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NOTE

Mixed germ cell-stromal testicular neoplasia in a crucian carp (*Carassius carassius* L.): a case report

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Abstract
This report documents a case of spontaneously occurring gonadal neoplasm in a crucian carp (*Carassius carassius* L.) caught in the wild. On the basis of the histopathological findings, the neoplasm was diagnosed as a mixed germ cell-stromal testicular neoplasia (gonadoblastoma).

Teleosts generally resemble other vertebrates in their basic susceptibility to developing neoplastic lesions. A large variety of neoplasms of various tissues have been recognized and described in a broad range of freshwater and marine species of fish (Hayes and Ferguson, 1989). The aetiology of tumours is generally complex and many of the factors that contribute to the initiation and growth of tumours remain unknown. Factors contributing to the formation of tumours in fish include viruses, chemical or biological compounds, physical agents, hormones, and the age, sex, genetic predisposition and immunological competence as predisposing factors of the host (Roberts, 1989). Tumours can arise in virtually any tissue of the body but they are more frequent in tissues such as skin, gills, liver and gut, where cell proliferation is normally active and may be exposed to noxious substances (Roberts, 1989). In contrast, neoplasms of reproductive tissues, especially germ-cell neoplasms (seminoma in males and dysgerminoma in females), are generally considered to be rare in fish (Masahito and Ishikawa, 1997). Cases of these neoplasms have been described in several fish species, such as African lungfish *Protopterus annectens* (Nigrelli and Jakowska, 1953), *P. aethiopicus* (Masahito *et al*., 1984) and *P. dolloi* (Hubbard and Fletcher, 1985), bagrid catfish *Hemibagrus macropterus* (Majedd and Wang, 1994), zebrafish *Danio rerio* (Smolowitz *et al*., 2002), barbel *Barbus barbus* (Palikova *et al*., 2007) and Japanese medaka *Oryzias latipes* (Hawkins *et al*., 1996). Non-germ gonadal cell neoplasms have also been described in fish including leiomyoma in yellow perch *Perca flavescens* (Budd *et al*., 1975) and large-mouth bass *Micropterus salmoides* (Herman and Landolt, 1975), testicular mesothelioma in blue shark *Prionace glauca* (Borucinska *et al*., 2003),

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ovarian carcinoma in koi carp *Cyprinus carpio* (Raidal *et al.*, 2006) and tumours of Sertoli cells origin in hybrids of carp and goldfish *Carassius auratus* (Leatherland and Sonstegard, 1978; Down and Leatherland, 1989).

This report describes a spontaneously occurring gonadal tumour in a wild adult male crucian carp *Carassius carassius* L. The fish (total length 41 cm, body weight 1480 g) was caught in the river Esla in the Province of Leon (NW Spain; 42°23'N, 5°33'W) and presented lethargy, skin haemorrhage, abdominal enlargement and distension and an ulcer on the left side of the body that communicated with the abdominal cavity (Figure 1a). The fish was collected alive and submitted refrigerated to the Veterinary Faculty of Leon (Spain) for its study, where it was received in less than 12 h. At necropsy, haemorrhage in the abdominal wall and in most of the internal organs was observed. In addition, a single large multinodular mass was located in the abdominal cavity partly enveloped by mesenteric membranes (Figure 1b). This mass, measuring 19 x 12 cm and weighing 500 g, was white, moderately firm with a solid and white to pale yellow cut surface. Several necrotic cores were observed and no infiltrative growth was seen in the surrounding tissues. Moreover, there was no evidence of metastasis to other organs.

Samples from skin ulcer, abdominal wall musculature, intestine, kidney, liver and heart were collected aseptically and inoculated onto tryptone soy agar and MacConkey agar for bacterial isolation. The plates were incubated at room temperature and bacterial identification was carried out by using Gram staining, biochemical tests and an API 10S identification kit (BioMérieux®, Marcy l´Etoile, France). The tumour mass was removed from the fish and several samples were preserved in 10% neutral-buffered formalin and, after 48 h fixation, they were routinely processed and paraffin-embedded. Tissue sections of 3 μm were stained with haematoxylin and eosin (H&E) and Masson´s trichrome stains. In addition, selected tissue sections were stained with periodic acid-Schiff (PAS). The nuclear diameter of different types of tumour cells was measured with the Soft Imaging System Analysis®, Version 3.0 (Soft Imaging System GmbH, Münster, Germany). Paraffin blocks and tissue sections are archived in collection of the Service of Pathological Diagnosis of the University of León.

Providencia stuartii and *Proteus vulgaris* were isolated from skin ulcer, abdominal wall musculature, intestine, kidney, liver and heart. These bacteria, frequently present in aquatic environments, may have penetrated into the abdominal cavity through the ulcer and reached the blood stream, spreading to all the organs of the fish.

