DISEASE IN WILDLIFE OR EXOTIC SPECIES

Renal Lesions in Cetaceans from Brazil


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Summary

This study reports the occurrence of renal lesions in cetaceans from the coast of Brazil subjected to necropsy examination between 1996 and 2011. The animals (n = 192) were by-caught in fishing nets, were found dead on beaches or died despite attempted rehabilitation. Kidney samples were evaluated grossly and microscopically and, depending on the histopathological findings, immunohistochemical and ultrastructural analyses were conducted. Due to autolysis, a diagnosis was reached in only 128 animals, of which 82 (64.1%) had kidney lesions. Cystic renal disease was the most common lesion observed in 34 cases (26.6%) and these were classified as simple cysts in eight cases (6.3%), polycystic kidney disease in one rough-toothed dolphin (Steno bredanensis), secondary glomerulocystic disease in 16 cases (12.5%) and primary glomerulocystic disease in nine cases (7%). Other lesions included membranous glomerulonephritis (28 cases; 21.9%), membranoproliferative glomerulonephritis (20 cases; 15.6%), lymphoplasmacytic interstitial nephritis (21 cases; 16.4%), lipoidosis (19 cases; 14.8%), glomerulosclerosis (8 cases; 6.3%) and pyogranulomatous nephritis (five cases; 3.9%); two of the latter were associated with the migration of nematode larvae. Additionally, tubular adenoma was identified in a Franciscana (Pontoporia blainvillei). The pathological implications of these lesions are discussed according the cause of death, age or sex of the animals. Furthermore, the lesions were compared with those of other marine and terrestrial mammals, including man.

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Keywords: cetaceans; cystic renal disease; kidney; pathology

Introduction

Specialized kidneys in cetaceans are an important adaptation to their marine environment (Hedges et al., 1979; Ortiz, 2001). Because of their reniculate morphology and increased medullary thickness, the kidneys play a key role in salt excretion and water conservation (Hedges et al., 1979). In cetaceans and other marine mammals, pathological changes have been described in the lung (Gonzales-Viera et al., 2011; Venn-Watson et al., 2012), liver (Jaber et al., 2004), thyroid gland (Cowan and Tajima, 2006) and pituitary gland (Cowan et al., 2008), but there has been no specific study of renal pathology in these species.

Renal infections are uncommon in cetaceans, and when occur they tend to be related to systemic processes (Sweeney and Ridgway, 1975). Pasteurella spp. is a significant bacterial pathogen that can infect the kidneys of cetaceans, causing nephritis as a consequence of acute septicemia (Dunn et al., 2001).

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Similarly, there are reports of renal mycosis in cetaceans secondary to systemic dissemination of *Candida albicans* (Reidarson et al., 2001), zygomycetes (Robeck and Dalton, 2002) or *Rhizomucor* spp. (Wünschmann et al., 1999). Nematodes such as *Crassicauda* spp. are observed occasionally in kidneys and other tissues, with the tail of adult stages reaching into the calyx to eliminate eggs through the urine (Dailey, 2001). Finally, interstitial nephritis associated with a novel alphaherpesvirus was diagnosed in a Blainville’s beaked whale (*Mesoplodon densirostris*) (Arbelo et al., 2012) and in a striped dolphin (*Stenella coeruleoalba*) with systemic herpesvirus and morbillivirus co-infection (Soto et al., 2012).

Idiopathic nephritis has been described in a number of cetaceans (Howard, 1983; Di Guardo et al., 1995; Cornaglia et al., 2000); however, this change may have had multiple possible causes that were not clarified (Howard, 1983). Even though cetaceans are known to accumulate a number of chemical carcinogens in their tissues (Martineau et al., 2000), reports of primary renal tumours are less common than those arising in other tissues (Newman and Smith, 2006). Additionally, cystic kidneys of different sizes have been observed in dolphins and other marine mammals and these cysts were considered to be developmental (Howard, 1983).

The aim of the present study was to describe the gross, microscopical, immunohistochemical and ultrastructural features of renal lesions in cetaceans from Brazil.

### Materials and Methods

Studied animals were found ashore or incidentally captured along the coast of seven Brazilian States between 1996 and 2011: Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Rio de Janeiro, Pernambuco and Ceará (Table 1). Necropsy examinations were undertaken by veterinarians or biologists and all tissue samples were deposited in the Marine Mammal Tissue Bank (BTMM) at the Laboratório de Patologia Comparada de Animais Selvagens (LAPCOM), Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil. Information about the gender, age and cause of mortality were provided by the staff who performed the examinations. The ages of the animals were estimated based on the total body length or by counting the number of growth layers in the dentine (Kasuya and Brownell, 1979).

