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Glomerulocystic kidney in two red piranhas *Pygocentrus nattereri*

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ABSTRACT: Glomerulocystic kidney (GCK) is defined by a dilatation of the Bowman's space (greater than 2 times the normal size) of more than 5% of all glomeruli. Although GCK has been occasionally documented in dogs, cats, and humans with renal failure, in fish, reports of spontaneous GCK are rare. For the present study, 2 captive adult red piranhas *Pygocentrus nattereri* from a closed population were submitted for post-mortem examination. Clinical history included lethargy, inappetence, dyspnea, and altered buoyancy. Macroscopically, the fish displayed coelomic distension and ascites. The kidneys were markedly enlarged and dark yellow. Histologically, Bowman's space was noticeably dilated, occasionally with atrophic glomerular tufts. Degeneration and necrosis of the tubular epithelium, infiltration, and nephrocalcinosis were also present. To the authors' knowledge, this present study is the first report of spontaneously occurring GCK in red piranhas and freshwater fish in general. Despite being rare, GCK is a condition with the potential to impair the health of fish and mammals, and further studies are needed to shed new light on this condition.

KEY WORDS: Glomerulocystic · Kidney · GCK · Piranha · *Pygocentrus* · Fish · Histopathology

1. INTRODUCTION

Teleost fishes have successfully adapted to diverse environments with significant variations in salinity. Their success is mainly attributable to their capacity for osmoregulation (Larsen & Perkins 2001), a task carried out by the kidney (Reimschuessel & Ferguson 2006), together with the gills and the intestine (Larsen & Perkins 2001). In freshwater environments, the internal osmolality of fishes surpasses that of the ambient water (Larsen & Perkins 2001). Therefore, the kidney must excrete large volumes of osmotically accumulated water through high glomerular filtration rates (Reimschuessel & Ferguson 2006, Takvam et al. 2021) while reabsorbing ions (Takvam et al. 2021). For that reason, freshwater nephrons typically have well-vascularized and developed glomeruli compared to marine nephrons (Reimschuessel 2001).

Renal cysts have been described in various fish species. Polycystic kidney disease (PCKD) often affects goldfish *Carassius auratus* and is characterized by renal tubular dilation (Besse et al. 1959, Munkittrick et al. 1985). Inherited genetic mutations are the most common cause (Besse et al. 1959), but obstructive lesions (Reimschuessel & Ferguson 2006) and chronic chemical injury (Munkittrick et al. 1985) may also cause PCKD. Even though tubular dilation is the main feature of PCKD, concurrent dilation of the Bowman's space has been observed in some cases (Lennerz et al. 2010). Few cases of glomerular cysts have been documented in fish, and in most reports only an average of 2–3 cysts were observed. However, in a marine long-

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horn sculpin *Myoxocephalus octodecemspinosus* they constituted one-third of the total glomeruli (Grafflin 1937).

Glomerulocystic kidney (GCK) is a term used when dilation of Bowman's spaces (greater than 2 to 3 times the normal size) affects at least 5% of the glomeruli (Lennerz et al. 2010). Contrary to PCKD, reports of GCK are infrequent in fish. A case was documented in a group of related captive turbot *Scophthalmus maximus* with associated mortality, but the mechanisms involved remain unclear (Schmidt-Posthaus 2017). In mammals, GCK has been occasionally reported in dogs (Ramos-Vara et al. 2004), cats (Harkin et al. 2003), and humans (Lennerz et al. 2010). Although primarily affecting neonates and juveniles, this condition also affects adults and often progresses to renal failure. The mechanisms responsible for glomerular cysts formation are not well understood. Mutations in genes expressing proteins in the primary cilia (i.e. uromodulin) (Gascue et al. 2011, Phillips & Al-Khawaja 2013), intra-renal obstruction, or exposure to certain drugs (Lennerz et al. 2010) are among the most recognized theories. To our knowledge, we document the first case of spontaneous GCK in 2 red piranhas *Pygocentrus nattereri*, a type of freshwater fish.