Histologically, the neoplasm exhibited two cellular growth patterns, intratubular and diffuse. Thus, in some fields, a proliferation of neoplastic cells showing differentiation into spermatogenic cells arranged in well-defined tubules separated by connective tissue septa was observed (intratubular pattern) (Figures. 1c, d, e). The tubules contained closely packed large polyhedral spermatogonium-like cells with lightly stained round nuclei (nuclear diameter 6-7 μm) with 1-2 prominent nucleoli (Figures 1d, e). Furthermore, nests of smaller neoplastic cells with condensed nuclei that

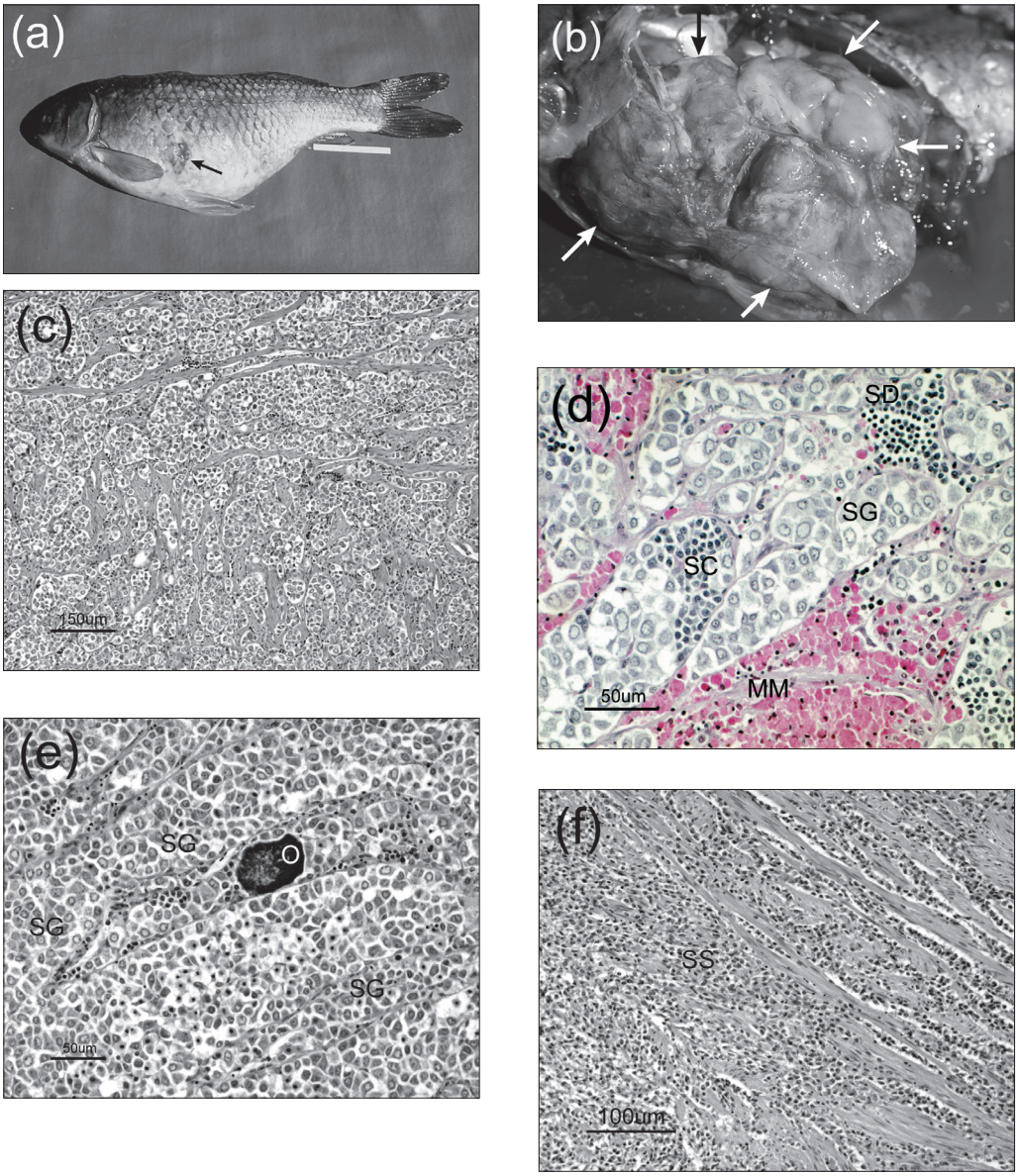
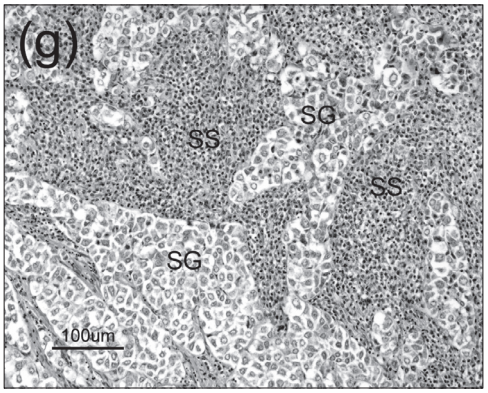
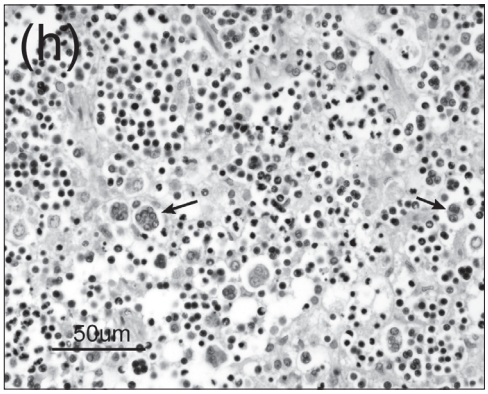


