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Short title: Lambl's excrescences in teleost fish

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ABSTRACT

Lambl's excrescences (LE) are fibrous strands typically occurring at coaptation lines of cardiac valves. Although LE have been largely reported in human medicine, information on LE occurrence in veterinary medicine is still scarce. The present study aimed to investigate the presence of LE in different freshwater fish and in swordfish. A total of 185 hearts were collected from different fish species and samples of different cardiac areas (sinus venosus, atrial wall with sinoatrial valves, ventricular wall with atrioventricular valves and bulb with bulboventricular valves) were submitted to histopathological evaluation. LE were detected in 6/103 freshwater fish (5.8%) and in 19/82 swordfish (23.2%). LE developed in atrioventricular, sinoatrial and bulboventricular valves. All the affected valves also showed endocardiosis. Based on the results of the present work, teleost fish, specifically swordfish, could be proposed as a novel spontaneous model for the study of LE pathogenesis.

KEYWORDS: Lambl's excrescences, teleost fish, cardiac valves, histology, endocardiosis

1. INTRODUCTION

Lambl's excrescences (LE) are fibrous, fine, mobile, filiform fronds that typically occur at coaptation lines of cardiac valves (Ammannaya, 2019). They have been mainly observed on the atrial side of the mitral valve (68-76%) (Aziz and Baciewicz, 2007; Takahashi et al., 1993) and on the ventricular side of the aortic valve (38-50%) (Inam et al., 2019; Patel et al., 2020). Rarely, they have also been observed on prosthetic, pulmonary and tricuspid valves (Kalavakunta et al., 2010; Leitman et al., 2014; Roldan et al., 1997). The pathogenesis is still unclear but it seems that high pressure blood-flow can provoke a tear in the endocardium of valves' closure lines. Deposition of fibrin over this injured endocardium causes endothelial overgrowth, resulting in the development of papillary outgrowths that partially detach from the surface, hyalinize, and ultimately become fibrosed. This process can be favored by the presence of endocardiosis, a myxomatous degeneration of the cardiac valves characterized by cellular proliferation and matrix degeneration with deposition of glycosaminoglycans and proteoglycans (Cooper and Spitsbergen, 2016a).

Moreover, the increase ofng shear forces with age can further contribute to LE development (Ammannaya, 2019). Pathological significance of LE remains controversial (Voros et al., 1999). Though mostly asymptomatic, in human medicine LE have been associated with thromboembolic events (i.e embolic stroke, cerebrovascular accidents and acute coronary syndrome) or with congenital heart pathologies (Amin et al., 2019; Inam et al., 2019; Jo et al., 2016; Kariyanna et al., 2018; Phillips et al., 2018).

Despite being investigated in human, very few reports of LE are available in animals. In veterinary medicine, filiform valvular fronds compatible with LE have been reported on the atrial surface of atrioventricular valves of horses (*Equus caballus*) (Else & Holmes, 1972;

Guarda et al., 1997; Passantino et al., 2018), dogs and pigs (Guarda et al., 2013). In marine mammals, LE have been described in the valve cusps of beached striped dolphins' hearts (*Stenella coeruleoalba*) (Scaglione et al., 2013).

To the best of our knowledge, there are no reports of LE in fish. Therefore, the aim of our study was to investigate, for the first time, the presence of LE in different freshwater fish and in swordfish.

2. MATERIALS AND METHODS

2.1 Animals

A total of 185 hearts were collected from 12 different species of freshwater fish and from swordfish (Table 1). Freshwater fish were caught in the riverine and lacustrine water of western Po Valley (Italy) by professional fishermen as per current regulations regarding animal welfare during slaughter (REG CE n. 1099/09). Only 2-3 years old adult fish were included in the study. Swordfish for human consumption were fished in the Ligurian Sea (Italy) by professional fishermen following the current regulations for conservation of the Mediterranean swordfish stock (minimum size, REG UE n. 191/2018) and welfare conditions during slaughter (REG CE n. 1099/09).

