized (Magleby et al., 2019). Herein, we present the diagnosis and successful treatment of *S. delphini*-caused cutaneous lesions on a common bottlenose dolphin (*Tursiops truncatus*) under human care.

This 49-y-old female common bottlenose dolphin displayed a moderate, acute, focal, vesicular dermatitis in the blowhole (dorsum and peduncle) that recurrently ulcerated despite routine wound management. Swabs from the external area of the blowhole near the ulcerative lesions (Figure 1a) were subjected to bacteriological and fungal investigations. The sample for bacteriological studies was cultured on agar plates in aerobic or anaerobic conditions. The isolates were subjected to a protein–peptide extraction protocol based on formic acid–acetonitrile (Bruker Daltonik, Bremen, Germany) to obtain matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) profiles. A complete antiobiogram (amoxicillin–clavulanic acid, ampicillin, ceftiofur, doxycycline, enrofloxacin, gentamicin, penicillin G, tetracycline, tiamulin) was performed on obtained isolates.

The isolate was identified to the species level as *S. delphini* (MALDI-TOF MS-based identification log [score] value, > 2.00). The antiobiogram performed on isolates revealed penicillin G resistance. No fungal growth was observed in the swabs.

Sloughing ulcerated skin was opportunistically collected for virological (e.g., cetacean herpesvirus, papillomavirus, poxvirus) studies using specific PCR protocols (VanDevanter et al., 1996; Bracht et al., 2006; Mengual-Chuliá et al., 2012) and for histological and immunohistochemical studies. The skin sample collected for histology was fixed in formalin for 48 h and routinely processed.

The skin sample was negative for cetacean herpesvirus, papillomavirus, and poxvirus. Histological examination showed a moderate, suppurative dermatitis characterized by a multifocal infiltration of the basal layers of the stratum spinosum of the epidermal papillae and the external areas of the dermal papillae by neutrophils (Figure 1c). For the immunohistochemical study, deparaffinized and rehydrated samples were incubated in horse serum followed by the primary antibody (rabbit polyclonal anti-*Staphylococcus* spp., 1:100 dilution,

incubation 2 h at 37ºC). Commercial reagents were used for the secondary antibody (ImmPRESS®-VR Horse Anti-Rabbit IgG Polymer Kit; Vector Laboratories LTD, Peterborough, UK) and chromogen (ImmPACT® NovaRED™ Peroxidase Substrate; Vector Laboratories LTD). Archive tissue samples from a domestic pig experimentally infected with *S. aureus* was used as the positive control. For negative controls, the primary antibody was replaced by a commercial universal negative control reagent.

Immunohistochemical assessment revealed a multifocal cytoplasmic granular immunoreaction for *Staphylococcus* spp. in neutrophils infiltrating the basal layers of the stratum spinosum and less often in those located in epidermal papillae (Figure 1d). Based on these results, a topic antimicrobial treatment using gentamicin (ophthalmologic formula) twice daily for 1 mo was implemented, which showed a progressive resolution of the lesions and no recurrence 1 y after the end of the treatment (Figure 1b).

Free-range dolphins and those under human care are increasingly being diagnosed with staphylococci infections, most of which are zoonotic. While *S. delphini* was first identified in cutaneous lesions of managed dolphins (Varaldo et al., 1988), there have been no further reports in this group, although it is seen in other species, including humans. Furthermore, the pathology of *S. delphini* in dolphins remains to be described. The characterization of gross and histological lesions induced by *S. delphini* may be highly valuable for diagnostic purposes in managed cetaceans as well as contributing to the understanding of *S. delphini* pathogenesis in these species.

Although incisional biopsy may be the ideal procedure for obtaining samples for histopathological studies, less invasive techniques should be