### Gross and Histological Examination

Gross findings, when present, were recorded on standard forms. Kidney samples were fixed in 10% neutral buffered formalin and processed routinely. Briefly, the tissues were dehydrated through graded alcohols, embedded in paraffin wax, sectioned (3 μm) and stained with haematoxylin and eosin (HE). When appropriate, serial sections were stained by Masson’s trichrome, periodic acid–Schiff (PAS), Brown and Bren, Congo red and Ziehl Neelsen stains. The inflammatory, degenerative and cystic findings

### Table 1

Information on the cetaceans investigated according their sex, age class and origin

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of animals</th>
<th>Sex</th>
<th>Age class</th>
<th>Origin</th>
</tr>
</thead>
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<tr>
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<td>2</td>
<td>M</td>
<td>Adult</td>
<td>By-catch</td>
</tr>
<tr>
<td><em>Megaptera novangliae</em></td>
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<td>M</td>
<td>Juvenile</td>
<td>Stranded</td>
</tr>
<tr>
<td><em>Delphinus capensis</em></td>
<td>2</td>
<td>M</td>
<td>Calf</td>
<td>Rehabilitation</td>
</tr>
<tr>
<td><em>Globicephala macrocephalus</em></td>
<td>3</td>
<td>M</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
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<td>M</td>
<td>Adult</td>
<td>NR</td>
</tr>
<tr>
<td><em>Kogia sima</em></td>
<td>2</td>
<td>M</td>
<td>Juvenile</td>
<td>NR</td>
</tr>
<tr>
<td><em>Lagenodelphis hosei</em></td>
<td>2</td>
<td>M</td>
<td>Calf</td>
<td>NR</td>
</tr>
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<td><em>Mesoplodon europaeus</em></td>
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<td>NR</td>
</tr>
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<td><em>Pepoconephalus electra</em></td>
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<td>Adult</td>
<td>By-catch</td>
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<tr>
<td><em>Physeter macrocephalus</em></td>
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<td>Calf</td>
<td>Rehabilitation</td>
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<td>Rehabilitation</td>
</tr>
<tr>
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<td>M</td>
<td>Calf</td>
<td>NR</td>
</tr>
<tr>
<td><em>Stenella longirostris</em></td>
<td>4</td>
<td>M</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td><em>Steno bredanensis</em></td>
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<td>M</td>
<td>Adult</td>
<td>NR</td>
</tr>
<tr>
<td><em>Tursiops truncatus</em></td>
<td>6</td>
<td>M</td>
<td>Juvenile</td>
<td>NR</td>
</tr>
</tbody>
</table>

| Total                    | 192               | 110 | 80  | 2   | 67  | 92  | 20  | 13  | 101 | 51  | 35  | 5   |

M, male; F, female; NR, Not recorded.
were recorded according to established criteria (Hartman, 1989; Maxie and Newman, 2007; Newman et al., 2012).

Immunohistochemistry

Immunohistochemistry (IHC) was performed to identify special features of the renal lesions. Briefly, selected formalin-fixed tissues were sectioned (3 μm) and antigen retrieval was conducted by incubating sections in citrate buffer (10 mM, pH 9.0) for 5 min in a pressure cooker. Primary antibodies used were mouse monoclonal anti-human pan-cytokeratin (AE1/AE3, M3515, clone AE1/AE3; Dako, Glostrup, Denmark) and anti-human vimentin (M7020, clone Vim 3B4; Dako) diluted 1 in 2,000 and 1 in 3,000, respectively. The EnVision™+ system (Dako) was used as a secondary reagent, and labelling was ‘visualized’ with 3,3’-diaminobenzidine (Dako). Sections were counterstained with Harris’ haematoxylin.

Transitional epithelium of the renal pelvis and the fibromuscular band at the corticomedullary junction of reniculi were used as positive internal controls for AE1/AE3 and vimentin, respectively. As negative controls the primary antibodies were replaced by 1% bovine serum albumin.

