Opis przypadku *Case report*

Cutaneous iridophoroma and bacterial infection in a male Siamese fighting fish (*Betta splendens* Regan, 1910)

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Summary

A Siamese fighting fish (*Betta splendens*) showed a whitish, well-demarcated mass on various sites on the body and on the head. Based on histopathological examination, a multifocal iridophoroma was diagnosed in the fish. On H&E-stained slides the iridophores contained fine to coarse golden-brown intracytoplasmic pigment granules, which exhibited white-blue birefringence with polarized light. Additionally, Gram-negative bacteria (*Aeromonas hydrophila* and *Aeromonas veronii*) were found in the diseased fish. The present report adds to the sparse literature describing iridophoromas in fish with bacterial infection, spontaneously.

Keywords: iridophoroma, fish, bacteria, infection

The Siamese fighting fish (*Betta splendens* Regan, 1910) inhabits South and Southeast Asia and is currently assessed as 'Vulnerable' on the IUCN Red List (2023), which indicates medium risk of extinction in the wild. It is currently one of the most popular ornamental fish worldwide. Fish have various forms of chromatophores: light-absorbing chromatophores (black/brown melanophores, yellow xanthophores, blue cyanophores, and red/orange erythrophores), light-scattering chromatophores (leucophores) and light-reflecting chromatophores (iridophores) (1, 4, 8). Neoplasms of pigmented cells in fish are mainly of three phenotypes: melanophoromas, erythrophoromas and iridophoromas (7). Iridophoromas are considered rare tumours of fish, most commonly reported as cutaneous masses (9, 10). They have been described in both marine (15, 16) and freshwater fish species (12, 13). Data on bacterial infections among fish with iridophoromas are limited. The aim of the present study was to describe the clinical finding and microscopic features of an iridophoroma in a Siamese fighting fish with bacterial infection.

Case description

One privately owned Siamese fighting fish with tumour-like skin lesions was submitted for examination to the Department of Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin. The live fish was killed with an overdose of buffered tricaine methanesulfonate (MS-222) at 200 mg/L. As the examinations and sample collection were done during routine veterinary practice, Ethics Committee approval was not required. Swabs from the kidney and body cavity were collected aseptically for bacterial isolation and characterization. The samples were streaked onto tryptic soya agar (TSA) (Sigma-Aldrich, USA) and Oxoid *Aeromonas* ampicillin agar (Oxoid CM833 with 5 µg/ml of ampicillin), and the plates were incubated at 28°C. The resulting colonies were identified by MALDI-TOFMS (matrix-assisted laser desorption/ionization – time of flight mass spectrometry) (Bruker Daltonik GmbH, Bremen, Germany) as described by Pastuszka et al. (11). The biochemical profile of the isolates was determined using the API-20E test (BioMerieux, France) according to the manufacturer's instructions.

For light microscopy, the tumours were fixed in 10% buffered formalin for 24 h. Then the tissues were dehydrated

in ethanol, equilibrated in xylene and embedded in paraffin according to standard histological techniques. The sections (5 µm) were stained with haematoxylin and eosin (H&E). Selected fields were photographed using a Nikon Eclipse E-600 microscope coupled with a Nikon DS-Fi1 digital camera (Nikon Instruments, Tokyo, Japan). The diagnosis of iridophoroma was based on morphological findings in light microscopy (10, 12).

Results and discussion

Iridophores are colour-generating cells which reflect light using stacks of platelets made from guanine. They are generally located above the melanophores within the dermal chromatophore unit (1, 6). Uncontrolled proliferation of iridophores can lead to iridophoroma. White iridophoromas have been described in fish (10, 12, 13, 15). Dong et al. (3) described Siamese fighting fish farmed in central Thailand suffering from two common syndromes, provisionally named skin nodule syndrome (SNS) and big belly syndrome (BBS). Iridophoromas resembling SNS have been observed in many fish, e.g. *Thymallus thymallus* (13), *Rastrelliger kanagurta* (15), and *Betta splendens* (10, 12). A mixed chromatophoroma (benigniridomelanocytoma) in a specimen of *B. splendens* was described by Shivley et al. (14). Ciambrone et al. (2) also described a chromatophoroma (iridophoroma or leucophoroma) in *B. splendens*. The true causative agents of these chromatophoromas remain unclear. A genetic predisposition to developing iridophoromas in fish has been postulated (9).

