Bacteria and yeast normal microbiota from respiratory tract and genital area of bottlenose dolphins (*Tursiops truncatus*)

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The information available regarding microbiological aspects of bottlenose dolphins describes pathogenic bacteria associated with stranded dolphins. There are only few reports describing normal microbiota in captive animals. Bacteria, viruses, fungi and protozoa constitute the normal microbiota, many of these microorganisms are part of the environment, and this co-existence is usually beneficial for the host. Nevertheless, normal microbiota could become opportunistic pathogens when the animals are stressed, immunosuppressed, or under antimicrobial medical treatment. The bottlenose dolphin is very popular in sea parks, however scarce knowledge about their normal microbiota, pathogenic potential and possible zoonotic relationship had not been determined jet. This review helps to understand the significance of achieving a database of these microorganisms with the purpose of a wide comprehension of their role in dolphins and people who works in straight contact with them.

Keywords normal microbiota; bacteria; yeast; marine mammals; dolphins

1. Normal microbiota

1.1 Definition

Normal microbiota is the term comprising bacteria, virus, protozoans and fungi which dwell in different anatomical regions supporting a tight relationship with the animal and human homeostasis (Ingraham and Ingraham, 2000; Sorum and Sunde, 2001; Tlaskalova *et al.,* 2004). These microorganisms are adapted to live within the host without causing disease or damage (Tlaskalova *et al.,* 2004). Likewise, normal microbiota can be transitory or permanent. Transitory microbiota colonize for short periods and it is originated from environmental microorganisms; and long-lasting microbiota is the one that persist for long periods of time, and is similar among individuals of the same specie (Ingraham and Ingraham, 2000; Hernández *et al*., 2004).

Microorganisms are ubiquitously in the environment and continuously in contact with animals and human; this interaction arises from birth trough ingestion or inhalation and elapse through the animals life-course. Each anatomical region creates its own selective environment where few microorganisms are favored above others (Tlaskalova *et al*., 2004). Therefore, microorganisms from internal and external surfaces such as oral cavity, gastrointestinal, respiratory and genitourinary tracts, conjunctiva and skin of healthy animals are considered normal microbiota (Sorum and Sunde, 2001; Ingraham and Ingraham, 2000; Tlaskalova *et al*., 2004). Some of these microorganisms are non-pathogens as dwellers of a specific region, but they can turn to be pathogens if they are moved to another region (Levinson and Jawetz, 2000).

1.2 Composition and function

Normal microbiota establishes through the interaction between the host and the environment. It is determined by such factors as age, race, hormones, diet, stress, sexual behavior, medication, season, geographical location, lodging, population density, animal contact and cleaning procedures (Sorum and Sunde, 2000). Natural microbiota will conform a complex mechanism over the mucous surface and skin where their interaction provide resistance against pathogenic microorganisms due to the substances, antimicrobial peptides produced, nutriment competition and the production of extracellular enzymes which act as inhibitors. Besides, they interfere the opportunist pathogens assault (Sorum and Sunde, 2000; Levinson and Jawetz, 2000; Tlaskalova *et al*., 2004), and takes in charge of the digestion of metabolizable substrates, vitamin production, stimulation of the immune system, and development of mucous cells. Moreover, the intestine mucous intervenes in the development and expansion of the lymphoid tissue, the maintenance and regulation of the immune response, and the intestinal traffic regulation (Kelly and Conway, 2005).

1.3 Interaction with the immune system

The response of the immune system grants certain tolerance toward normal microbiota, this response consists of three phases: (1) detection of the microorganism, (2) translation of the microorganism recognition to an appropriate signal and (3) induction of the appropriate effectors response (Kelly and Conway, 2005). These chain, mainly developed by

diverse families of receptors and a signal transduction system involved in the recognition of preserved molecules among microorganisms, some of these receptors are toll-like receptors (TLR), and Nod1 and Nod2 (nucleotide-binding oligomerisation domain proteins) (Kelly and Conway, 2005; Portnoy, 2005). The coexistence of normal microbiota is usually beneficial to the host thought the ceaseless interaction between both, becoming a significant role in the coevolution for their joint survival (Steiner *et al*., 2000).

