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Case Report

Lichtheimia ramosa Mucormycosis in a Bottlenose Dolphin

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Abstract

5 years male subadult bottlenose dolphin (*Tursiops truncatus*) named "Pato" belonging to the oceanic dolphin family was maintained in *Friguia* Public Park Dolphinarium in Tunisia for two years dating from January 2010. The patient exhibited acute anorexia and gradual weight loss. The animal developed respiratory failure and died on September 2011 despite treatment including antibiotics and corticosteroids. Over the course of illness, fungal infection was not suspected. Post mortem histopathologic examination revealed the presence of irregular, thick, non-septate and fragmented hyphae consistent with a zygomycete infection. *Lichtheimia ramosa* was identified by morphological characteristics on fungal culture of tissues necropsy and confirmed by PCR sequencing ITS-5.8s-ITS2 ribosomal RNA gene. This is believed to be the first report of *Lichtheimia ramosa* mucormycosis in bottlenose dolphin.

Keywords: Pulmonary; Mucormycosis; Bottlenose Dolphin; *Lichtheimia ramosa*

Introduction

Mycoses are a significant cause of stranding in bottlenose dolphins (*Tursiops truncatus*) and are well documented in both captive and free-ranging individuals [1]. *Aspergillus (Asp) fumigatus* and less frequently *Asp niger* and *Asp terreus* are the more frequent causes of fungal pneumonia in marine mammels [2a]. Even though zygomycetes are more rarely observed in, marine mammals (bottlenose dolphins, harbor porpoises and killer whales) but they often cause rapid fatal disease. Common sites of infection by zygomycetes are the skin, the respiratory system and to a lesser extent the central nervous system [1,2b]. These infections are mainly observed in debilitated animals.

The present report describes a case of pulmonary mucormycosis in a juvenile bottlenose dolphin (*Tursiops truncatus*) caused by *Lichtheimia ramosa*. To our knowledge, it is the first case to be reported in a marine mammal.

Case Presentation

5 years male subadult bottlenose dolphin named "Pato" and belonging to the oceanic dolphin family has been maintained in *Friguia* Public Park Dolphinarium, Tunisia, for two years dating from January 2010.

In November 2010, the animal exhibited an acute anorexia and gradual weight loss. Blood analysis showed increased WBC and neutrophils' count (25.3 10^3/µl, 91% respectively), increased Lactic Dehydrogenase (LDH), gamma glutamyl transferase (γGT), alkaline phosphatase (AP), amylase and lipase enzymes and Total Protein's (TP) levels. Ciprofloxacine (1.75g/kg per os daily for 12 days) and ceftazidime (3.5g per os daily for 10 days) were administered. After a transient improvement, the state of the animal worsened again on January 2011. Hematological and serum biochemical analyses revealed persistent increasing levels of γGT, amylase and lipase enzymes. Corticosteroids were initiated on January 7th 2011 and continued for 8 months (Dexamethasone [2 to 15 mg daily] for 4 days, then continued for 3 months [0.5 to 10 mg] and followed by Prednisone (55 to 110 mg daily for 5 months). On January 14th, fecal culture grew *Echerichia coli* and *Pseudomonas aeroginosa*. *Pseudomonas aeroginosa* was also isolated from the nasal swab. Despite these results, antibiotics were not administered. Over the next 7 months, Pato exhibited alternant periods of anorexia with perturbation of attention and transient improvement.

On August 2011, one month before death, the animal state dramatically worsened. Hematological and biochemical examinations revealed an elevated WBC count, marked thrombocytopenia and a marked increase of AST (540U/l), ALT (277 U/l), γ GT (413U/l), AP (670U/l), amylase, lipase enzyme and TP levels (9.6g/dl). In addition, blood levels of sodium and chloride were increased (164mEq/l and 129 mEq/l respectively). It is worth mentioning that the level of Total Bilirubin (TB) was increased all over the period of captivity of the animal, while Blood Urea Nitrogen (BUN), glycemia and creatinine concentrations were within reference intervals. Further fecal culture grew *Pseudomonas aeroginosa*. Enrofloxacine was initiated (525 mg/kg/day for 6 days) followed by Amikacine (0.75 g/kg/day increased to 1.5g for 7 days) and by Ceftazidime (3.5 g/kg/day for 6 days). Despite antibiotics and corticosteroids treatment, Pato developed respiratory failure and died on September 7th 2011.

Necropsy examination was performed. Samples taken from the lung, trachea and lymph nodes showed an irregular, thick, non-septate and fragmented mycelial filaments (Figure 1) and some necrosis foci inside a granulomatous chronic inflammation.

Direct mycological examination of lung specimens showed large and non-septal hyphae. Fungal culture grew mycelia colonies identified as *lichthemia* sp on the basis of morphological characteristics (Figure 2). Colonies were white with a cottony texture. Reverse was uncolored. Microscopically, colonies were consisted of large, non-septate

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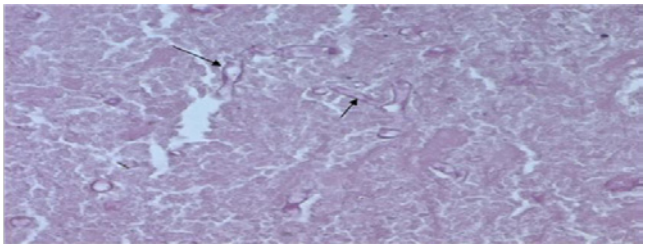

Figure 1: Irregular, thick, non-septate and fragmented hyphae (arrows). Pulmonary tissues necropsy (periodic acid-Schiff reaction) X400.

