Polycystic Kidney Disease in Rough Toothed Dolphins (Steno bredanensis) found in the Paraná coast, Southern Brazil

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ABSTRACT

Background: Renal cystic diseases (RCD) are characterized by cystic structures on renal parenchyma associated with obstructive lesions, membranous disruptions, and/or growth disturbances. The polycystic kidney disease (PKD) shows specific pathological characteristics, related to mutations on PKD1 and/or PKD2 chromosome locus on humans. In Persian cats and bull terriers the condition is like the human “adult-onset” PKD, while in Perendale sheep the “childhood”-like is described. In cetaceans, RCD are reported, however the characterization of PKD is scarcely described. This report aims to describe two cases of PKD and one of RCD in stranded Steno bredanensis, and to discuss the disease associated factors.

Cases: Four rough-toothed-dolphins were found stranded in the Paraná coast, southern Brazil between 2016 to 2018, through the Santos Basin Beach Monitoring Project (PMP-BS), one of the systematic monitoring programs required by Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) for the environmental licensing process of oil production and transport by Petrobras in the presalt province. In three animals histological sampling was performed. One of them (animal 3) was found alive, presenting altered buoyancy with lateralization to the right and signs of pneumonia. The blood analysis showed anemia and leukocytosis. The animal showed poor clinical prognosis, and even with supportive treatment, came to death four days after the rescue. Routine autopsies were performed on all animals. Animals 1 and 2 presented macroscopically enlarged kidneys containing disseminated cystic structures in the parenchyma. On microscopic examination, the cortical region showed diffuse cystic structures delimited by variable thickness of fibrous tissue, usually compressing adjacent glomeruli, without concomitant inflammatory process. In these cases, the death was associated with the end stage renal disease. Animal 3 showed grossly few cystic structures, well delimited and replacing some reniculi. Tracheitis, granulomatous pneumonia, esophagitis, gastritis, enteritis and papilloma on penis and palate were observed. Microscopically, the cysts were lined by a single layer of columnar to cuboidal epithelial cells surrounded by extensive fibrotic tissue. Multifocal tubular necrosis was also noticed. Multifocal moderate nonsuppurative encephalitis with parasitic eggs and bacterial granulomatous hemorrhagic pneumonia were observed. In this case, the death was associated with the lesions in the nervous system.

Discussion: Data concerning polycystic kidney disease on cetaceans and wild animals is limited, and no primary genetic pathway was associated. In the present study, the gross and histological aspects observed on two animals (1 and 2) are similar to the characteristics found in the human adult form of PKD, while the characteristics observed on animal 3 are consistent with usual cystic disease. In addition, the animals are aged like humans where the end stage renal disease occurs in patients around 70 years old. The kidney histological aspects observed in all animals are similar, however, animal 3 showed no renomegaly, a characteristic of PKD. Considering the genetic pathway involved in humans and some animal’s breeds, investigation on gene mutations in S. bredanensis could help to define if this is also a genetic disorder and increase the knowledge about PKD.

Keywords: kidney, delphinidae, PKD, diagnostic pathology, cystic disease, rough toothed dolphin.
INTRODUCTION

Cystic alterations are the most common kidney lesions on Brazilian cetaceans, including simple cysts, multiple cysts, primary and secondary glomerulocystic diseases, PKD representing less than 1% [5]. Renal cystic diseases in mammals are grossly characterized by one or more cystic structures within the renal parenchyma, and usually have the pathogenesis associated with obstructive tubular lesions, membranous disruptions and/or growth/genetic disturbances [4]. The condition known as polycystic kidney disease (PKD) brings forward a complete singular and genetic related pathogenesis on several mammal species [4,9,20], including some cetaceans [5,19].

The PKD is an inherited autosomal genetic disorder affecting humans and animals. In humans, two types of PKD are reported, the autosomal recessive (ARPKD), named “childhood”, culminating with intrauterine or born death, and the autosomal dominant (ADPKD), known as “adult-onset” which is characterized by cystic formations during adult life, leading to end stage renal disease [1,4,18].

Beach monitoring programs are an interesting tool to evaluate animal health. Standardized analyzes contribute to establish the main lesions and eventually, the causes of death in marine animals. The rough-toothed dolphin, Steno bredanensis belongs to the subfamily Steninae [3] and shows high genetic particularities when compared to other species of Delphinidae family. In view of PKD pathogenicity, the highly genetical association, the gross and microscopic findings on 2 of 4 S. bredanensis stranded in Paraná coast, the aim of this report is to describe 2 cases of PKD and 1 case of renal cystic disease in S. bredanensis and discuss associated factors.