Ultrastructure

Fragments (1 mm³) of selected paraffin wax-embedded tissues were used for transmission electron microscopy (TEM). These were reprocessed and fixed in 3% glutaraldehyde, then embedded in Epon Poly/Bed 812-Araldite 502 resin and polymerized at 100°C. Ultrathin sections (70 nm) were contrasted with uranyl acetate and lead citrate and examined using a JEOL® JEM-1011 transmission electron microscope.

Results

Kidney samples from 192 cetaceans (minimum of three reniculi per animal) were collected for microscopical examination during necropsy examination. Eighteen species were included in this study (Table 1), of which Franciscana (Pontoporia blainvillei) and Guiana dolphin (Sotalia guianensis) were the most common, with 125 and 22 individuals, respectively. In gender evaluation, 110 cetaceans were males, 80 were females and the genders of two animals were not recorded. Of the 192 animals, there were 92 juveniles, 67 adults, 20 calves and 13 with unrecorded ages. A total of 101 were by-caught in fishing nets, 51 were found stranded and 35 died during rehabilitation; in five cases the source was not recorded.

Gross Lesions

The most frequent gross lesions were simple cysts, present in eight cetaceans (seven P. blainvillei and one S. coeruleoalba). The cysts were distributed randomly only in the cortex of some reniculi, were well demarcated, ranged from 0.2 to 0.7 cm in diameter and were filled by a gelatinous and slightly brown substance (Fig. 1). Additionally, a rough-toothed dolphin (Steno bredanensis) had bilateral polycystic disease, in which both kidneys were enlarged (approximately 30 × 15 cm) and had multiple cysts (2–8 cm in diameter) with only a few reniculi retaining normal appearance (Fig. 2).

Histopathology and Immunohistochemistry

Microscopically, 64 samples had varying degrees of autolysis and were unsuitable for diagnosis. Of the remaining 128 samples, 82 (64.1%) had at least one recognizable renal lesion (Table 2) and 46 had no detectable lesions (35.9%).

The main finding was cystic disease in 34 samples (26.6%), which were microscopically classified into four categories. Simple cysts (n = 8; 6.3%) were located in the cortex, lined by a simple flat to cuboidal epithelium and were filled with an eosinophilic substance (Fig. 3). IHC showed the cyst lining was positive for AE1/AE3, confirming their tubular epithelial origin. Secondary glomerulocystic disease (SGCD) occurred in 16 cases (12.5%) and was characterized by multiple glomerular cysts with variable quantities of eosinophilic substance in the Bowman’s space (Fig. 4). These lesions were considered secondary because of their association with glomerulonephritis and lymphoplasmacytic interstitial nephritis with
varying degrees of interstitial fibrosis. Primary glomerulocystic disease (PGCD) was present in nine cases (7%) with features similar to SGCD; however, it was not associated with intra- or extraglomerular lesions (Fig. 5). Polycystic kidney disease (PKD) was diagnosed in the aforementioned rough-toothed dolphin with bilateral polycystic disease; cysts had varying diameter, glomeruli were absent and a focal area of squamous metaplasia was positive for AE1/AE3 expression (Fig. 6).

Membranous and membranoproliferative glomerulonephritis occurred in 28 (21.9%) and 20 cases (15.6%), respectively. These lesions were characterized by diffuse thickening of the glomerular capillary wall, which was more evident with PAS staining. The difference between these lesions was that membranoproliferative glomerulonephritis had variable proliferation of glomerular cells (Fig. 7). Lymphoplasmacytic interstitial nephritis was observed in 21 cases (16.4%), presenting as an interstitial infiltration of lymphocytes and plasma cells accompanied by variable fibrosis (Fig. 8). Eight cases (6.3%) were diagnosed with varying degrees of glomerulosclerosis, ranging from focal to collapsing (Fig. 9), and in all of these there was interstitial fibrosis and mononuclear cell infiltration. Pyogranulomatous nephritis was observed in five cases (3.9%) and was characterized by necrosis, infiltration of neutrophils, epithelioid and giant cells. In two of these cases nematode larvae were seen within blood vessels (Fig. 10). Tubular adenoma was identified histologically in only one by-caught Franciscana and was composed of tubular proliferation of well-differentiated epithelial cells, which were positive for AE1/AE3 and negative for vimentin (Fig. 11). Additionally, 19 cases (14.8%) had varying degrees of tubular lipidosis.