1.4 Relation with stress and diseased

When stress causing factors are severe or persistent and the animal is unable to adapt, a harmful imbalance is generated in the animal, which consequences can be notice at the immune, psychic, metabolic and reproductive level (Aubin and Dierauf, 2001). Finally, crowning in a functional alteration of the immune system and turn the animal vulnerable to infectious disease that will turn to be the development of a pathological condition (Aubin and Dierauf, 2001).Captive and free living marine mammals are subjected to a series of events that can cause stress, for example; environmental degradation, boats heavy traffic and excessive noise, social environment, predation, fishing, food competition, parasitism, capture, transportation, isolation, overcrowding, inadequate facilities and excessive noises (Aubin and Dierauf, 2001; VanBressem MF *et al*., 2009).

Diverse studies have been done in dolphins *T. truncates* to determine those physiological signs of stress; neutrophylia, eosinopenia, lymphopenia, leukocytosis, hyperglycemia, cortisol increment and aldosterone in whey, erythrocytes sedimentation increment, hypoferremia, urine's osmolarity increment and changes in the normal microflora of the respiratory tract (Aubin and Dierauf, 2001). At this point, when they are exposed to environmental, physiological, nutritional or immunological altered conditions, members of the normal microbiota turn to be opportunistic and cause disease (Tlaskalova *et al*., 2004). This condition is met specially in immunosuppressed individuals; likewise, this condition is met under wide spectrum antimicrobial treatment (Tlaskalova *et al*., 2004).

Chlorine and other antimicrobial components added to water, as well as the antimicrobial treatment can alter normal microbiota and trigger pathogens dissemination which will display advantages allowing them to establish and infect immune depressed animals (Buck, 1980). The interaction between host and microorganisms is a fragile and fluctuating line; its unbalance can provoke host death, latency states, microorganism eradication, colonization or commensalism. This unbalanced condition frequently fall into disease development. In that manner, virulence is a variable dependent on the host susceptibility, background and on the interaction´s nature between host and microorganism (Casedevall and Pirofski, 2001).

1.5 Importance in marine mammals

While rehabilitation of stranded or captive animals normal microbiota data supports straightforwardly a wide understanding of the bacteria role on dolphins with physiological disorders, and allows the assessment of the general health status of free-living marine mammals, it leads to the delineation of methods intended to assess and prevent disease in captive dolphins, along with the appraisal of the human transmission risk. The scarce evidence of those microorganisms associated with this specie results in misunderstandings, therefore efforts should be emphasized due to the last year's apogee of dolphin's interactions with humans in practices such as swimming with dolphins, and dolphins oriented therapy particularly because participants are primarily senile people, children and pregnant women. All these population is under immunosuppression menace. Besides, it is worthwhile on the improvement of biosecurity measures leading to better management in captivity and rehabilitation.

1.6 Considerations for the microbiological sampling

To accomplish a normal microbiota study, it should be done with proper regard to the following key points; dolphins must not be dealing with any clinical pathology, neither infection, nor under antibiotic treatment at least during the former three weeks before the sampling scheme. It is profitable to organize simultaneous studies in order to support the animal general health status as cytology, complete blood count, biochemistry test, general examination and file full review. Under captivity conditions trained animals are a prerequisite for friendly sampling through operant conditioning and the execution of chemical containment. These will allow a no more than 10 minutes of non-stress management per dolphin ensuring lumped parameters under physiological levels.

In order to obtain good respiratory tract samples, no water contamination should be achieved. The blowhole periphery must be cleaned with a sterile gauze, followed by the placement of a sterile bag or container of broader size which surpass by approximately 2 cm the blowhole wide. Two strong expirations will give deep respiratory track as shown in Fig 1. It is better to sample the second blow in order to catch it without water. Finally, the sample is identified, and transferred by means of sterile hyssops.

For vaginal or preputial samples dolphins should be rotated into the back-ventral position so as to expose the dolphins genital area through the water level, again the area must be cleaned by means of sterile gauzes and the genital split is gently lay aside with the fingers (it is recommended to wear sterile gloves) Fig. 2, then proceed to introduce a

sterile hyssop into the vagina or into the foreskin, with circular movements, two samples should be taken from each dolphin, and identify each sample.

Samples should be maintained under cooling and should be processed in a maximum of 24 hours period.

Fig. 1. How to place the container upon dolphin's blowhole, sampling.

Fig. 2. Dolphin's genital area detached allowing clear and successfully obtaining an optimum microbiologic sample.

2. Microorganisms associated with bottlenose dolphins

In the last years, bacterial diseases, morvilivirus infection and fitotoxins had been recognized to be main reasons of marine wild mammal's mortality around the world (Dunn *et al*., 2001; Van Bressem MF *et al*., 2009). Additionally, pathogenic bacteria have been identified in unrecognized or newly infected hosts. For instance, *Mycobacterium* species present high morbidity and mortality in captive and free-living populations of marine mammals.