CASES

The animals analyzed in this study were obtained between January 2016 and December 2018 through the Santos Basin Beach Monitoring Project (PMP-BS), one of the systematic monitoring programs required by Brazilian Institute of Environment and Renewable Natural Resources (IBAMA) for the environmental licensing process of oil production and transport by Petrobras in the presalt province. The sampling area was the coast of Paraná state (25°44'S and 48°29'W), southern Brazil. This area is characterized by a coastline of 105 km, including Baía de Guaratuba in the south and the Paranaguá Estuarine Complex (PEC) in the north. A field permit was granted by the Ministry of Environment-MMA (ABIO 640/2015).

During the period of the study 4 specimens of *Steno bredanensis* were found stranded; one (animal 3) of these was alive and extremely debilitated, in poor prognosis condition, and died 4 days later. All other animals analyzed were within middle (animals 1 and 2) or advanced (animal 4) decomposition stage. They were submitted to routine necropsy and tissues fragments sampled for histopathological analysis, stained with hematoxylin and eosin (HE) [1,2,3,4]. Additionally, Masson’s Trichrome [1,2,3,4,5,6] and Periodic Acid Schiff - McManus [1,2,3,4] stains were also performed to characterize connective tissue of renal lesions. Teeth samples were also collected to determine the age of the animals by dentine Growth Layer Groups (GLG) analysis. Determination of age is one of the parameters evaluated in cetaceans analyzed in PMP-BS following a technique previously described [8].

Except for animal 4, that showed high degree of autolysis, precluding gross examination and a proper sampling for histological analysis, the other 3 animals had gross and microscopic findings described for each individual, and clinical signs were included when available.

**Case 1.** The necropsy findings in a 26-year-old male dolphin, were a fecaloma in the large intestine and bilateral nephromegaly. The right kidney had approximately 60.0 x 33.0 cm and the left 35.0 x 15.0 cm. The capsule surface of both kidneys was light brown to pale and irregular. On the sectioned surface, the renal parenchyma was almost completely replaced by multiple large cysts (approximately 90%), well delimited, with variable size and surrounded by marked fibrotic tissue; only a few reniculi showed normal morphology (Figure 1 A-B). The renal microscopic findings (Figure 2 A-D) confirmed the autolysis seen on gross examination accompanied by numerous bacterial colonies. Marked and multifocal tubular ectasia/cysts was also present, randomly distributed on cortical region surrounded by mild to extensive fibrosis, evidenced by Masson’s trichrome stain. Usually near to glomerulus, some proximal tubules were surrounded by mild fibrous tissue. A mild thickness of Bowman’s capsule and glomerular atrophy were evidenced by PAS-McManus stain.
Case 2. The necropsy of 30-year-old male dolphin, revealed myocardial abscess, prescapular lymph node necrosis and bilateral nephromegaly. The left kidney had approximately 35.0 x 18.0 cm and the right 32.0 x 23.0 cm, with capsular surface pale to light brown and irregular. On cut, the renal parenchyma was pale, almost completely replaced by multiple well delimited cystic structures of variable sizes and surrounded and interspersed by fibrous tissue (Figure 1C-D); less than 10% of reniculi showed normal gross morphology. At microscopic analysis (Figure 2 E-F), there were cystic formations and multifocal tubular ectasia on cortical region, randomly distributed but usually close to glomerulus. The cystic dilatations were surrounded by delicate to extensive fibrotic tissue, characterized on Masson’s trichrome stain.

Case 3. A 22-year-old male dolphin, was found alive, lethargic, and was forwarded to the Marine Animal Rehabilitation Center of Beach Monitoring Program of Paraná. The animal presented altered buoyancy with lateralization to the right and signs of pneumonia. The blood analysis showed anemia and leukocytosis. The therapy was based on penicillin7 (10,000 UI/kg), dexamethasone8 (0.1 mg/kg) and hydration with lactated Ringer’s solution. In addition, supportive therapy associated with fish feeding was performed, but the animal died after 4 days.