2. MATERIALS AND METHODS

For diagnostic purposes, 2 adult male wild-caught red piranhas from a closed population (n = 13) at Berne Animal Park were submitted alive and separately, within one month, to the Institute for Fish and Wildlife Health (FIWI), University of Bern, Switzerland. Clinical history included lethargy, dyspnea, inappetence, weight loss, and altered buoyancy. Both fish were housed in a 25 000 l recirculating indoor freshwater tank with spotted pike-characin *Boulengerella maculata* and oscar *Astronotus ocellatus*, operating since 2013. The temperature was set at 24.8°C (±0.5°C), and water parameters were regularly monitored (pH 7.4, hardness 5.4°KH, phosphate <0.5 mg l^{-1}, and nitrate <1.0 mg l^{-1}). The diet consisted of rudd *Scardinius erythrophthalmus* and complete food for aquarium fish (Top Aquaristik Züchter-Bits and Züchter-Sticks, Switzerland).

At FIWI, both fish were euthanased with 150 mg l^{-1} buffered 3-aminobenzoic acid ethyl ester (MS 222®, Argent Chemical Laboratories). The fishes were measured and a complete post-mortem examination was carried out. The presence of external and intestinal parasites was assessed by direct microscopic examination of wet mounts of skin, first left branchial arch, and intestinal content. To examine the presence of bacteria, spleen, liver, kidney, and ascitic fluid samples were cultured on blood agar plates (Biomerieux) for 48 hours at 22°C. The growth of bacteria was checked daily. Samples from the kidney, liver, and gallbladder were placed in 10% neutral buffered formalin for tissue fixation. Formalin-fixed tissues were processed for paraffin embedding, sectioned at 4 µm, and stained with hematoxylin and eosin (HE), Periodic acid–Schiff (PAS), Grocott methenamine silver (GMS) and Ziehl-Neelsen (ZN) for histologic processing and pathological assessment. Additionally, liver, spleen, and kidney samples were frozen for PCR targeting *Mycobacterium* spp. DNA was extracted using DNeasy® Blood & Tissue Kit (QIAGEN) according to manufacturers' instructions. DNA concentration and quality were checked with NanoDrop™ One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific). Conventional PCR was performed according to Roth et al. (2000), amplifying a 200 to 300 bp part (depending on the *Mycobacterium* strain) of the 16S–23S spacer. The products were checked on a 1.5% agarose gel for amplification and molecular weight and sent for sequencing (Microsynth AG, Balgach). Sequencing results were determined by BLAST-n based on a search in the GenBank database (www.ncbi.nlm. nih.gov).

3. RESULTS

Both piranhas had similar lengths (24.0 and 24.4 cm). On post-mortem examination, Fish 1 showed marked coelomic distension (Fig. 1). Macroscopically, the kidney was moderately enlarged, yellow to tan-brown, and with a soft consistency. The liver was moderately decreased in size and light brown. Attached to the liver was a well-demarcated and partially pedunculated 5.0 cm × 4.8 cm × 4.3 cm mass, which was a brown and dark-green color. It was lobulated and cavitated, and filled with red to dark brown fluid.

Fig. 1. Piranha with coelomic distension (arrowheads)

The mass was identified histologically as hepatocellular carcinoma. Fish 2 had a moderate amount of yellow, translucent fluid in the coelomic cavity and depletion of the perivisceral adipose tissue. The kidney showed the same macroscopical features as described for Fish 1.

The wet mounts revealed no parasites or other significant findings in both cases. In addition, the bacteriological examination yielded no bacteria. PCR was positive for *Mycobacterium* spp. in Fish 1, and sequencing showed 95–96% similarity to *Mycobacterium florentinum*.