Of equal importance, brucellosis another disease known to infect terrestrial mammals had been documented in marine mammals all over the world (Higgins, 2000; Dunn *et al*., 2001; Bourg *et al*., 2007). Diverse factors hamper the quantification of the bacterial diseases burden into morbidity and mortality of wild life marine mammals, nevertheless increasing research efforts are heading to this area.

In contrast with free-living dolphins, fungi and bacterial infections are common reason of dolphin death in captivity. A significant proportion of cetacean diagnosed with respiratory tract problems develop pulmonary abscesses, from which the most frequently associated bacteria are *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and scarcely Gram negative bacteria. However, bacterial diseases turn to be the outcome of strong parasitism under captivity and free living dolphins (Dunn *et al*., 2001).

Aerobacter spp., *Staphylococcus* spp., *Streptococcus* spp., *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa* had been reported to be cause of pneumonia. They are associated to signs such as depression, halitosis and breathing difficulty (Higgins, 2000; Medway, 1973; Medway, 1980). Bacterial primary or secondary infections associated to pneumonia are frequently developed by free living cetacean, particularly in small sized ones causing the animal death due to Gram negative bacteria such as *Edwarsiella* spp., *Klebsiella* spp., *Salmonella* spp., *Vibrio* spp., *Aeromonas* spp., *Pseudomonas* spp. y *Pasteurella* spp. (Kawashma *et al*., 2004). Many of these microorganisms can be part of the normal microbiota and under immunosuppression the opportunistic infection arises.

2.1 Bacterial diseases

Aeromonas spp.

Aeromonas spp., comprise microorganisms with wide distribution in the aquatic environment (Hatha *et al*., 2005), diverse studies focus on mobile species because they had been identified as pathogens for several animals and humans (Hatha *et al*., 2005). Fish intestine lodge a great quantity of these microorganisms, principally *A. hydrophila*, *A. caviae* and *A. sober*. Among these three species, *A. hydrophila* has been identified as the etiology of a wide disease variety including fish systemic and localized disease (Loghothetis and Austin, 1996; Hatha *et al*., 2005). *A. hydrophila* cause, in dolphins *T. truncates,* ulcerous dermatitis, pneumonia and septicemia (Cusick and Bullock, 1973), it has also been isolated from the tegumentary system, liver, lung, spleen, kidney and heart blood of the bottlenose dolphin (Cusick and Bullock, 1973; Higgins, 2000).

Brucella pinnipediae and *Brucella cetaceae*

In the early nineties, *Brucella* was isolated from corpses of seals, dolphins, porpoises and whales (Bourge *et al.,* 2007; Foster *et al*., 2002). Previous characterization of these *Brucella* strains showed genetically differences among marine and terrestrial isolations, and revealed new species, which were proposed as *Brucella pinnipediae* and *Brucella cetaceae*. Moreover evidence had pointed out the existence of subspecies within these groups (Bourge *et al*., 2007; Dawson *et al*., 2008).

The serological evidence of *Brucella* spp., exposition in several cetaceans has been extensively documented, including *T. truncatus*. *Brucella* isolation from marine mammals and strain biotyping were first reported simultaneously in 1994. Ross *et al*. (1994) reported *Brucella* isolation from *post-mortem* samples of marine mammals stranded near Scotland coasts, namely; common seal (*Phoca vitulina*), porpoise (*Phoca enaphocoena*) and common dolphin (*Delphinus delphis*) (Foster *et al*., 2002); meanwhile Ewalt *et al*. (1994), published a *Brucella* isolation from an aborted fetus of a bottlenose dolphin (*T. truncatus)* in the EUA (Dunn *et al*., 2001; Higgins, 2000; Foster *et al*., 2002). Even though the scarce number of isolations, there is strong serological evidence of this infections wide scope, its prevalence, the variety of infected species and its wide geographical distribution (Dawson *et al.*, 2008).

Clostridium perfringens

Clostridium perfringens an anaerobic bacterium is the causal agent of clostridiasis. Its entry route are skin injuries, causing myositis and in unattended cases the animal death. It has been isolated from different marine mammals as dolphin (*Tursiops truncatus*), whale (*Orcinus killer whale*), and California sea lion (*Zalophus californianus*) from myositis injuries from the area in which intramuscular injections were applied (Buck and Shepard 1987). Additionally, another report described sudden death of a female dolphin due to clostridial infection of injuries infringed by another dolphin. Bacterium was isolated from dorsal muscle, blood, left heart ventricle, and thoracic and abdominal fluid. Finally, *C. chauvoei* and *C. novyi* had been also isolated from marine mammal's tissues (Buck and Shepard 1987).

