Epidemiology of tattoo skin disease in captive common bottlenose dolphins (Tursiops truncatus): Are males more vulnerable than females?

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Epidemiology of tattoo skin disease in captive common bottlenose dolphins (*Tursiops truncatus*): Are males more vulnerable than females?

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**ABSTRACT**

Clinical and epidemiological features of tattoo skin disease (TSD) are reported for 257 common bottlenose dolphins held in 31 facilities in the Northern Hemisphere. Photographs and biological data of 146 females and 111 males were analyzed. Dolphins were classified into three age classes: 0–3 years, 4–8 years, and older than 9 years. From 2012 to 2014, 20.6% of the 257 dolphins showed clinical TSD. The youngest dolphins with tattoo lesions were 14 and 15 months old. TSD persisted from 4 to 65 months in 30 dolphins. Prevalence varied between facilities from 5.6% to 60%, possibly reflecting variation in environmental factors. Unlike in free-ranging Delphinidae, TSD prevalence was significantly higher in males (31.5%) than in females (12.3%). Infection was age-dependent only in females. Prevalence of very large tattoos was also higher in males (28.6%) than in females (11.1%). These data suggest that male *T. truncatus* are more vulnerable to TSD than females, possibly because of differences in immune response and susceptibility to captivity-related stress.

**KEYWORDS**

Tattoo skin disease; common bottlenose dolphin; *Tursiops truncatus*; captivity; epidemiology

Common bottlenose dolphins (*Tursiops truncatus*) in captivity are affected by several cutaneous diseases, with the subacute form of erysipelas and tattoo skin disease (TSD) being the most notorious (Dunn, Buck, & Robeck, 2001; Geraci, Hicks, & St Aubin, 1979; Geraci, Sauer, & Medway, 1966; Sweeney & Ridgway, 1975). Erysipelas is caused by the pathogenic bacterium *Erysipelothrix rhusiopathiae*, and in the dermatologic form, it is characterized by gray, elevated rhomboid lesions that occur over the entire body. The pathogenesis in this form presumably involves neutrophilic infiltration, bacterial microthrombosis of the dermal vasculature, and epidermal infarction leading to necrosis. Prompt antibiotic treatment is needed to avoid septicemia and death (Dunn et al., 2001; Geraci et al., 1966; Sweeney & Ridgway, 1975).

In odontocetes, TSD is characterized clinically by irregular, variably extensive, gray or black stippling of the skin (Flom & Houk, 1979; Geraci et al., 1979; Van Bressem, Van Waerebeek, Reyes, Dekegel, & Pastoret, 1993). Macroscopically, a presumptive diagnosis of TSD can be made based on visual inspection of high-resolution photography (Van Bressem et al., 2009). Histologically, there is focal vacuolar degeneration of cells in the *stratum intermedium* and compaction of adjacent cells in this layer. The overlying *stratum externum* is increased in depth by the proliferation of flattened cells extending into the epidermis rather than as an epidermal extension. The vacuolation of some epidermal cells and compaction and hyperplasia of others accounts for the gross appearance of pale foci with dark tattoo-like marks.

Viral replication occurs in a transitional zone between the swollen vacuolated *stratum intermedium* and the compacted periphery, as evidenced by eosinophilic cytoplasmic inclusion bodies.
visible on light microscopy and enveloped dumbbell-shaped virions on electron microscopy (Flom & Houk, 1979; Geraci et al., 1979). In more chronic lesions, there is focal pitting and disruption of the surface layer, allowing entry of bacteria and other opportunists (Geraci et al., 1979). The etiologic agents are poxviruses of the subfamily Chordopoxvirinae, tentatively classified into the genus Cetaceanpoxvirus, which is still awaiting acceptance by the International Committee on Taxonomy of Viruses (Bracht et al., 2006; King, Lefkowitz, Adams, & Carsten, 2012).

Cetacean poxviruses (CPVs) are genetically and antigenically more closely related to the orthopoxviruses than to the parapoxviruses (Barnett et al., 2015; Blacklaws et al., 2013; Bracht et al., 2006; Fiorito et al., 2015; Van Bressem, Van Waerebeek, & Bennett, 2006). TSD is generally not a life-threatening disease (Flom & Houk, 1979; Geraci et al., 1979; Smith, Skilling, & Ridgway, 1983). However, though its histological features and epidemiological pattern have been thoroughly investigated (Flom & Houk, 1979; Geraci et al., 1979; Smith et al., 1983; Van Bressem, Gaspar, & Aznar, 2003; Van Bressem & Van Waerebeek, 1996; Van Bressem et al., 2009), its pathogenesis remains poorly understood.

