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CASE REPORT

Non-clinical

Cutaneous nerve sheath tumours, non-infectious polycystic kidney disease, renal granulomas, nephrocalcinosis and crystals: Incidental pathological findings in pond-living goldfish (*Carassius auratus*, Linnaeus)

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Abstract

In an adult male goldfish (*Carassius auratus*, Linnaeus), an unusual combination of diseases was detected. The owner noted a bilateral, asymmetrical distention of the abdomen, multiple cutaneous masses and an altered swimming behaviour over the course of time; diagnostic work-up was not requested and euthanasia elected. Grossly, the cutaneous masses were whitish, discrete, extremely friable. Histologically, the dermis was multifocally expanded by moderately cellular, unencapsulated neoplastic tissue, composed of dermal spindle cells. The celomic cavity opening revealed a gelatinous multicystic mass, corresponding to the kidney, which replicated several cysts compressing the residual parenchyma. Histologically, sparse, late-stage granulomas, basophilic intratubular deposits and crystals were also detected. No mycobacterial DNA was detected in tissue with granulomas. No parasites were detected in the histological sections examined. Diagnoses of cutaneous nerve sheath tumours, polycystic kidney disease, renal late-stage granulomas, nephrocalcinosis and crystals were formulated. The authors had proposed to recapitulate each single condition.

KEYWORDS

crystals, FFPE samples, PCR, goldfish, granuloma, nephrocalcinosis, Polycystic Kidney Disease (PKD)

BACKGROUND

This case can be considered quite uncommon, as an adult goldfish (*Carassius auratus*) was affected by a concurrent combination of pathologic conditions, multiple cutaneous nerve sheath tumours (NST), polycystic kidney disease (PKD), renal granulomas, nephrocalcinosis and intratubular crystals. In particular, the relationship among NST and non-infectious PKD was discussed, as well as the possible causes of the nephrocalcinosis and renal crystals.

CASE PRESENTATION

The owner of a 10-year-old goldfish (*C. auratus*) noted a severe, bilateral, non-symmetrical distention of the abdomen. The external observation of the fish also revealed the presence of multiple, whitish to pinkish, cutaneous masses, located on the dorsal aspect of the body and at the level of the fins, especially the dorsal one. In addition, an altered swimming behaviour over the course of time was noticed. The fish was fed a commercial feed (Koi Colour Nature sera).

In consideration of the clinical presentation, which suggested an underlying progressive systemic disorder, the owner refused any diagnostic work-up and elected for euthanasia. At the arrival to the diagnostic laboratory, the fish was euthanased with a lethal dose of anaesthetic (2-phenoxyehanol), and immediately examined.

INVESTIGATIONS

Regarding water quality, a sample was taken and analysed: the Cl, sulphates and Na were low, as well as the N forms, with few nitrates, low ammonia. It was classified as 'deep, fossil water' by the laboratory that carried out the analyses (Gruppo C.S.A. Rimini, Italy).

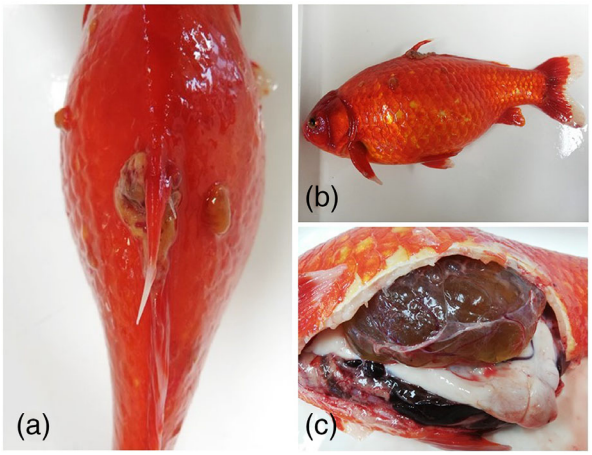

FIGURE 1 Goldfish (*Carassius auratus*), gross findings related to integument and renal parenchyma. (a) Integument. The masses were located on the dorsal aspect of the body; they were whitish and pinkish, discrete, ranging from 0.5 to 4 cm in diameter. (b) The celomic cavity appeared bilaterally and asymmetrically dilated. (c) Kidney. A large, cystic mass, displacing and compressing the other viscera, was evident. The appearance and the texture of the tissue was gelatinous.