Erysipelothrix rhusopathiae

Pleomorphic Gram positive bacilli, causal agent of erysipela in pigs and turkeys, which in captive cetacean has been identified following fish contaminated ingestion. Cetaceans are the most susceptible marine mammals. Two presentations had been described; the first one is the septicemic, which frequently culminates in sudden death without specific signs. This presentation was reported in several marine mammals including the atlantics spotted dolphin (*Stenella plagiodon*), Atlantic white-beaked dolphin (*Lagenorhynchus albirostris*), bottlenose dolphins (*Tursiops truncatus*), Atlantic spotted dolphins, Risso dolphins (*Grampus griseus*), and the pacific white sided dolphins(*Lagenorhynchus obliquidens*) (Medway, 1973); the second is the dermatological form characterized by skin greyish rhombuses shaped lesions in the dorsal trunk, the snout and on the melon, besides anorexia, weakness and black colored excrement. This last presentation was reported in an 18-month-old bottlenose dolphin (Medway, 1973; Higgins, 2000). The microorganism can be isolated from lymphnodes, lung and liver. In the septicemic form under autopsy, dropsy, intestinal multifocal petechiae, ecchymoses, hemorrhages, splenomegaly and skin injuries were described (Medway, 1973; Higgins, 2000). Therefore considerable controversy has arisen regarding the appropriateness of vaccination regimes in captive marinas (Medway, 1973).

Helicobacter cetorum

Helicobacter species are microaerobic, motile, fusiform, or slightly curved-to-spiral, gram-negative bacteria that over the last decade have been linked to gastritis with and without ulcers in a wide variety of animals and humans (Harper *et al*., 2003; Goldman *et al*., 2009). In marine mammals, *H. cetorum* has been isolated from main stomachs and feces of stranded and captive cetaceans, and it was suggested that this specie may be involved in the development of gastritis in this animals (Harper, 2003). *H. cetorum* infection has been detected by PCR of feces or gastric fluid in 9 of 14 captive cetaceans and 13 of 18 stranded wild cetaceans, including Atlantic bottlenose dolphins (*T. truncatus*), Atlantic white-sided dolphins (*Lagenorhynchus acutus*), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*), and a captive beluga whale (*Delphinapterus leucas*) (Harper, 2003). Oral–oral and fecal–oral routes may also be implicated in transmission of *Helicobacter* infection in marine mammals; both gastric and enterohepatic *Helicobacter* species can survive for prolonged periods in water, which also supports the role of environmental transmission in the marine environment (Goldman *et al.,* 2009). Gastric ulcers have been reported for decades in wild and captive dolphins, the clinical signs include inappetence, anorexia, abdominal tenderness, depression, and occasional unresponsiveness (Harper *et al.*, 2000). Complete blood count may reveal a leukocytosis and anemia if bleeding is present. This clinical syndrome is consistent with gastritis and peptic ulcer disease (PUD) (Harper *et al.*, 2000). *H. cetorum* infection has been demonstrated in clinically healthy captive cetaceans and others with signs including chronic regurgitation,

intermittent inappetence, weight loss, lethargy and in some cases infections associated with gastritis and presence of spiral-to-curved bacteria in inflamed tissues (Harper *et al.,* 2003).

Nocardia spp.

Nocardia are filamentous gram positive bacteria, that are generally in the environment, manure, water and soil, easily inhaled or aspirated (Dunn, *et al.*, 2001). In cetaceans pathogenic nocardias as *N. asteroids* and *N. brasiliensis,* had been reported (Fowler, 1993), frequently developing pulmonary or extrapulmonary disease forms which have been described in nine cetacean species (Fowler, 1993). *N. asteroids* have been associated also with necrosis and pyogranulomatous lymphadenitis, pleurisy, encephalitis and mastitis. *N. paraguayensis*, is the causal agent of mycetoma, where ulcerous injuries can be observed along the oral cavity (Fowler, 1993).

Pasteurella multocida

Pasteurella cause disease in ruminants and rabbits, nevertheless in dolphins appears as an hemorrhagic bronchopneumonia (Medway, 1973), and resembles an acute or subacute blood poisoning. Frequently, death happens without clinical signs, or only few hours of anorexia or other behavior signs as lethargy and declined swimming (Dunn *et al*., 2001). Other authors have mentioned *P. multocida* as cause of enteritis, ending in animal death due to bacteremia and intestinal hemorrhage, also *Mannheimia haemolytica* has been reported to cause hemorrhagic tracheitis (Dunn *et al*., 2001).

