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Multiple, life-compatible, congenital physical deformities in association with splenic lymphangiomatosis in *Zoogoneticus tequila* (Webb & Miller, 1998)

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ABSTRACT

The present case describes an adult female *Zoogoneticus tequila* showing a congenital, marked shortening with left deviation of the upper and lower jaws, in association with microphthalmia of the left eye. The fish was normally fed and had a normal reproductive behaviour. After spontaneous death occurred at an advanced age, the fish was submitted for necropsy examination, which revealed the presence of an oval, 1 cm × 0.5 cm × 0.5 cm in size, cystic structure containing clear amber fluid, located in the coelomic cavity, in place of the spleen. Histopathological examination revealed multiple cystic spaces empty or filled with a slightly eosinophilic, homogenous, proteinaceous material, and lined by flattened, vimentin-positive endothelial-like cells. Residual parts of splenic tissue were also admixed with cystic spaces, suggesting a final diagnosis of cystic lymphangiomatosis of the spleen, which has not been previously described in fish. This is the first report of multiple, life-compatible, congenital physical deformities in association with splenic lymphangiomatosis in *Zoogoneticus tequila*.

1. Introduction

Congenital malformations are not uncommon in fish and can be derived from a number of causes. Physical deformities can involve jaw and skull malformations, as well as gill or spinal anomalies. Defects of genetic origin or those associated with incubation temperature or hormonal effects are apparent from early in hatchery development, whereas anomalies of husbandry or nutritional origin may occur at any stages of development. Defects of eye are also common in embryos, with abnormally small eyes (microphthalmia) being particularly frequent[1].

On the other hand, cystic lymphangiomatosis of the spleen is a very rare entity described in humans, which occurs secondary to developmental malformation of the lymphatic system[2-4]. In veterinary medicine, lymphangiomatosis has been also rarely observed in different organs of dogs[5,6], but not in the spleen. To the best of our knowledge, no reports of this congenital disease are available for fish species.

The Mexican goodeid, *Zoogoneticus tequila* (Webb and Miller, 1998) (*Z. tequila*) is considered a nearly extinct fish in the wild and it is maintained in captivity by the non-profit international "Goodeid Working Group" represented by zoological institutions and aquarists in North America, Mexico and Europe[7]. The unique, currently existing Italian colony was founded in 2007 by one of the authors and a limited number of pathologies has been recorded so far within this colony[8,9]. *Z. tequila* is a viviparous species and females give birth to free-swimming fry after an intraovarian gestation.

2. Case report

The present case describes an adult female *Z. tequila* showing a congenital, marked shortening with left deviation of the upper and lower jaws, in association with microphthalmia of the left eye (Figure 1). These defects were apparent at the fry stage, immediately after birth. The fish was normally fed and had a normal reproductive behaviour. After spontaneous death occurred at an advanced age, the fish was submitted for necropsy examination, which revealed the presence of an oval, 1 cm × 0.5 cm × 0.5 cm in size, cystic structure containing clear amber fluid, with multiple, brownish superficial foci, located in the coelomic cavity, in place of the spleen (Figure 2).

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All experimental procedures involving animals were conducted in accordance to all institutional and national guidelines for the care and use of experimental animals and approved by the institutional review board of the Faculty of Veterinary Medicine, University of Teramo (Italy).

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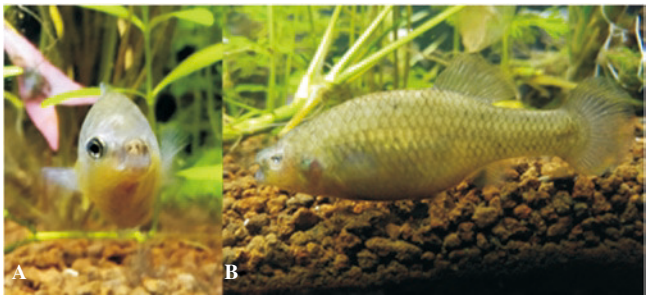

Figure 1. Adult female *Z. tequila* showing a congenital, marked shortening with left deviation of the upper and lower jaws (A), associated with an abnormally small left eye (B).