Figure 2: Macroscopic feature of *Lichtheimia ramosa* in culture on Sabouraud dextrose agar medium.

branching hyphae with funnel-shaped apophysis. Identification was further confirmed by PCR and sequence analysis. The following primers were used (ITS1: 5' GTTCCTCACAGTTTTGTGCA 3'/ ITS4: 5' TTGATTTCAGATCTAGATGT 3') targeting the *ribosomal Internal Transcribed Space* (ITS) of ribosomal DNA. These primers amplify a 735 bp fragment. The sequence alignment of the strain in GenBank database showed 100% homology with *lichtheimia ramosa* registered as *lichtheimia ramosa* strain SN-A2 18S HQ285688.

Discussion

Fungal diseases are well documented in captive and free ranging bottlenose dolphins (*Tursiops truncatus*) [3,6]. Common sites of infection include the skin and respiratory tract [7,8]. Systemic and central nervous system infections have been more rarely reported [1,9].

Pulmonary Aspergillosis is the most frequent mycosis in cetaceans [10]. Among the non-Aspergillus species, zygomycetes, known as ubiquitous opportunistic soil inhabitants, are the most commonly reported. Prognosis of zygomycosis is usually poor because of the rapid dissemination of the disease and it's fatal outcome [11].

Predisposing factors include traumatic tissue damage and prolonged corticotherapy [11,12]. In our patient, the long-term use of antibiotics and corticosteroids together with the opportunistic *Pseudomonas aeroginosa* infection may have acted as predisposing factors and have accelerated the fatal outcome. Mucormycosis in dolphins has been reported to mainly involve central nervous system [1], lungs [13] and skin [14]. Systemic infection has also been reported [8]. Our patient developed a pulmonary infection known to range between the most frequent localizations.

Follow up laboratory testing revealed a high WBC count all over the course of our dolphin disease. Leukocytosis has been reported to be a common finding in infected bottlenose dolphins whatever the infection agent [15]. Increased albumin concentrations, and increased AST, ALT, γGT activities observed in our dolphin are consistent with previous reports on aggressive mycoses [14]. On the other hand, it is known that corticosteroids treatment and skeletal muscle damage from overexertion can further enhance AST and ALT amounts [16].

Ionic perturbations, as those shown our patient, are less documented [2b].

In suspected cases of mucormycosis, diagnosis can be achieved by the demonstration of characteristic hyphae in biopsy specimens. However, in many cases the disease is only diagnosed on post mortem examination of necropsy specimens as the case reported herein. The species identification requires sporulation on culture media. However, cultures can be time-consuming and unreliable as some fungal species do not grow readily [7]. On the other hand, identification based on macroscopic and microscopic mycological characteristics may be difficult and misleading. Therefore, molecular techniques are the best alternative for an accurate identification of the pathogenic species.

Histopathological examination of the necropsy specimens taken from our dolphin showed irregular, thick, non-septate and fragmented hyphae consistent with a zygomycete.

Mycological examination showed the fungus to belong to the *Lichtheimia* genus; further identified as *Lichtheimia ramosa* by PCR sequencing ITS-5.8s-ITS2 ribosomal RNA gene. In previous reported cases of dolphin mucormycosis, molecular techniques used for the identification of the causal species include semi nested PCR and sequencing [17], PCR ITS1-ITS2 [13] and sequencing of D1/D2 of 26 rRNA [18]. Nearly all reported mucormycosis cases in dolphins were caused by one of the following species: *Apophysomyces elegans* [5,8,14,19]; *Saksenaea vasiformis* [14]; *Rhizopus arrhizus* [syn. *R. oryzae*] [13] and *Cunninghamella bertholletiae* [2b,18]. To our knowledge, our study is the first to report a zygomycosis case caused by *Lichtheimia ramosa* in a cetacean. *Lichtheimia ramosa* has already been reported as a causal agent of rhinocerebral zygomycosis in cattle [20] and systemic infection zygomycosis in bovines and other warm-blooded animals [21]. In humans, members of the genus *Lichtheimia* are the second or the third more frequent agent of mucormycosis in Europe and Worldwide, following *R. arrhizus (oryzae)* which is the leading species worldwide [17] and in Tunisia [22].

In our dolphin fungal infection was not suspected over the course of the disease so that no antimycosics were administrated. Early diagnosis, aggressive surgical removal of the infected tissue and the use of posaconazole [19] would have been necessary for a successful outcome.

Conclusion

The present case emphasizes the pathogenic potential of *Lichtheimia ramosa* zygomycosis in marine mammals, as well as the

potential role of opportunistic microflora, particularly *P. aeroginosa* in immunocompromised bottlenose dolphins. Because of the rapid and aggressive nature of the growth of these organisms, early detection and diagnosis of zygomycosis is critical for successful therapy.

Human and Animal Rights

This study did not contain any experiments involving human subjects. In addition, no animal experiments were performed in this study. The animal described in the present article was submitted as a necropsy sample after its death, and all of the procedures applied to the animal were approved by the Committee on the Ethics of Animal Experiments of Obihiro University of Agriculture and Veterinary Medicine. All applicable international, national, and institutional guidelines for the care of animals were followed.

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