Pseudomonas aeruginosa

Gram negativa bacteria that can be found in must of natural environments including water bodies, plants, soil, waste water, and humid environments. Occasionally as part of the intestinal microbiota or in human skin acting as oportunistic or nosocomial pathogen of chronic bronchoneumonia (Gyles *et al*., 2004). *P. aeruginosa*, generally requieres an immunological alteration in the host in order to establish as an infection and developing pneumonia, bacteremia, urinary tract and surgical wound infections in humans and other animals. In dogs it is related to otitis and to dermatitis in lambs (Gyles *et al.*, 2004). In bottlenose dolphins it causes bronchopneumonia, dermatitis, osteomyelitis and septicemia. Also, it is present in skin necrosis and ulceration, respiratory problems and depression. *Pseudomonas* has been isolated from the respiratory and digestive system of belugas (Dunn *et al*., 2001); in contrast in marine wolves it is regarded as part of the normal microbiota (Dunn *et al.*, 2001).

S. aureus

In bottlenose dolphins under captivity or free-life, it is considered a part of the normal microbiota (Streitfeld and Chapman, 1976). It can be isolated from lungs with bronchopneumonia signs, in respiratory mixed infections and from the tegumentary system of dolphins or seal of Greenland (*Phoca groenlandia*), likewise from the digestive system of belugas (*Delphinapterus leucas*) (Streitfeld and Chapman, 1976).

Streptococcus spp.

A coconut shaped, Gram positive bacterium, implied in mortality and morbidity among marine mammals. Many species had been reported to be isolated from *T. truncatus*, such as *S. zooepidemicus* and B- hemolytic *Streptococcus* from skin abscesses, bronchopneumonia, pyelonephritis, myocarditis, metritis, blood poisoning and osteomyelitis (Evans *et al*., 2006); Evans (2006) mentioned that even though it does not represent a treat for free-living animals it should be taken on account in captivity specially in confined areas, due to the fact that an infected animal can transmit directly the bacterium to other marine mammals or fish (Evans *et al*., 2006). Moreover, two *S. pneumonia* had been recovered from lung tissue biopsies of captive dolphins with asymptomatic death, but autopsy showed pneumococci (Van der Linden, 2009).

Vibrio spp.

Vibrios are Gram negative bacteria in high densities in water and marine organisms like corals, fish, mollusks, grass of the sea, sponges, shrimp and zooplankton, environments like estuaries, coasts, sediments and acuaculture (Thompson *et al.*, 2004). In dolphins *T. truncatus;V. fluvialis, V. alginolyticus, V. parahaemolyticus, V. vulnificus, V. cholerae* (without antigen O) and nonclassified vibrios have been described in healthy and sick individuals, in Hawaii they have been isolated routinarely from dregs, blowhole and pharynx (Schroeder *et al*., 1985; Beck and Rice, 2003).

In other dolphin species, the vibrios have been associated to necrotic hepatitis, acute focal bronchopneumonia, and active dermatitis in fins, subcutaneous tissue and face top, *V. alginolyticus* cause necrotic stomatitis and *V.*

Parahaemolyticus has been isolated from lung, which can be the entry route through which vibrios invade the vascular system across the intestinal mucous, the lung and oral wounds (Schroeder *et al*., 1985; Tangredi and Medway, 1980). These same species can be wound contaminants, causing animal death due to septicemia (Dunn *et* al., 2001), and animal keepers had been pointed out in potential zoonotic risk (Schroeder *et al*., 1985; Tangredi and Medway, 1980).

3.4 Mycotyc Diseases

The mycelia fungi and yeast are usually opportunists or secondary invaders; these microorganisms are a risk for animal health of immunosuppressed animals (Reidarson*et al*., 2001). *Aspergillus fumigatus, Candida* spp*., Cryptococcus neoformans, Fusarium* spp. and zigomicetos (*Apophysomyces elegans, Rhizomucor pusillus, Saksenaea vasiformis*), are considered pathogenic opportunists. Under the same conditions endemic pathogens as *Blastomyces dermatitidis, Coccidioides immitis* and *Histoplasma capsulatum* are capable of infecting healthy animals (Reidarson*et al*., 2001). These fungi manage to invade the host through inhalation, trauma, or ingestion, and finally lodge at the lungs, skin or gastrointestinal tract (Reidarson *et al.,* 2001). Due to the fact that the fungi are poorly transmissible among animals, mycoses are frequently endemic and rarely cause epidemic events (Reidarson *et al.,* 2001). They have been reported twice as zoonosis; *Blastomyces dermatitidis* in a dolphins *T. truncatus* and in *Loboa loboi*, the Amazon dolphin (Reidarson *et al*., 2001).