In free-ranging Peruvian dusky dolphins (Lagenorhynchus obscurus), common dolphins (Delphinus capensis and D. delphis), T. truncatus, and Hector’s dolphin (Cephalorhynchus hectori hectori), TSD prevalence levels varied significantly with age and were higher in juveniles compared with calves; this finding was attributed to the progressive loss of maternal immunity (Van Bressem & Van Waerebeek, 1996; Van Bressem et al., 2009). Juveniles also had a higher probability of suffering from TSD than did adults, presumably because more adults had acquired active immunity following infection. A worldwide study of 1392 free-ranging odontocetes, comprising 17 species, suggested that the epidemiological pattern and severity of TSD are indicators of population health (Van Bressem et al., 2009). In captive common bottlenose dolphins, environmental conditions and general health may influence TSD expression, persistence, and recurrence (Geraci et al., 1979; Smith et al., 1983).

Recent reports of severe and persistent TSD in captive dolphins in popular media prompted this investigation using methodology designed to document dermatitis in free-ranging dolphins (Murdoch et al., 2008; Sanino, Van Bressem, Van Waerebeek, & Pozo, 2014; Van Bressem et al., 2003; Wilson, Thompson, & Hammond, 1997). Here we report the clinical presentation and epidemiology of TSD in captive common bottlenose dolphins at several facilities in the Northern Hemisphere.

**Material and methods**

**Dolphins**

High-quality images of 146 female and 111 male bottlenose dolphins held in 31 dolphinariums and marine parks in the United States (n = 18; F1–F18) and Europe (n = 13; F19–F31) from 2008 to 2014 were examined for the presence of TSD (Table 1). Images and biological data for each dolphin were initially retrieved from the Ceta-Base inventory, an online database that maintains a historical record of captive cetaceans (http://www.cetabase.org/captive/cetacean), in agreement with the managing director. Whenever possible, biological data were further checked using the Marine Mammal Inventory (http://www.nmfs.noaa.gov/pr/permits/inventory.htm), a database of all marine mammals held in permanent captivity under the National Oceanic and Atmospheric Administration (NOAA) Fisheries jurisdiction and maintained by the National Marine Fisheries Service. Additional photographs of the dolphins published on Flickr were also checked for the presence of tattoo skin lesions (often abbreviated as “tattoos”) and for the precise date and location. Dolphins were classified into three age categories (0–3 years, 4–8 years, and older than 9 years), approximating the life stages of “calves and young juveniles,” “juveniles and subadults,” and “adults,” respectively (Read, Wells, Hohn, & Scott, 1993).
Table 1. Prevalence (Prev) of tattoo skin disease in captive common bottlenose dolphin communities held in facilities in the Northern Hemisphere.

<table>
<thead>
<tr>
<th>Facilities</th>
<th>Males Nt</th>
<th>Npos</th>
<th>Females Nt</th>
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<th>Prev (%)</th>
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<td>Total</td>
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<td>35</td>
<td>146</td>
<td>18</td>
<td>257</td>
<td>53</td>
<td>20.6</td>
</tr>
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</table>

Note. The corresponding facilities for each code are provided in the Materials and Methods section. Affected facilities are indicated in italics.

Nt = total number of dolphins for whom high-quality images were available. Npos = number of dolphins positive for tattoo skin lesions.

^aPresence of very large tattoo skin lesions in dolphins housed at these facilities.

Codes used for facilities were F1, Baltimore National Aquarium, MD; F2, Brookfield Zoo, IL; F3, Clearwater, FL; F4, Dolphin Cove/Dolphin Plus Bayside, FL; F5, Dolphin Plus/Island Dolphin Care, FL; F6, Dolphin Research Center, FL; F7, Gulf World Marine Park, FL; F8, Gulfarium, Marine Adventure Park, FL; F9, Indianapolis Zoo, IN; F10, Institute for Marine Mammal Studies, MS; F11, Miami Seaquarium, FL; F12, SeaWorld San Antonio, TX; F13, Six Flags Discovery Kingdom, CA; F14, Texas State Aquarium, TX; F15, Discovery Cove, FL; F16, Mirage Dolphin Habitat, NV; F17, SeaWorld Orlando, FL; F18, SeaWorld San Diego, CA; F19, Acquario di Genova, Italy; F20, Boudewijn Park, Belgium; F21, Loro Parque, Spain; F22, Marineland Antibes, France; F23, OltreMare, Italy; F24, Selwo Marina, Spain; F25, Tampereen Sarkanniemi, Finland; F26, Tiergarten Nurnberg, Germany; F27, Zoo Aquarium de Madrid, Spain; F28, Parc Astérix,
Tattoo skin disease