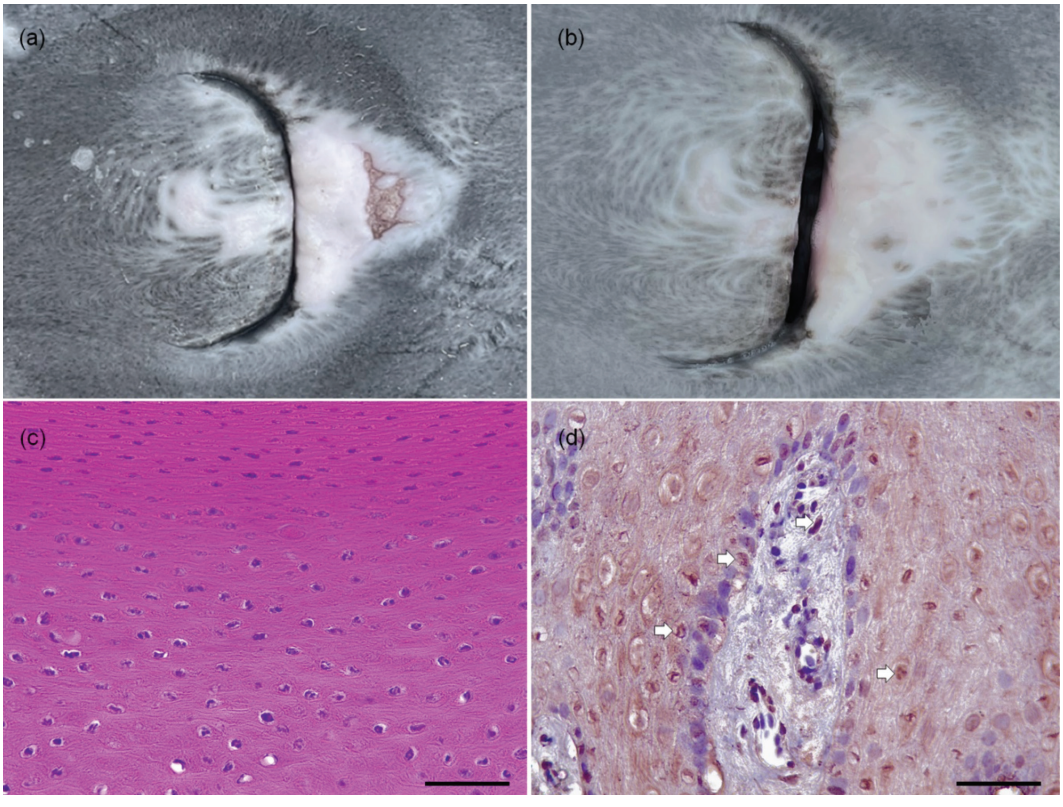

Figure 1. *Staphylococcus delphini* cutaneous lesions in a bottlenose dolphin (*Tursiops truncatus*) under human care: (a) the initial vesicles tend to rupture leading to a moderate subacute focal ulcerative dermatitis of the blowhole. Recurrence explains local fibrosis; (b) dermal ulcers are resolved after topic antimicrobial treatment, and there is no fibrosis and recurrence of lesions 1 y after treatment; (c) moderate acute multifocal suppurative dermatitis in the basal layers of the stratum spinosum of the epidermis (haematoxylin-eosin; scale bar = 100 μm); and (d) multifocal cytoplasmic brown granular immunoreaction in the neutrophils infiltrating the basal layers of the stratum spinosum and less often in the epidermal papillae (arrows) (rabbit polyclonal anti-*Staphylococcus* spp. antibody; scale bar = 50 μm).

considered—especially in aged cetaceans under human care. In this study, we took advantage of a sample from the ulcerated skin for virological and histopathological studies, thereby avoiding stress, anaesthesia, and secondary infections of the surgery site. The sample proved diagnostic for histopathology, showing the usefulness of the opportunistic collection of the detached skin as a first step in those cases in which incisional biopsy may not be recommended.

The pathogenesis of *S. delphini* dermatitis in small odontocetes is unknown. The histopathological examination discussed herein showed a suppurative dermatitis associated with intraepithelial cleft formation. *S. delphini* is known to produce exfoliative toxin, trypsin-like serine protease cluster, and enterotoxin C (Vrbovská et al., 2020). This mechanism explains the degradation of intercellular junctions in the epidermis triggering vesicle formation and ulceration.

Immunohistochemical detection of staphylococci has not been employed before in the diagnosis of dermal bacterial infections in dolphins. We localized staphylococci intracellularly in neutrophils and epithelial cells. The intracellular location of *Staphylococcus* observed herein is explained by the surface protein Y which facilitates *S. delphini* to internalize into phagocytic cells (Maali et al., 2020). Immunohistochemistry brings together the bacteriological and histopathological results and may be used as an ancillary tool in suspected cases of cutaneous bacterial infections in managed cetaceans.

The resolution of full-thickness cutaneous ulcers in a common bottlenose dolphin caused by *S. delphini*, described in this short note, was achieved using topic antimicrobial treatment. This treatment promoted lesion healing as observed in the skin that displayed fibrosis 1 y after treatment. Furthermore, we showed the importance of performing an adequate diagnostic workflow based on bacterial culture and identification for successful long-time management.

MALDI-TOF MS seems to be an accurate tool to identify *S. delphini* infections in biological samples from dolphins. Concordantly, MALDI-TOF MS has been suggested as the best option for distinguishing members of the *S. intermedius* group (Canver et al., 2019). Our results also highlight the need to monitor antimicrobial resistance to successfully manage dermal invasive infections in cetaceans under human care. Further studies should establish risk factors predisposing managed aquatic mammals to staphylococcal dermatitis.

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