Ultrastructural Evaluation

Ultrastructural evaluation was performed in three of the best preserved tissues from cases of membranoproliferative glomerulonephritis. Lesions were characterized by thickening of the glomerular capillary basement membrane, moderate intramembranous electron-dense deposits in the Bowman’s capsule.

Table 2

Renal microscopic diagnoses according to the cetacean species

<table>
<thead>
<tr>
<th>Species</th>
<th>MGN</th>
<th>MPGN</th>
<th>LPIN</th>
<th>PGN</th>
<th>GS</th>
<th>Lipidosis</th>
<th>Simple cysts</th>
<th>PKD</th>
<th>PGCD</th>
<th>SGCD</th>
<th>Tubular adenoma</th>
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</table>

MGN, membranous glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; LPIN, lymphoplasmacytic interstitial nephritis; PGN, pyogranulomatous glomerulonephritis; GS, glomerulosclerosis; PKD, polycystic kidney disease; PGCD, primary glomerulocystic disease; SGCD, secondary glomerulocystic disease.
and glomerular tuft hypercellularity with hypertrophy of endothelial and mesangial cells (Fig. 12). In one case with glomerulosclerosis there was an increase in mesangial matrix and moderate deposits of electron-dense material in the glomerular capillary basement membrane, with the foot processes of the capillary basement membrane fused and marked tortuosity and narrowing of the glomerular capillary lumen. Glomerulosclerotic lesions were accompanied by marked electron-dense deposits in the Bowman’s capsule and bundles of electron-dense fibrillar structures with transverse striation consistent with interstitial collagen (Fig. 13).

**Discussion**

The most frequent finding in this study was cystic renal disease, which occurred as one of four different types. Simple cysts represent a common incidental finding in man and most domestic and laboratory animals, and generally do not have clinical significance. However, in rare cases they may result in haemorrhage, secondary infection or rupture or may lead to development of carcinomas arising from the wall of the cyst (Hartman, 1989; Maxie and Newman, 2007; Newman et al., 2012; Alpers and Fogo, 2013).

The simple cysts with tubular origin observed in this study had similar features to those observed in laboratory animals (Jackson et al., 2008). In dolphins,
this lesion has been considered a development anomaly as it often occurs in the absence of urinary obstruction (Howard, 1983).

Polycystic kidney disease (PKD) was observed in an adult male rough-toothed dolphin that stranded on the central coast of São Paulo State. In domestic animals, PKD is generally considered a hereditary disease that may occur as either an autosomal dominant condition that affects predominantly adults. PKD in animals is similar to the disease in man, which is associated with defects in the \textit{PKD1} and \textit{PKD2} genes (Hartman, 1989; Alpers and Fogo, 2013), or arises as an autosomal recessive condition that affects mostly calves (Maxie and Newman, 2007; Newman et al., 2012). Because the individual dolphin was an adult and did not have any evidence of urinary obstruction, it is possible that the case was analogous to the autosomal dominant condition described in domestic ungulates (Maxie and Newman, 2007; Newman et al., 2012). However previous toxicological study revealed that this individual dolphin had considerably higher levels of polychlorinated biphenyls (PCBs; $\sum 26.8 \mu g/g$ lipid) and dichlorodiphenyltrichlorothane (DDT) pollutants ($\sum 118 \mu g/g$ lipid) than other cetaceans in the same region, and concentration levels similar to those observed in cetaceans from industrialized countries (Yogui et al., 2010). Because PCBs have been found to be associated with the occurrence of PKD in laboratory animals (Maxie and Newman, 2007), another possibility is that this lesion developed due to an unusually high exposure to pollutants by this individual.
The squamous metaplasia observed in this case is also similar to that observed in cases of PKD in domestic ferrets (Jackson et al., 2008).

To our knowledge, SGCD and PGCD have not been reported previously in dolphins. In this study, SGCD was considerably less frequent in Franciscana than in other cetaceans examined. It must be noted, however, that the Franciscana carcasses in this study were more frequently obtained from incidental by-catch than other cetaceans (91 versus 10). By-caught cetaceans are more likely to be in better health than those that die during rehabilitation or are found dead on beaches, (Geraci and Lounsbury, 2005), which may indicate that the Franciscanas in this study were less frequently exposed to the predisposing factors of SGCD.