On necropsy, few well delimited, whitish, showing a tick capsule and without fluid content, renal cysts on parenchyma, measuring 1.0 to 2.0 cm in diameter were observed (Figure 1 E-F). Tracheitis, granulomatous pneumonia, esophagitis, gastritis, and enteritis were also observed. The hard palate and penis showed a well-defined mass measuring, respectively, 6.0 cm x 1.0 cm and 1.3 cm, white and rough (papilloma). The microscopical findings were renal tubular necrosis with intraluminal hyaline cylinders and the presence of multifocal cysts, in the parenchyma and between the reniculi, delimited by marked fibrotic tissue and lined by stratified cuboidal epithelial cells. Multifocal moderate nonsuppurative encephalitis with parasitic eggs, bacterial granulomatous hemorrhagic pneumonia, lymphoplasmacytic enterocolitis, severe and diffuse granulomatous otitis, with intralobular parasitic eggs and lymphoid depletion in the spleen and lymph nodes were also observed. The genital and oral papillomatosis was confirmed, and parasite eggs were also found in lung, lymph nodes and hyoid bone.

DISCUSSION

Renal diseases affecting marine animals are few reported, and data concerning polycystic kidney disease is scarce. In Brazil, a previous study with 192 stranded cetaceans reported an occurrence of 26.6% of renal cystic alterations. Among these animals, only one, also a Steno bredanensis, showed gross and microscopic PKD consistent characteristics [5] presenting high similarity with animal 1 and 2 described in this report. Interestingly, in the present study three of the 4 S. bredanensis stranded and evaluated showed cystic alterations.

Even on terrestrial wildlife mammals few PKD reports were found in available databases. The species reported are springbok (Antidorcas marsupialis) [8], racoons (Procyon lotor) [7], slender lorises (Loris lydekkerianus) [16], pygmy hippopotamus (Hexaprotodon liberiensis) [15], Brazilians agoutis (Dasyprocta leporine) [13], and European roe deer (Capreolus capreolus) [2] and domestic ferrets (Mustela putorius furo) [10]. The majority of the reports concerning ADPKD on animals, includes some breeds of pets, such as Persian cats and Bull Terriers, and laboratory mice [14].

In the present study, the gross and microscopic findings in animal 1 and 2 are like previous reports describing PKD in wild species [2,7] characterized a markedly size increase, on both kidneys, an irregular subcapsular surface, and the presence of numerous cystic structures on cut surface. On the other hand, animal 3, presented macroscopically only a few sparse cystic structures, resulting in no increase of the renal size. As reported in slender lorises, no other cystic alterations associated with “adult-onset” PKD in humans was seen in this case [16]. Microscopically the cystic structures displaced renal parenchyma and are lined by cuboidal epithelial cells [2,7] surrounded by variable thickness of fibrous tissue [15].

Primarily, PKD pathogeny on humans is related to mutation on PKD1 and PKD2 gene locus, which has also been described in several animal species cases of ADPKD [1,6,12]. However additional factors, as renal ischemic injury and environmental disturbances were described involving cystic formation, progression, and severity [1, 17]. In addition, molecular and morphological disturbances on renal tubular cells have been related to different signaling pathways that showed no association with mutations [1], but with external factors such as pollution, chemical compounds, or therapeutic drugs [4,5].
Figure 1. Gross morphology of the *Steno bredanensis* kidneys. A- Animal 1: marked enlargement of right kidney and thickening of the capsule. B- Animal 1: the parenchyma is replaced by large cysts of different sizes and surrounded by fibrosis. C & D- Animal 2: diffuse loss of renal usual architecture, replaced by numerous cystic formations, well delimited by fibrous tissue, yellow-pale and various sizes. Rare focus of normal parenchyma can be seen (arrows). E & F- Animal 3: cysts, with different sizes and thick capsule (arrows), and on cut surface view (arrowhead).
In the human “adult-onset” PKD, about 50% of end stage renal disease patients are around 70 years old [1,18]. Animals 1 and 2 were 26 and 30 years old, respectively, a declining age for this species, considering that the time life expectancy is around 32 years old [11]. Therefore, the age of these animals and the severity of the renal disease showed a similarity with the genetic human renal disease PKD. Animal 3 was younger, in comparison to the other cetaceans, and showed mild to moderate cystic disease accompa-
nied by a parasitic encephalitis and lesions in other systems. In this case, the death was associated with the lesions in the nervous system and not to an end stage renal disease.

Taken together, our results indicate that the gross and microscopic findings in 2 specimens are consistent with PKD. Moreover, the species affected is the same reported in a previous study [5]. Considering these aspects, a hereditary character, inherent to this species, should be considered as a primary pathway for PKD in *S. bredanensis*, similarly to other affected species. However, exposure to environmental pollutants cannot be excluded as a contributive factor. Therefore, the lack of genetic analysis on this report hampers the full comprehension and characterization of this condition on *S. bredanensis* and any other cetacean’s species. This way, further studies are needed to elucidate properly and deeply the pathogenesis of marine mammal’s polycystic kidney disease.


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