Tattoos were identified on the basis of their macroscopic appearance—that is, irregular, dark gray to black, cutaneous lesions with a stippled pattern. They were previously shown to be associated with poxviruses in free-living and captive *T. truncatus* by electron microscopy and/or polymerase chain reaction (Bracht et al., 2006; Flom & Houk, 1979; Geraci et al., 1979; Van Bressem & Van Waerebeek, 1996). We calibrated lesion size relative to the maximum diameter of the dolphin’s eye (cornea) and height of the dorsal fin. “Small” lesions (< 15 mm) corresponded to affected areas smaller than half the corneal diameter; “medium-sized” lesions (15–50 mm) had a diameter less than twice the corneal diameter; and “large” tattoos (51–115 mm) measured more than twice the corneal diameter and up to half of the dorsal fin height (Cartee, Brosemer, & Ridgway, 1995; Sanino et al., 2014; Van Bressem et al., 2015). “Very large” tattoos measured at least half of the dorsal fin height (> 115 mm). The age at first detection, the topography, and the number of lesions were also examined.

The minimal duration of the disease was examined using serial images of 30 positive dolphins taken from 2008 to 2014. We calculated TSD prevalence levels in the different facilities for 2012 to 2014 and examined the influence of sex and age categories on prevalence. Statistical significance of differences in prevalence (α = 0.05) was verified with chi-square tests.

Results

**Characteristics of tattoo skin disease in captive dolphins**

Tattoos were typical in all affected dolphins and were often associated with tooth rakes inflicted by conspecifics in both sexes (Figure 1). They were mostly distributed on the head, throat, flanks, and back (Figure 1) and ranged in number from 1 to more than 20 on the visible body parts. Hence, these are minimum numbers. Tattoo size varied from small (5 mm) to very large (> 750 mm; Figure 1). The very large lesions were found in 10 males and 2 females ranging in age from 9 years to 29 years (Figure 2) and housed in the United States and in Europe (Table 1), respectively. Minimal TSD persistence varied from 4 to 65 months (x̄ = 32.8; SD = 21.0) in 30 dolphins while it was more than 22 months in those with very large lesions (Figure 3). The youngest dolphins with TSD were 14- and 15-month-old females born in The Netherlands in 2013. These calves had very few tattoos that varied in size from small to medium, and in 1 calf, the skin lesions persisted for at least 4 months.

**Epidemiology of tattoo skin disease in captive dolphins**

From 2012 to 2014, 53 of 257 (20.6%) *T. truncatus* held in 31 marine parks in the United States and Europe had active TSD (Table 1). Six facilities were affected in the United States, and 4 were affected in Europe (Table 1). Clinical TSD prevalence varied significantly (χ² = 15.05, df = 4, p = 0.05) between facilities with 10 or more dolphins, from 5.6% (n = 18, F13) to 60% (n = 20, F17; Table 1). TSD affected 18 females and 35 males (Table 1). Prevalence was significantly (χ² = 14.2, df = 1, p < 0.001) higher among males (31.5%, n = 111) than females (12.3%, n = 146). Tattoo prevalence varied with age categories in females and was the highest (23.1%) among the 4- to 8-year-olds (Figure 4). However, this variation was not statistically significant (χ² = 3.4, df = 2, p = 0.18).

In males, prevalence levels were high (greater than 25%) in all three age categories (Figure 4). Among affected dolphins, prevalence of very large tattoos was higher in males (28.6%, n = 35) than in females (11.1%, n = 18), though this result was not statistically significant (χ² = 2.08, df = 1,
Prevalence of very large lesions varied among three facilities that housed more than 10 TSD-positive dolphins. It reached 50% at F17 (n = 12) and 16.7% at F18 (n = 12), but was 0 at F30 (n = 17).