Histological findings in the kidney were similar in both fish, although the severity and extent of lesions were more pronounced in Fish 2. Approximately 70% (Fish 1) to 90% (Fish 2) of the glomeruli had cystic dilation of the Bowman's spaces. These showed an increase of at least 3 times the normal size (Figs. 2a,b & 3). The glomerular cysts were either spherical, oval, or polygonal, and the parietal epithelium of the Bowman's capsule in affected glomeruli was squamous to cuboidal. A few cysts were partially divided by thin trabeculae of epithelial cells. Two or more tufts were also seen in a single cyst (dysplastic glomerulus). Within the cysts were either well-preserved or atrophic glomerular tufts composed of a few cells in a grapelike arrangement. On occasions, due to the diminutive nature of the tuft, the glomeruli were not readily identifiable. The cysts were also filled with eosinophilic proteinaceous fluid and debris or ap-

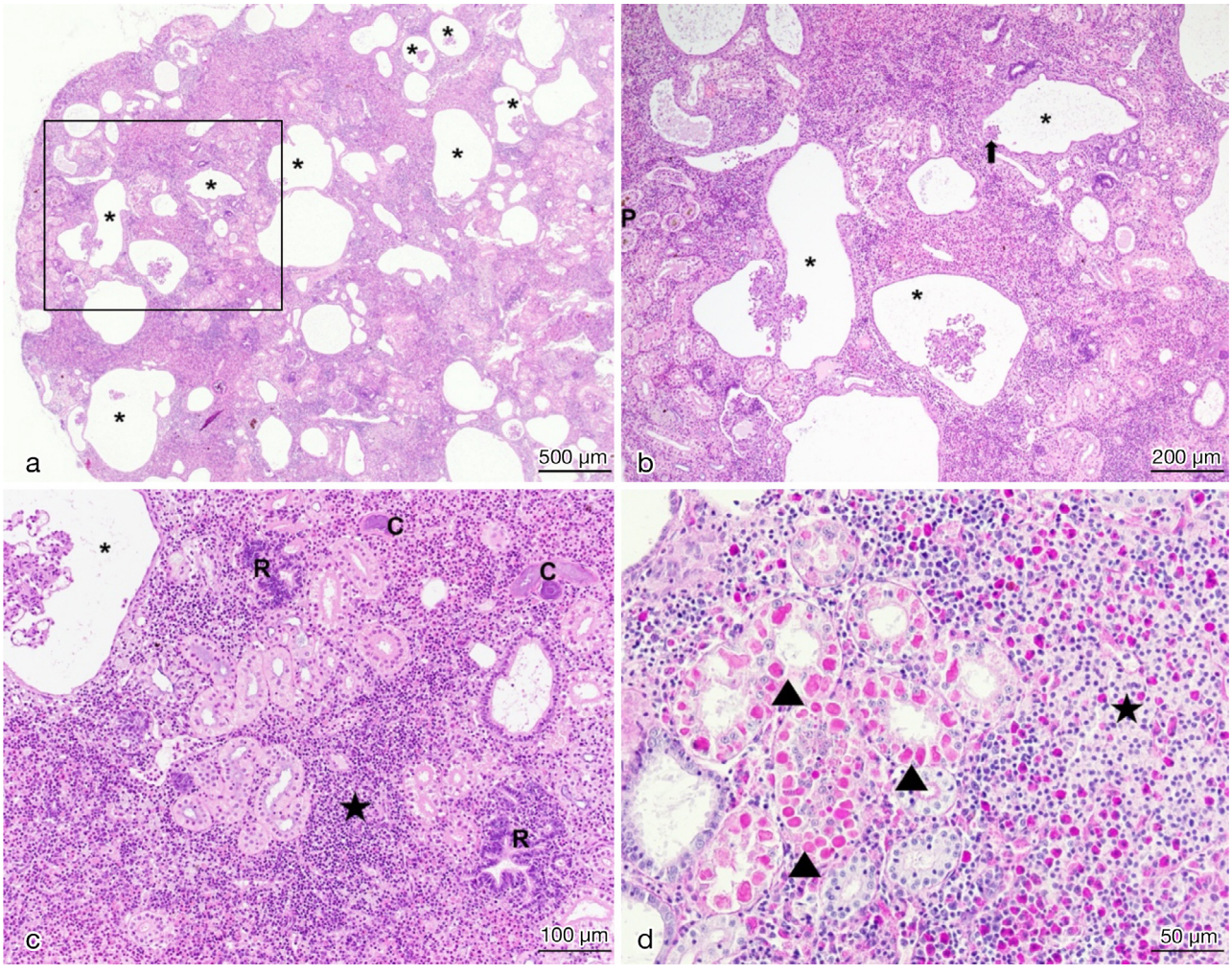

Fig. 2. Kidney. (a) Glomerulocystic kidney with multifocal dilation of the Bowman's spaces (*) (HE stain). (b) Magnification of the framed area in (a): dilated Bowman's space (*), atrophic glomerular tuft (arrow), and pigmented deposits (P) (HE stain). (c) Concurrent findings (magnification of lower right quadrant of (b)): multifocal infiltration (star) by macrophages, eosinophilic granular cells and lymphocytes (HE stain), tubular neogenesis (R), and calcification (C). (d) Hyaline degeneration of the tubular epithelium (arrowhead) and interstitial infiltration by macrophages and eosinophilic granular cells (star) (PAS stain)

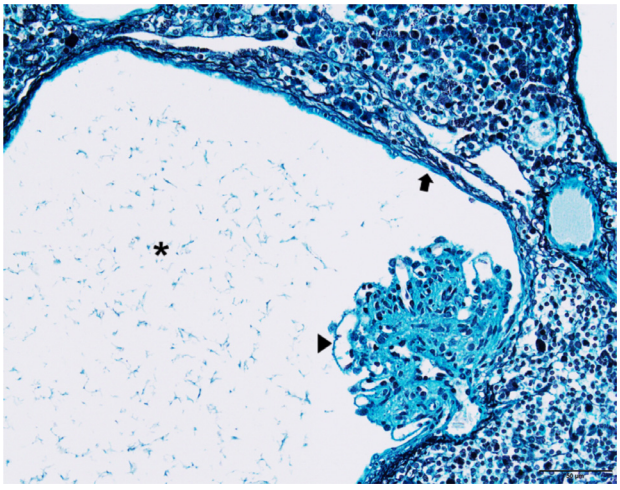

Fig. 3. Kidney (GMS stain). Detail of a glomerulus with dilated Bowman's space (*) lined by cuboidal to squamous epithelium (arrow) and glomerulus with dilated capillaries (arrowhead)