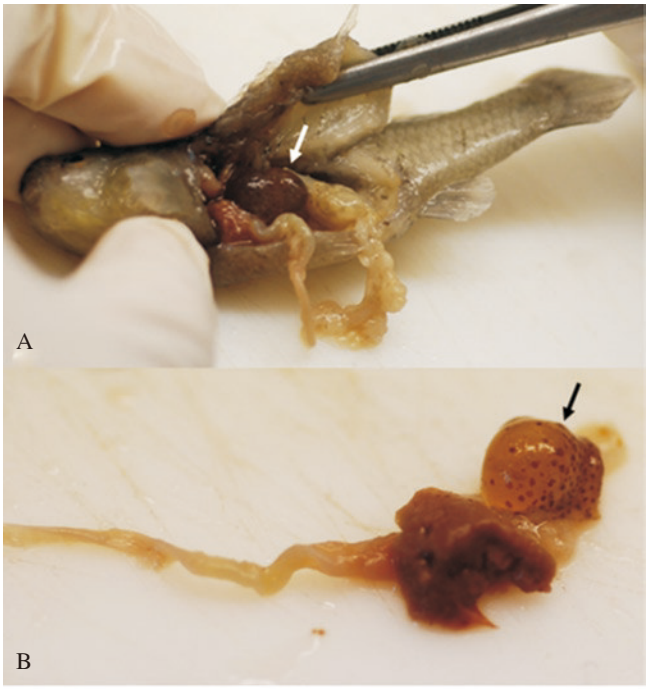

Figure 2. A: Coelomic cavity of the adult female *Z. tequila* showing an oval cystic structure in place of the spleen, containing clear amber fluid (arrow); B: Isolated cystic structure displaying multiple, brownish superficial foci (arrow).
The liver and part of the intestine are also visible.

The cystic structure, as well as the normal spleen of a spontaneously dead female old fish of the same species, were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin. Additional sections were also processed for immunohistochemistry using primary antibodies specific for pan-cytokeratin (pan-cytokeratin; 1 in 100 dilution, AE1/AE3, mouse monoclonal; Dako, Glostrup, Denmark) and vimentin (1 in 100 dilution, V9, mouse monoclonal; Dako). Labelling was identified by use of secondary biotinylated goat anti-mouse antibody (1 in 200 dilution; Vector Laboratories Inc., Burlingame, California, USA) and subsequently visualized using an avidin-biotin complex method (Vectastain® ABC Kit, Vector Laboratories) with 0.1% H_2O_2 in 3,3'-diaminobenzidine solution (Sigma-Aldrich, St Louis, Missouri, USA) as chromogen. Sections were counterstained with Mayer's haematoxylin (Merck, Darmstadt, Germany). Negative controls were performed in all instances by omitting the primary antibody from the staining schedule or replacing it by an antibody of irrelevant specificity. Sections of canine skin were used as positive controls.

Histopathological examination revealed the presence of multiple, large, irregular cystic spaces, empty or filled with abundant, slightly eosinophilic, homogenous, proteinaceous material, and lined by flattened endothelial-like cells. Marginally distributed, multifocal residual portions of parenchymal tissue, similar to that of normal spleen, were also associated with cystic spaces (Figures 3 and 4). Endothelial-like cells were diffusely positive for vimentin (Figure 4, inset) and negative for pan-cytokeratin.

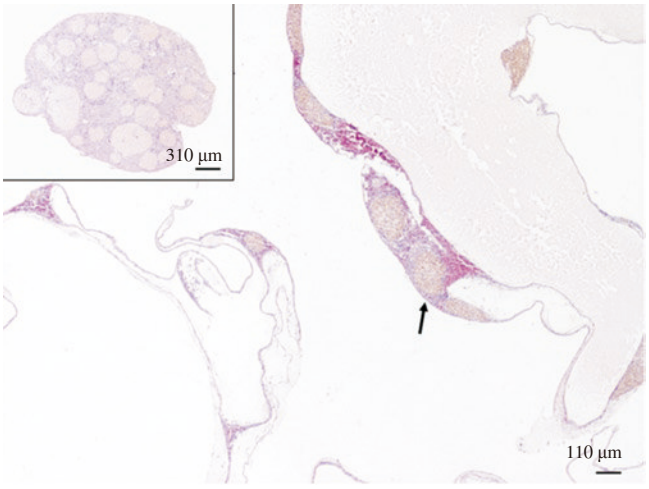

Figure 3. Histopathological examination of the abdominal cystic structure showing multiple, large, irregular cystic spaces, empty or filled with a slightly eosinophilic, homogenous, proteinaceous material, and lined by flattened endothelial-like cells.
Residual portions of splenic tissue are also visible (arrow). Inset: Microscopical appearance of normal spleen of *Z. tequila*, showing numerous melanomacrophage centres.