The fish examined in our study showed a whitish well-demarcated mass on various sites on the surface of the body and on the fins and head (Fig. 1a, b). Skin lesions appeared as intradermal nodules measuring 2-5 mm in diameter. Microscopically, there were poorly demarcated, moderately cellular, infiltrative neoplasms composed of fusiform cells arranged in irregular short interlacing bundles and streams. In H&E staining, the cytoplasm of the neoplastic cells contained fine to coarse golden-brown intracytoplasmic pigment granules (Fig. 1c), which exhibited white-blue birefringence with polarized light (Fig. 1d). The cells exhibited moderate anisocytosis and anisokaryosis and rare mitoses. Based on the histopathological examination, a multifocal iridophoroma was diagnosed in the fish.

Two bacterial isolates from the infested fish samples were identified using MALDI-TOFMS as *Aeromonas veronii* and *Aeromonas hydrophila*, with score values

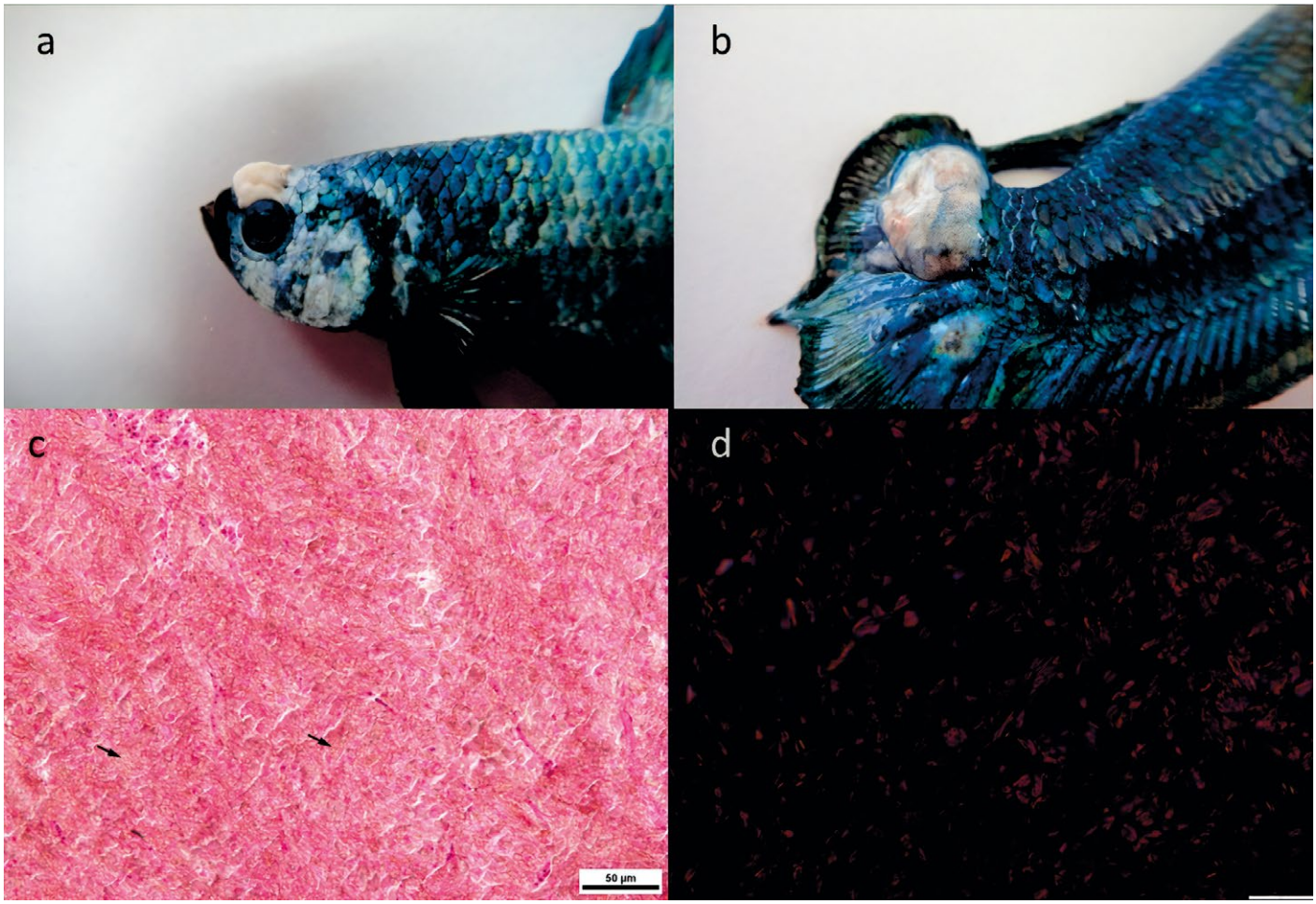

Fig. 1. Example of the distribution of skin lesion (Olszewska et al. 2020) (a, b). Photomicrography of the transverse cross section of the caudal peduncle shows tumour cells containing golden-brown pigment (black arrow) (c). Histological section of the tumour mass showing neoplastic cells with intracytoplasmic, birefringent, white-blue crystals under polarized light (d). H&E