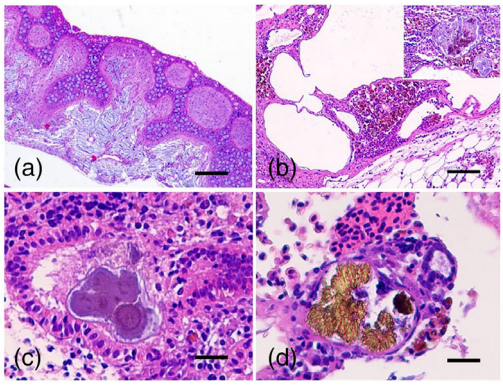

FIGURE 2 Histology. Skin (a) dermal nerve sheath tumour (NST). Renal parenchyma (b–d) polycystic kidney disease (PKD), granulomas, intratubular calcium deposits and crystals. Haematoxylin–eosin staining. (a) Integument. NST: the dermis was multifocally expanded by moderately cellular, unencapsulated neoplasm. Neoplastic cells were spindle-shaped, arranged in bundles, expanding up to the dermo-epidermal junction (bar = 500 μm). (b) Kidney, PKD. The normal parenchyma was compressed and replaced by multiple cysts, lined by attenuated epithelial cells and distended by clear space (bar = 500 μm). The interstitial tissue contained sparse, late-stage granulomas occupied by large necrotic centre (inset, bar = 100 μm). (c) Kidney, nephrocalcinosis. Basophilic deposits, consistent with calcium salts, were detected multifocally within the tubules. The tubular epithelial cells, arising from the metanephric blastema, showed signs of regeneration (bar = 50 μm). (d) Kidney, intratubular crystals showed a radial pattern, occupying almost all the lumen of the tubule (bar = 50 μm).

For histopathological purposes, samples of the cutaneous masses, the renal tissue and the main organs were collected, fixed in 10% neutral buffered formalin, dehydrated and embedded in paraffin. Formalin-fixed paraffin-embedded (FFPE) sections were cut at 3-μm thickness and stained with haematoxylin-eosin (H&E), Von Kossa (for staining crystals) and periodic acid Schiff (PAS) (to detect thickening of the Bowman's capsule).

FFPE sections including kidney tissue presenting granulomas were subjected to DNA extraction and polymerase chain reaction (PCR) analysis to investigate *Mycobacterium* sp. presence. DNA was extracted using the Purelink Genomic DNA kit (Invitrogen) following the manufacturer's instructions with minor modifications as previously reported.[1] DNA quality was evaluated through a PCR assay targeting a fragment of the thyroid-stimulating hormone gene.[2] Mycobacteria presence was investigated through a PCR method targeting the *Mycobacterium* sp. HSP65 gene.[3]

Grossly, the total length of the fish was 29 cm. The cutaneous masses mainly located on the dorsal aspect of the body were whitish and pinkish, discrete, ranging from 0.5 to 4 cm in diameter; they were extremely friable and prone to colliquation (Figure 1a). The celomic cavity appeared bilaterally and asymmetrically dilated (Figure 1b); after its opening, a bulging cystic mass, displacing and compressing the other viscera, was evident. The mass was composed of many large, clear, gelatinous fluid-filled cysts, ranging in size from microscopic to 4 cm in diameter; as a whole, the texture of the renal tissue was gelatinous (Figure 1c).

Histologically, the dermis was multifocally expanded by moderately cellular, unencapsulated neoplastic tissue, sharply demarcated by the basal membrane beneath the epidermal layer (Figure 2a). The cutaneous neoplasms were composed of interlacing bundles, storiform areas of cells supported by a delicate fibrillary eosinophilic matrix and blood vessels. Neoplastic cells were spindle, 15–25 μm long, with a moderate amount of fibrillary, eosinophilic cytoplasm. Nuclei were oval, variably elongated, containing one nucleolus and, in some regions, oriented parallel with their long axes. Mitotic figures were less than one at high power field. Bacterial aggregates, parasites and fungi were not detected in the histological samples examined. The H&E sections revealed that the normal renal parenchyma was markedly compressed and almost totally replaced by multiple 0.5–4 in diameter cysts. Cysts were lined by attenuated epithelial cells and distended by clear space (Figure 2b). Glomerular tufts were spared, whereas some of them showed a dilation of the Bowman's space and a progressive accumulation of clear eosinophilic fluid. No signs of Bowman's capsule thickening were detected with PAS staining. Several parts of the nephronal units that formed the tubules were dilated, but the epithelial lining was normal or only mildly attenuated. The interstitial tissue was rich in pigmented macrophages, arranged as single cells or in centres, and contained sparse, late-stage granulomas (Figure 2b, inset) according to a recent classification,[4] originating from the melano-macrophagic centres. Other findings were represented by multifocal, intratubular basophilic mineralisation (Figure 2c) and crystals radiating from the luminal tubules (Figure 2d) that were mildly stained with Von Kossa staining (not shown). Sparse thyroid follicles, a normal finding of the cyprinid kidney, were also present. No parasites in the histological samples of the renal tissue examined were detected. There were no histological abnormalities observed in the other tissues examined.