Figure 1. Gross and histological appearance of a gonadal neoplasm in a crucian carp (*Carassius carassius* L.). (a) Crucian carp showing abdominal swelling, skin haemorrhage and an ulcer in the trunk region (arrow). (b) Abdominal location and gross appearance of the tumour mass (19 x 12 cm). (c) Neoplastic cells arranged in an intratubular growth pattern (H&E). (d) Several spermatogenic stages (spermatogonia, SG; spermatocytes, SC; spermatids, SD) and melano-macrophage centre (MM) (PAS). (e) Well-defined tubules containing spermatogonia-like tumours cells (SG) and an oocyte-like cell (O) (H&E). (f) Spindle-shaped cells (SS) arranged in a diffuse growth pattern (H&E). (g) Sheets of spindle cells (SS) intermixed with destroyed neoplastic tubules containing spermatogonia-like neoplastic cells (SG) (H&E). (h) Multinucleated cells (arrows) interspersed with spermatogenic cells (PAS).





resembled spermatocytes (nuclear diameter 3-4 μm) and spermatids (of about 2 μm nuclear diameter) were also present in tubules (Figure 1d). Likewise, scattered intratubular oocyte-like cells were also recognized (Figure 1e). The diagnosis of spermatocytic seminoma was justified by the histological observation that the neoplasm exhibited stages of spermatogenesis. However, some authors (Smolowitz *et al.*, 2002) have pointed out that seminomas, which also contain large cells resembling oocytes interspersed in the neoplastic gonadal tubules, should be named dysgerminoma.

In the current case, there was in addition a further part of the tumour showing disorganized tubules and sheets of densely packed spindle-shaped cells arranged in a diffuse growth pattern which invaded the surrounding tissues (Figures 1f, g). Multinucleated cells with 4-5 nuclei per cell were observed (Figure h) and mitotic figures were rarely present in either growth pattern of the neoplasm. In addition, large necrotic areas and many PAS-positive melano-macrophage centres (Figure 1d) were also present, these being a probable indication of the large-scale degeneration and subsequent phagocytic activity that was under way, as has previously been described in gonadal neoplasms in cyprinid fish (Down and Leatherland, 1989).

On the basis of the histological observation that the neoplasm exhibited cells that resembled the cellular stages of spermatogenesis and oocyte-like cells interspersed in the neoplastic gonadal tubules coexisting in the same tumour with the sheets of cells indistinguishable from the stromal cells, a diagnosis of mixed germ cell-stromal tumour was established. The term gonadoblastoma has also been suggested as an acceptable diagnosis when both germinal and stromal tumour types are present (Down and Leatherland, 1989).

This type of gonadal neoplasm is common in carp hybrids, including ornamental koi carp, but is very rare in the parental species, such as carp (Down and Leatherland, 1989; Harshbarger, 2001). Regardless of the diagnosis, they could result from the difficulty of certain hybrid combinations to produce a gonad and they therefore represent

dysmorphogenesis instead of neoplasia (Harshbarger, 2001). In other cases, water pollution and exposure to both carcinogenic and endocrine disrupting substances have been related to gonadal tumour formation (Palikova *et al*., 2007). Likewise, mixed germ cell-sex cord stromal neoplasia has only rarely been reported in tumour surveys of domestic animals (Kennedy *et al*., 1998). Whether this is because the tumour is, in fact, rare or whether testicular tumours have rarely been analyzed in such a way as to confirm the diagnosis is not clear (Kennedy *et al*., 1998). Other types of testicular neoplasms have been reported, such as spermatocytic seminoma described in Japanese medaka (Hawkins *et al*., 1996), carp-funa hybrids (Granado-Lorencio *et al*., 1987), and Zebrafish (Smolowitz *et al*., 2002) as well as Sertoli-cell tumours found in carp-funa (Granado-Lorencio *et al*., 1987) and carp-goldfish hybrids (Down and Leatherland, 1989).

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