peared empty due to the processing. Concurrent findings (Fig. 2c,d) included tubular dilation (affecting approximately 20% of the tubules), moderate hyaline and vacuolar degeneration, and necrosis of the tubular epithelium. Multifocally, the tubules were expanded by either granular to globular green-brown material or radiating, sharp, acicular, colorless crystalline deposits. Moderate (Fish 1) and marked (Fish 2) deposition of basophilic, granular to fragmented material (mineral) was likewise observed in the tubular lumina (nephrocalcinosis) and in the core of granulomas. The interstitial tissue and tubules were multifocally and markedly infiltrated by numerous macrophages, pigmented macrophages, eosinophilic granular cells (EGCs), and lymphocytes. Multifocal granulomas were also observed. In addition, there was an increased number of small islands of intensely basophilic nephrons with pilling up of the epithelium (nephron neogenesis). Intralesional acid-fast bacteria were detected in Fish 2. In Fish 2, eosinophilic and clear cell foci in the liver (preneoplastic lesions) and a neuroendocrine neoplasm in the corpuscles of Stannius in the kidney were diagnosed.

4. DISCUSSION

Glomerulocystic kidney (GCK) is defined by the dilation of Bowman's spaces greater than 2 to 3 times the normal size and affecting at least 5% of the total glomeruli (Lennerz et al. 2010). In this present study, >70% of the glomeruli in the fishes were affected by dilation of the Bowman's spaces at least 3 times greater than the standard size. Within the Bowman's spaces were occasionally atrophic glomerular tufts and proteinaceous fluid, as described in mammals (Harkin et al. 2003, Ramos-Vara et al. 2004, Lennerz et al. 2010).