The FFPE kidney sample presenting granulomas resulted negative to the PCR for *Mycobacterium* sp.

Diagnoses of multiple cutaneous NST, PKD, renal late-stage granulomas, nephrocalcinosis and crystals were formulated.

DIFFERENTIAL DIAGNOSIS

Kidney enlargement disease (KED).

DISCUSSION

This case was considered quite uncommon, as it was affected by a concurrent combination of pathologic conditions, each of them considered per se quite common in *C. auratus*.

The cutaneous masses grossly presented as multiple, dermal, variably pigmented growths, histologically corresponding to spindle cell tumours, and previously called schwannomas or neurofibromas.[5] Recently, these types of tumours were renamed as NST,[6] but some descriptions with the previous names of multiple cutaneous tumours are still available in the literature.[7,8]

KED is a parasitic, chronic, progressive disease affecting the renal tissue of *C. auratus* and some species of carp (*Carassius gibelio*). It is caused by the myxozoan *Hoferellus carassii* that, for the vital cycle, requires two aquatic oligochetes as intermediate hosts. Unfortunately, there is no known treatment for this condition; management for this pathogen involves decreasing the area for oligochaete worms that reside in the pond.[9] Histologically, the parasite may trigger papillomatous cystic hyperplasia of the renal tubular epithelium and subsequent degeneration of the interstitium. Glomeruli are rarely involved. In the late phase of the disease, the tubules appear markedly dilated and filled with fluid.[10] The hypothesis of PKD having a parasitic origin was excluded in this case, as no parasites such as myxozoans were detected in the histological sections examined.

Non-infectious PKD is a common pathologic condition in fish, occurring with a kidney enlargement that could swell, occupying almost all of the celomic cavity. The most common clinical signs are lethargy, ascites, variably symmetrical and bilateral abdominal swelling and loss of balance.[11] Death occurs in animals affected by a severe form of PKD. The surviving animals show a progressive deterioration of general condition, which can be attributed to the loss of renal function. In the majority of cases, it is supposed that cysts can have an autosomic genetic origin,[12] according to a recessive mode of transmission.[11] Fluid-filled cysts can be readily imaged using ultrasound examination; the cysts can be aspirated for fluid analysis using ultrasound guidance. Laparoscopic renal biopsy can also be performed in an anaesthetised animal. Fluid-filled cysts that are causing buoyancy or swimming problems can be drained, using ultrasound-guided fluid centesis. Fluid removal will only give temporary relief and the fluid will return anywhere from several days to several months, depending on the severity of the renal pathology. The condition is progressive and leads to the eventual death of the animal. There are currently no known medical treatments.[9] In the non-infectious PKD here described, the absence of clinical scale protrusion could suggest a partially conserved function of the nephrons. Nevertheless, the lack of any clinical, diagnostic imaging and haematological data excludes the possibility of confirmation of this assumption.

The relationship of the PKD in the paper with other previous descriptions is uncertain.[13] No myxosporean parasites were detected, and loss of tubular epithelium or papillary intraluminal cystic structures, as reported for KED, were found. The descriptions of PKD appear to closely parallel our findings in that the cysts have a more simplified structure, there is some glomerular involvement and we were unable to identify an obvious aetiologic agent.