Discussion

We applied techniques used to document TSD in free-ranging dolphins to study the epidemiology of infection in 257 captive common bottlenose dolphins in facilities in the Northern Hemisphere. The authors previously validated this approach by comparing electron microscopy and molecular diagnostic data with clinical presentation (Blacklaws et al., 2013; Duignan, 2000; Van Bressem & Van Waerebeek, 1996; Van Bressem et al., 1993). The disease was detected at 10
facilities in the United States and Europe, with prevalence levels varying from 5.6% to 60% during 2012 to 2014 (Table 1).

Differences in prevalence may reflect variation in environmental conditions, such as housing, water treatment methodology, crowding, noise, confinement, excessive or insufficient sun exposure, the presence of aggressive individuals (Dierauf, 1990; Fair et al., 2014; Geraci et al., 1979; Waples & Gales, 2002), and/or the circulation of different strains of CPVs with variable virulence in the affected facilities. By comparison, in three free-ranging T. truncatus communities, prevalence levels varied from 4.5% (Strait of Gibraltar, Spain, n = 334) and 5.1% (Southeast Pacific Ocean, Peru, n = 79) to 21.9% (Sado Estuary, Portugal, n = 32; Jiménez-Torres et al., 2013; Van Bressem et al., 2003). The dolphins residing in the Sado Estuary are regarded as a community under chronic physiological stress due to severe environmental degradation and high Polychlorinated biphenyl (PCB) exposure (Augusto, Rachinas-Lopes, & dos Santos, 2012; Jepson et al., 2016).
The earliest TSD infection among captive *T. truncatus* was detected in calves aged 14 and 15 months old. Younger dolphins may still be protected by maternal immunity, which was proposed to explain the significant age-related variation in prevalence encountered in several species of free-ranging odontocetes (Van Bressem & Van Waerebeek, 1996; Van Bressem et al., 2009). Tattoo lesions were often associated with tooth rakes, suggesting that the virus could be transmitted directly by bites or that a damaged epidermis may facilitate infection. If so, excessive, aggressive behaviors may lead to a higher occurrence of TSD in facilities where social stress is high. TSD persisted for months and even years in some individuals, and the lesions progressively grew to a very large size. This pattern is consistent with CPVs that have developed immune evasion strategies, as has been reported for other poxviruses (Shiler, 2015; Yousif & Al-Naeem, 2012). A long period of infectivity would favor viral persistence in marine parks. Further investigations on the pathogenesis of TSD in captive dolphins are clearly necessary.

At six facilities, very large lesions were found in 10 males and 2 females aged 9 to 29 years old (Table 1). In all cases, the very large tattoos were observed in dolphins who had shown clinical TSD for more than 22 months. Prevalence of such unusual lesions was high among TSD-positive dolphins kept at F17 (50%, n = 12) and F18 (16.7%, n = 12). Overall, TSD prevalence was also high in these facilities (Table 1), suggesting environmental and/or social conditions that favor TSD occurrence and persistence. Both housed more than 30 dolphins, with a high, but variable, proportion of males (> 50%) in F17.

Two other notable cases of very large tattoos were detected at F16 and F29. There, the presence of very large lesions was associated, perhaps spuriously, with excess (F16) or lack (F29) of exposure to natural sunlight. Excessive sun exposure has also long been recognized as an environmental stressor in captive dolphins (Dierauf, 1990), and mitochondrial DNA damage associated with ultraviolet-induced microscopic lesions and apoptosis has recently been documented in three whale species (Bowman, Martinez-Levasseur, Acevedo-Whitehouse, Gendron, & Birch-Machin, 2013). In free-ranging *T. truncatus*, very large tattoos were observed only in the Sado Estuary community that is under chronic physiological stress (Augusto et al., 2012; Jepson et al., 2016; Van Bressem et al., 2003).

Very large tattoos may be confused with infarctive epidermal necrosis caused by *E. rhusiopathiae* (Dunn et al., 2001; Geraci et al., 1966; Melero et al., 2011). However, the dermatitis of erysipelas lacks the characteristic stippled pattern of tattoos; has a rhomboid, square, or rectangular shape (cf. diamond skin disease); and runs a very different clinical course, potentially leading to septicemia and death without antibiotic treatment (Dunn et al., 2001). Erysipelas is generally a historic disease of captive dolphins, because most facilities have eliminated clinical disease through vaccination (Lacave, Cox, Hermans, Devriese, & Goddeeris, 2001). TSD is a progressive, persistent disease in
captive dolphins, with no documented associated mortality but also no reported treatment. There is no evidence of zoonotic transmission of TSD (Van Bressem et al., 2009). However, considering that several nonhuman animal poxviruses can cause diseases in humans (Haller, Peng, McFadden, & Rothenburg, 2014; Hicks & Worthy, 1987), it would be sensible to take protective measures when handling TSD-infected cetaceans and to avoid all physical contact with dolphinarium visitors.