Renal infections are uncommon in cetaceans and, when they occur, tend to be more often associated with infections in other systems (Sweeney and Ridgway, 1975). Membranous and membranoproliferative glomerulonephritis are primary glomerular lesions that are associated with deposition of immune complexes in the glomerular capillary basement membrane, resulting from recurrent antigenaemia from parasitic and microbial infections (Alpers and Fogo, 2013). These lesions often become more prevalent with age and the original cause of the lesion may not always be evident (Maxie and Newman, 2007; Newman et al., 2012; Alpers and Fogo, 2013). These lesions are uncommon in cetaceans (Howard, 1983; Gulland et al., 2001); however, they have been
reported in other marine mammals such as pinnipeds (Bergman et al., 2001) and polar bears (Ursus maritimus) (Sonne et al., 2006, 2007). In polar bears, these lesions are often associated with exposure to organohalogenated compounds and mercury; these pollutants are immunosuppressive and favour recurrent infections after which immune complex deposition occurs (Sonne et al., 2006, 2007). In addition to the deposits on the glomerular basement membrane, cases with a membranoproliferative reaction presented with marked glomerular hypercellularity of mesangial cells. In man and domestic animals, this occurs as a result of immune complex deposition and activation of the complement system, causing the proliferation of glomerular cells and leucocyte infiltration (Newman et al., 2012; Alpers and Fogo, 2013). In cetaceans, membranoproliferative glomerulonephritis has been reported in a beaked whale and may have had the same causes as in terrestrial animals (Howard, 1983).

Interstitial lymphoplasmacytic nephritis has been observed in man and results from both infectious and non-infectious causes (Alpers and Fogo, 2013). In domestic animals, this lesion often has haematogenous origin, being secondary to a systemic or glomerular process (Maxie and Newman, 2007; Newman et al., 2012). In the present study, virtually all cases of lymphoplasmacytic interstitial nephritis were associated with some level of glomerulonephritis, suggesting a similar pathogenesis. Five cases were diagnosed with pyogranulomatous nephritis, of which two were associated with nematodes and the remaining three cases did not have any evident causes. In domestic animals, pyogranulomatous nephritis is generally related to a variety of infectious agents (Newman et al., 2012). Although nematodes of the Crassicaudidae family (e.g. Crassicauda spp. and Placentoma spp.) are known to infect the kidneys of cetaceans (Dailey, 2001), the nematodes observed in this study were smaller than those generally reported for Crassicaudidae, which are usually long and thick (Howard et al., 1983; Dailey, 2001). Additionally, the nematodes found in this study were observed inside blood vessels, similar to those observed in granulomatous lesions associated with larval migration of Toxocara canis in dogs and cattle (Maxie and Newman, 2007; Newman et al., 2012).

Glomerulosclerosis is the terminal stage of glomerulonephritis and other chronic renal diseases, with functional failure of the nephron (Newman et al., 2012). It has been reported in a long-finned pilot whale (Globicephala melas) and in California sea lions (Zalophus californianus) and its pathogenesis in marine mammals is thought to be similar to that of terrestrial mammals (Cowan, 1966; Howard, 1983). In this study, all cases of glomerulosclerosis were accompanied by glomerulonephritis and lymphoplasmacytic interstitial nephritis with variable degrees of fibrosis, suggesting they corresponded to terminal stages of glomerulonephritis caused by immune complex deposition.

The only neoplastic process observed in this study was a tubular adenoma in a by-caught juvenile male Franciscana. This tumour has been reported previously in a by-caught bottlenose dolphin (Tursiops truncatus) and, as in this study, it was considered an incidental finding (Migaki et al., 1978). It is believed that this is the first neoplasm reported in Franciscana.

Finally, all cases with renal lipidosis also had hepatic lipidosis (unpublished observations), suggesting that these processes had a common aetiology. In domestic animals and non-human primates, hepatorenal lipidosis is part of a syndrome not yet completely understood (Laber-Laird et al., 1987; Stalker and Hayer, 2007). In cetaceans, this lesion has been associated with a toxic process or nutritional deficiency (Howard, 1983).

In conclusion, this study provides the results of an investigation of renal pathology in cetaceans from the Brazilian coast and may be useful for future comparisons, as kidney pathology is still poorly understood for these species. Furthermore, this work presents conditions not previously reported in dolphins, such as primary and secondary glomerulocystic diseases, and the first report of a tumour in a Franciscana. Overall, our findings corroborate the general interpretation that renal pathological processes in cetaceans are parallel with, and have similar pathogenesis and mechanisms to those in terrestrial mammals.

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