GCK is not a disease but a phenotype that comprises distinct entities. In humans, it may have a genetic origin (i.e. a variant of PCKD, hereditary or syndromic) or result from an obstructive or sporadic process (ischemia or drug-induced) (Lennerz et al. 2010). Despite this, the exact mechanism of glomerular cysts' formation remains unclear. In experimental studies, mutation of the gene *Col4a1m* encoding the α1 chain of collagen IV, a major basement membrane component, resulted in GCK in adult mice (Chen et al. 2016). Likewise, the inactivation of Wwtr1, a protein regulating several transcriptional factors, led to the loss of ciliary integrity and GCK (Lennerz et al. 2010, Jun et al. 2022). In recent years, numerous cystic disease syndromes have been associated with mutations in genes expressing proteins in the primary cilia (Bissler et al. 2010). Primary cilia are single, immotile organelles located at the apical aspect of many cell types, such as the epithelial cells lining the Bowman's capsule and the renal and biliary ducts (Deane & Ricardo 2012, Ross & Pawlina 2016). They act as chemosensors, osmosensors, and mechanosensors, and are essential for normal tissue morphogenesis (Ross & Pawlina 2016). Teleost fishes also have ciliated epithelial cells in the kidney, but these cilia are numerous and highly motile, unlike that of mammals (Deane & Ricardo 2012). Despite the differences, however, defects of the motile renal cilia may cause a cystic kidney phenotype in zebrafish (Drummond 2005). Glomerular cysts were also observed in zebrafish mutants targeting *stk3*, a kinase-regulating transcription coactivator Yap1/Wwtr1, the main effectors of the Hippo signaling pathway (Ren et al. 2021). The Hippo signaling pathway is involved in organ size and development, restricting cell proliferation, and promoting apoptosis of excess cells (Halder & Johnson 2011), indicating that the importance of cilia is conserved in vertebrates and providing a valuable model for studying renal cilia (Drummond 2005). Additionally, mutations of the hepatocyte nuclear factor 1β, necessary for the development of the pronephric tubule, also have been shown to cause glomerular cysts in zebrafish (Drummond 2005). Likewise, the inactivation of the polycystic kidney disease 2 gene has been shown to cause glomerular cysts without affecting cilia motility, suggesting multiple mechanisms for cyst formation (Sullivan-Brown et al. 2008). Interstitial inflammation or obstruction

may also lead to tubular occlusion, increased pressure in the Bowman's space, and consequent formation of glomerular cysts (Lennerz et al. 2010). Conversely, it has also been suggested that in mammals, the continuous leakage of cystic fluid into the interstitial tissue acts as a stimulus for inflammation and fibrosis, which would explain the end-stage renal failure often seen in GCK (Carson et al. 1987). In this present study, concurrent nephrocalcinosis, intratubular crystal deposition, infiltration, degeneration, and necrosis of the tubular epithelium were observed, but the origin of the GCK and associated renal disease is unclear. Inherited genetic mutations or obstruction caused by intratubular nephrocalcinosis are the most likely factors. However, a combination of different factors cannot be ruled out. Considering the importance of primary cilia in kidney and liver development, changes in the liver are also expected. In mammals with GCK, changes in the liver, such as hepatocellular adenoma and hepatic cysts (Lennerz et al. 2010), have been documented. A connection between the histopathological changes in the kidney and liver in both fish in this present study can only be speculated, and additional studies should be conducted. The detection of *Mycobacterium florentinum* was most likely unrelated without further significance, not rare in captive fish.

To the authors' knowledge, this is the first report of spontaneously occurring GCK in red piranhas and freshwater fish in general. Even though concurrent pathologies have been observed in both fish, the severity and extension of the histopathological changes in the kidney, compatible with end-stage renal disease, were most likely the cause for the marked health impairment. Despite being rare, GCK is a condition with the potential to impair the health and well-being of fish and mammals. Given the similarities between fishes' and mammals' nephrons (Reimschuessel 2001, Drummond 2005) and the still limited knowledge of the pathogenesis of GCK, the use of fish models may be an effective tool to shed new light on this condition.

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