of 2.2168 and 2.2540 (Tab. 1). On TSA media, *Aeromonas* colonies were round, shiny, and white, ranging in size from 2 mm to 3 mm, whereas on *Aeromonas* agar colonies were dark green and rounded. All isolates were motile and ONPG, ADH, LDC, CIT, IND, VP, GEL, GAL, AMY, OX and CAT positive (Tab. 1). According to the biochemical characteristics, the percentage of identification (%id) of strain N1 as *A. hydrophila*/*caviae*/*sobria* 2 was good (90.9%), with a T-index of 0.18, and that of strain N2 was excellent (99.8%), with a T-index of 0.62 (Tab. 1). The %id is an estimate of how closely the profile corresponds to the taxon relative to all other taxa in the database. The T-index is an estimate of how closely the profile corresponds to the most typical set of reactions for each taxon, as follows: excellent (%id ≥ 99.0, T ≥ 0.075), very good (%id ≥ 99.0, T ≥ 0.5), good (%id ≥ 90, T ≥ 0.25), and acceptable (%id ≥ 80.0, T ≥ 0).

Tab. 1. Characteristic and identification of bacteria strains isolated from diseased fish

Strains	Characteristics	Identification
MALDI-TOF-MS		
N1	Score value: 2.2168	*A. veronii*
N2	Score value: 2.2540	*A. hydrophlia*
API-20E		
N1	Positive: ONPG, ADH, LDC, ODC, CIT, TDA, IND, VP, GEL, GLU, MAN, SAC, AMY, ARA, OX, CAT, O/129 Negative: H_2S, INO, SOR, RHA, MEL	*A. hydrophila*/*caviae*/*sobria* 2 Code: 7367125 %id – 90.9, T = 0.18
N2	Positive: ONPG, ADH, LDC, CIT, IND, VP, GEL, GAL, AMY, OX, CAT, O/129 Negative: ODC, H_2S, URE, TDA, MAN, INO, SOR, RHA, SAC, MEL, ARA	*A. hydrophila*/*caviae*/*sobria* 2 Code: 7247005 %id – 99.8, T = 0.62

Explanations: ONPG – Ortho nitro phenyl-βD-galactopyranosidase; ADH – Arginine dihydrolase; LDC – Lysine decarboxylase; ODC – Ornithine decarboxylase; CIT – Citrate utilization; H_2S – Hydrogen sulfide; URE – Urease, TDA – Tryptophan deaminase; IND – Indole; VP – Voges Proskauer; GEL – Gelatinase; GLU – Glucose (fermentation/oxidation); MAN – Mannitol (fermentation/oxidation); INO – Inositol (fermentation/oxidation), SOR – Sorbitol (fermentation/oxidation); RHA – Rhamnose (fermentation/oxidation); SAC – Saccharose (fermentation/oxidation); MEL – Melibiose (fermentation/oxidation); AMY – Amygdalin (fermentation/oxidation); ARA – Arabinose (fermentation/oxidation); OX – Oxidase; CAT – Catalase; O/129 – Vibriostatic agent 2,4,diamino6,7-diisopropylpteridine

Our previous observations indicate that fish with cancer have a substantially higher mortality rate if they also have bacterial infections (data not shown). Our observations revealed that eliminating pathogens in the cancer disease process can prolong the life of diseased fish (data not shown).

In conclusion, microbiologically confirmed infections are poorly documented in fish with iridophoromas. The present report adds to the sparse literature describing iridophoromas in fish with bacterial infection. It should be assumed that the cancer process in the examined individual led to dysfunction of the immune system, which in turn resulted in a reduction of natural immunity barriers and susceptibility to bacterial infections, bacterial species that are still present in the fish environment. Further studies are still needed to explore the immunological interactions between infecting bacteria and iridophoroma in a single host.

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