Regarding renal granulomas, they were classified as late-stage, according to a recent classification,[4] based on the presence of a large necrotic centre. The necrotic regressive change may have interfered with the possibility of detecting any bacteria. To reinforce the use of molecular methods for diagnostic purposes, a recent study demonstrated that PCR is a rapid, specific and sensitive method for detecting *Mycobacterium marinum* in goldfish[14] and ornamental fish in general.[15] In this case, no mycobacterial DNA was detected in the FFPE kidney sections presenting granulomas, despite the extracted DNA being assessed as suitable for molecular investigation. In relation to water quality parameters, the scarce amount of natrium and chloride could have determined a higher effort for osmoregulation in hyperosmotic fish as cyprinids, maybe determining a higher activity of renal ultrafiltration and leading to a higher amount of carbonates in the ultrafiltrate. This could have determined their deposition within the tubular lumina as basophilic concretions detected at histology and termed nephrocalcinosis. The aetiology of nephrocalcinosis is still uncertain, and it is believed that it is related to dietary factors and/or alteration in water's physical and chemical parameters, particularly a prolonged exposure of fish to high levels of carbon dioxide, and a nutritional aspect where a magnesium deficiency and a selenium toxicity have been implicated.[16] The severity and prevalence of nephrocalcinosis may be particularly high in some ground waters, as occurred in this case report (the water was considered 'deep, fossil' by the laboratory).

Presence of crystals within the renal tubules of wild or aquarium fish is rarely reported, and linked with pyridoxine or vitamin A deficiency.[17] In a case report about PKD in discus (*Symphysodon aequifasciatus*) and Siamese fighting fish (*Betta splendens*), mineral deposits and oxalate crystals in the lumen of tubules were observed.[18] In our case, intratubular crystals, mildly stained with Von Kossa, were interpreted as oxalate crystals. It is only a matter of the reader's speculation whether the presence of spirulina in the feed, as well as other components that were not subjected to investigation, could have favoured the crystals' formation, in a predisposed animal. Regarding speculation about what was the first condition that occurred, the variability in the rate of disease progression among human patients with autosomal dominant PKD suggests an environmental component in pathogenesis; new findings show that deposition of calcium oxalate crystals activates PKD-associated signalling pathways to flush out lodged crystals, but also triggers cyst formation.[19] However, in our goldfish, the massive cystic degeneration of the kidney overcame the presence of crystals, suggesting that this could have been a primary and maybe congenital event.

Concerning the connections with some diseases detected in this goldfish, a relation among NST and PKD could exist, as in human patients with familial neurofibromatosis called von Recklinghausen's disease, renal malformations such as cysts have also been described.[20]

- In goldfish, cutaneous nerve sheath tumours can occur together with polycystic kidney disease.

- Granulomas, a frequent histological finding in fish, not always are related to mycobacterial infection.
- Intratubular renal mineral deposits, consistent with nephrocalcinosis, can be related to sub-optimal water quality.
- The renal crystals detected can be oxalates and could be the consequence of nutritional imbalances.

In conclusion, the concurrent presence of pathological entities, namely NST, non-infectious PKD, renal granulomas, nephrocalcinosis and intratubular crystals, was described in an adult goldfish. The absence of myxozoan parasites excluded a KED. Some of the observed conditions could have resided on sub-optimal water quality for this fish species. However, the lack of anamnestic data cannot confirm any hypothesis and recalls the necessity to include an accurate diagnostic work-up.

AUTHOR CONTRIBUTIONS

Antonio Casalini provided the anamnestic information of the goldfish. Francesca Errani took the gross pictures and performed the molecular investigations for mycobacteria detection. Luciana Mandrioli and Albamaria Parmeggiani searched the literature and wrote the first draft of the manuscript. Sara Ciulli edited manuscript drafts.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

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ETHICS STATEMENT

The owner consented to the fish euthanasia for humane reasons.

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IMAGE QUIZ

Figure 1 Multiple cutaneous nerve sheath tumours.
Figure 2d Kidney. Intratubular radiating crystals.

MULTIPLE-CHOICE QUESTION

What's the likely diagnosis?

POSSIBLE ANSWERS TO MULTIPLE-CHOICE QUESTION

Figure 1 Choose among cutaneous multiple fibroma, nerve sheath tumour and epidermal papilloma.

Figure 2d Choose among nephrocalcinosis or melamine deposits.

CORRECT ANSWER

Figure 1 Multiple nerve sheath tumour.

Figure 2d Nephrocalcinosis.