Aspergillus fumigatus

It is the mycotic infection more frequently reported in marine mammals (Medway, 1973; Reidarson *et al*. 1998). In some dolphins this microorganism has caused necrotizing pneumonia and encephalitis (Sunday *et al*., 1992). It is characterized by for respiratory low system affection with pulmonary nodules, observed as plates by X- rays (Medway, 1973). Reidarson mentioned that bronchoscopy facilitates an early and definitive diagnosis, as well as an opportune treatment; unlike agar gel immunodiffusion which only identifies specific circulating antibodies, vice versa *Aspergillus fumigatus* are evident merely during active infection (Reidarson *et al*., 1998).

Candida albicans

The infection caused by *Candida* is another frequent mycosis in marine mammals (Buck, 1980; Dunn *et al.,* 1982; Higgins, 2000), and had been reported in respiratory tract, skin, oral cavity and oesophagus. The infection dissemination is frequently fatal, and it had been associated to death of many cetaceans under captivity (Sweeney and Ridgway, 1975; Sweeney *et al*., 1976; Buck, 1980; Medway, 1980; Dunn *et al.,* 1982; Higgins, 2000; Dunn, 2001). *C. albicans* is an opportunistic pathogen and is one of the most important species of *Candida*. Skin candidiasis is caused by *C. albicans*, mostly in wounds and mucocutaneous joints nearby blowhole and vagina. Additionally, it can be located in esophagus and gastric area, manifested by regurgitación, vomiting and head shaking (Medway, 1980).The pathognomonic lesion is the esophagus ulceration. Equally, it can realize a systemic invasion towards any organ; kidneys, central nervous system, and heart valves which are the most common targets (Reidarson *et al.,* 2001). The predisposing factors are fecal pollution and organic food waste (Reidarson *et al*., 2001). *C. albicans*, is considered an opportunist, part of the normal microbiota of the mucous where it resides. It has been isolated about 4 to 54% in free-living dolphins *T. truncatus* in Florida, figure similar to those of cetacean mantained in aquariums (Reidarson *et al*., 2001).

Loboa loboi

Loboa loboi is a non-culturable yeast, a dimorphic yeast from the Onygenales order and *Ajellomycetaceae* family (Van Bressem MF *et* al., 2009), and cause a chronic granulomatous dermatitis. Transmission is through direct contact and injuries can be located in any part of the animal body, histological injuries prove to be an acanthosis characterized by squamous cells proliferation (Sweeney and Ridway, 1975). This microorganism has not been isolated and its sistemic disemination has not been proved, its habitat is soil and vegetation allowing skin access by penetration or accidental trauma (Reidarson *et al*., 2001; Van Bressem MF *et al*.2009). Serological data indicates that dolphins and humans are infected with similar *L. loboi* strains. In humans, lobomycosis is a skin chronic fungal infection, endemic in rural regions in South and Central America. The disease has been localized in *T. truncatus* from the Indian River Lagoon, Florida, with prevalence levels oscillating between 6% (n =484) and 12.4% (n = 186) in the period 1996 to 2006 (Van Bressem MF *et* al., 2009).

Cryptococcus neoformans var. *gattii*

This microorganism has been founded in a male Atlantic bottlenose dolphin (*T. truncatus*) (Miller *et al.*, 2002). The clinical signs were tachypnea, transient dyspnea, mild tachycardia and development of multiple hyperechoic nodules, parenchymal consolidation, and pleura thickening (Miller *et al*., 2002). After treatment with itraconazole during 120 days the dolphin died, and the diagnosis was pleuritis and bronchopneumonia. The pathology findings included

pulmonary lymph nodes enlarged and spherical to ellipsoidal extensive coalescent granulomatous lesions (3- to 14-µm), throughout both lungs, mucicarmine-positive, encapsulated, budding cells consistent with *C. neoformans* (Miller *et al.*, 2002). The culture of lung tissue yielded colonies of *C. neoformans* and lastly it was identified as serotype B (Miller. *et al.*, 2002).

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