In this study, TSD prevalence was found to be 2.5 times higher in males than in females. This epidemiological pattern departs from that observed in free-ranging Delphinidae for whom there is no sex bias (Van Bressem & Van Waerebeek, 1996; Van Bressem et al., 2009). In the captive females, prevalence varied with age category and was the highest in individuals aged 4 to 8 years old. Similarly, in free-ranging Delphinidae, the highest TSD prevalence levels occurred in juveniles (Van Bressem et al., 2003; Van Bressem & Van Waerebeek, 1996; Van Bressem et al., 2009). However, among males, prevalence was high in all age classes. Males were also more likely to develop very large tattoo lesions than were females, though the differences were not statistically significant.

These data suggest that captive male *T. truncatus* are more vulnerable to TSD than females, possibly because of differences in immune response, because males perhaps are under greater social stress from conspecifics than are females, and because they may be more vulnerable to captivity-related environmental stresses (husbandry, water quality, confinement). Sexual maturation, reproduction, and seasonal changes in testosterone can lead to increased stress levels in males through competition and aggression for access to females (Connor & Smolker, 1995; Ridgway, 1972; Waples & Gales, 2002; Yamamoto et al., 2015). Numerous stress-related diseases, such as ulcerative gastritis, perforating ulcer, cardiogenic shock, and psychogenic shock have been reported in captive cetaceans (Marino & Frohoff, 2011). Interestingly, male *T. truncatus* kept in captivity in the United States and Portugal were also significantly more likely to be papillomavirus seropositive compared with females, while seroprevalence was not sex-dependent in dolphins free-ranging in Florida and South Carolina (Rehtanz, Ghim, & McFee, 2010). Altogether these data suggest that, generally, captive male bottlenose dolphins often suffer more stress-related diseases than do captive females.

Infection with CPV has long been recognized in facilities housing captive dolphins, and there have been many hypotheses regarding the transmission and maintenance of infection (Flom & Houk, 1979; Geraci et al., 1979; Smith et al., 1983). Poxviruses likely circulate between facilities, as dolphins are regularly transferred from one aquarium to another. This procedure logically increases the risk for transmission between facilities and favors a broader geographic distribution of poxviruses and other pathogens in captive dolphins. Furthermore, shipment of dolphins between facilities is a very stressful event that may lead to alterations of the neuroendocrine response and to immune response impairment (Noda, Akiyoshi, Aoki, Shimada, & Ohashi, 2007; Spoon & Romano, 2012) and thus increase disease susceptibility. Combined, these data suggest that a high TSD prevalence together with a significantly higher occurrence in males than in females and the presence of very large tattoos may indicate chronic stress in captive bottlenose dolphins.

**Conclusion**

TSD occurs at varying prevalence levels between facilities holding captive common bottlenose dolphin groups; the reasons for this finding should be further investigated. The CPVs circulating in captive dolphins need to be characterized and compared to those infecting free-ranging populations. Though these viruses are not known to cause clinical disease in humans, all physical contact between marine mammal park visitors and infected dolphins should be avoided. This avoidance may have implications for swim-with-dolphin programs. TSD may persist for months and even years, and some dolphins, especially males, develop very large tattoos over time. The role of specific captive environmental conditions (husbandry, crowding, social structure, light intensity, etc.) should be studied for potentially contributing factors.
Captive male *T. truncatus* have significantly higher TSD prevalence levels than females, with high prevalence levels in all age categories, and they are more frequently affected by very large tattoos. Males may be more vulnerable to environmental and social stresses than females. Assessment of behavior, improvement of environmental quality, reduction in the number of dolphins in each pool, and maintenance of appropriate groupings of age and sex classes should be attempted to reduce physiological and social stresses and ensuing aggressive behaviors. The potential correlation of TSD and tooth rakes provides a suspected inoculation route that needs to be further explored.

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