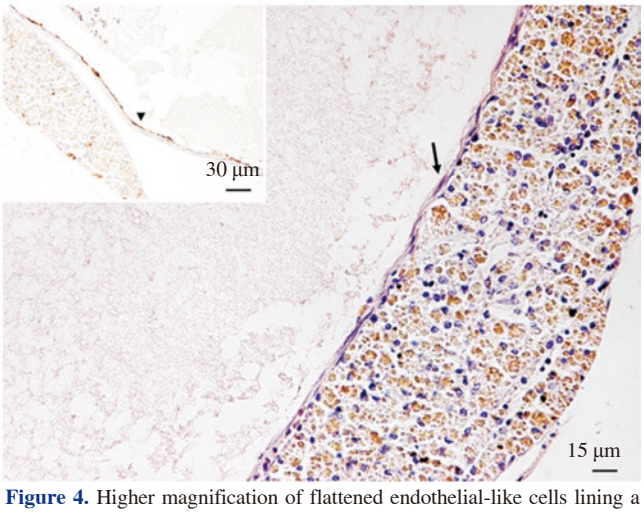

Figure 4. Higher magnification of flattened endothelial-like cells lining a cystic space (arrow).
Inset: Intense and diffuse vimentin immunoreactivity of endothelial-like cells lining the cystic structure (arrowhead).

3. Discussion

On the basis of the histological findings, reflecting the features described in human cases, a final diagnosis of cystic lymphangiomatosis of the spleen was made[2-4]. Vimentin immunoreactivity was also in line with this diagnosis, though the endothelial origin of cells lining the cystic structures could not

be immunohistochemically confirmed, since available antibodies directed against endothelial markers did not cross-react with fish tissues.

Cystic lymphangiomatosis of the spleen is an extremely rare pathological entity in humans, which is usually solitary and asymptomatic, though it may be also a part of a multiorgan (systemic) disease. Its pathogenesis is thought to be related to sequestration or obstruction of lymphatic vessels during embryogenesis, even though the exact cause is unknown[2-4]. The condition may present as solitary or multiple nodules (lymphangiomas), or as a diffuse growth (lymphangiomatosis), and consists of multiple cysts of varying sizes lined by flat endothelial cells, which contain eosinophilic serous fluid, and separated by fibrous connective tissue septa with rare blood vessels[4]. Based on the size of the dilated lymphatic channels, lymphangiomas may be histologically classified as capillary (super-microcystic), cavernous (microcystic), or cystic (macrocystic), though this nomenclature is not uniformly accepted among pathologists. The differential diagnosis of splenic lymphangiomas includes hemangiomas, true (splenic) epidermoid cysts, mesothelial cysts and parasitic cysts[3]. Hemangiomas, which occur not infrequently in fish are characterized by vascular channels lined by endothelium, but they are filled with red blood cells instead of amorphous proteinaceous material of lymphangiomas, as in our case[1,3]. Histological and immunohistochemical findings also exclude other types of splenic cysts, or a parasitic aetiology.

Although splenic lymphangiomatosis has not been documented in fish, multiple congenital splenic cysts were observed in two wild brown trout (*Salmo trutta*), where most of the cysts were lined by a single layer of endothelial cells, similarly to our case[10]. Reports of cystic conditions in fish, other than those associated with parasite infections, are rare, and the kidney, ovary and liver appear to be the mostly involved sites for non-parasitic cysts, which are usually congenital in origin, though their nature was not always clearly defined[1,11-15]. Since lymphangiomatosis is generally considered to be a congenital malformation of the lymphatic system, it should be taken into consideration among the possible differential diagnoses of congenital cystic conditions in fish, and histopathology is the essential step to establish the exact nature of cysts[4].

In fish, most congenital malformations are considered to be the result of genetic factors alone or in combination with unsuitable environmental factors during embryonic development. In this respect, several skeletal anomalies, as well as microphthalmia, are examples of conditions of presumed genetic origin. However, it is usually difficult or impossible to define the exact relationship between the different aetiologies and to identify the precise cause of the congenital defects occurring in fish[16]. Apart from the previously documented, very low incidence of skeletal defects, no other physical deformities were recorded in the present Italian colony, making a role of environmental deterioration or poor genetic diversity of the captive stock of *Z. tequila* in a causation of the lesions observed unlikely[8].

To the best of our knowledge, this is the first report of multiple, life-compatible, congenital physical deformities in association with cystic lymphangiomatosis of the spleen in *Z. tequila*.

Conflict of interest statement

We declare that we have no conflict of interest.

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