2.2 GROSS AND HISTOPATHOLOGICAL EVALUATION

After evisceration, hearts were immediately fixed whole in 10% neutral buffered formalin (pH 7) for 7 days prior to examination. A thorough macroscopical external examination was conducted. Hearts were then sectioned across the median longitudinal plan and samples of sinus venosus, atrial wall with sinoatrial valves, ventricular wall with atrioventricular valves and bulb with bulboventricular valves were collected. The obtained sections were routinely embedded in paraffin wax blocks, sectioned at 5 μm thickness, mounted on glass slides and stained with Haematoxylin & Eosin (H&E) for histopathlogical evaluation.

Additionally, histochemical stainings (Periodic Acid Schiff (PAS)-Alcian, Toluidine blue and Weigert-Van Gieson) were performed on selected sections (Mazzi, 1977). All the samples were reviewed by three veterinary pathologists at the Department of Veterinary Sciences, University of Turin (Italy). The observed lesions were systematically described and data were expressed as number of animals presented LE (N) and percentages (%).

3. RESULTS

3.1 Gross and Histopathological evaluation

At gross examination, freshwater fish hearts did not show any significant lesion. Histopathological analysis highlighted the presence of LE in 6/103 freshwater fish (5.8%, two black bullhead, one roach, one common carp, one tench, one wels catfish) (Table 1). Particularly, LE were observed in 6 atrioventricular valves (Figure 1a) and in one sinoatrial valve (Figure 1b). All the valves that showed LE also exhibited endocardiosis, characterized by thickening of the spongiosa, deposition of eosinophilic interstitial matrix, degeneration of the valve leaflets and proliferation of fibroblastic tissue.

In swordfish, macroscopical changes in shape and/or thickening of the heart valves compatible with endocardiosis were evident in 54 out of 82 cases (65.9%) (Table 1). With histopathological evaluation, 19 swordfish out of 82 (23.2%) had LE. In particular, LE were highlighted in 11 atrioventricular valves (Figure 2a), 6 bulboventricular valves (Figure 2b) and 2 sinoatrial valves. This histopathological finding was always associated with endocardiosis.

Histologically, both in freshwater fish and swordfish LE appeared as filiform projections, single or grouped (5-20) at the edges of the valve cusps, straight or bent towards the bloodstream with a variable size (1 mm thick and 1-10 mm long). They were composed of a central elastic connective-tissue core in continuity with the connective tissue of the valve. This densely hyalinized central core contained variable orientated collagen and elastic fibrils enclosed by a single layer of endothelium and it was lined by bland-looking, endocardial-type spindle cells. Collagen and elastic fibers in the LE stained intensely with Weigert-Van Gieson stain (Figure 1c-2c). Glycosaminoglycans in the regions of endocardiosis stained with PAS-Alcian (Figure 1d-2d) and Toluidine blue.

4. DISCUSSION

The presence of LE indicates a pathological finding generally observed at atrioventricular and semilunar valves' coaptation line. LE can be associated with old age or previous cardiac diseases (e.g. rheumatic heart disease, endocarditis, pulmonary or systemic hypertension), but LE can also occur in healthy individuals (Aziz & Baciewicz, 2007). Although a greater number of cases has been reported in humans (Voros et al. 1999), data on LE in veterinary medicine are still lacking. For these reasons, the aim of this work was to investigate the presence of LE in different fish species.

In the present study, no macroscopic lesions were recorded in freshwater fish while 54/82 swordfish presented macroscopical changes of the cardiac valves. At microscopic examination, LE were recorded in 5.8% of freshwater fish and 23.2% of swordfish. The exact pathogenetic mechanism underlying LE formation has not yet been fully understood neither in fish nor in other species. The most accepted theory regarding the development of LE proposes that they are organized thrombi. The continual striking upon the valves causes small tears in the endothelium, which leads to fibrin deposition. In high-pressure environments, thrombi are converted into flat, fibrous scars. This is followed by growth of an endothelial layer over the fibrous surface and condensation of the enclosed fibrin (Davogustto et al., 2015). Swordfish are fast-swimming fish that rely on their great speed and agility in the water to catch their prey (Videler et al., 2016). Hence, they can be possibly subjected to higher blood pressure compared to freshwater bottom feeder fish such as the common carp (Cyprinus carpio) or the tench (*Tinca tinca*. This higher turbulent blood flow in the cardiac chambers of swordfish could represent a possible explanation for, the higher percentage of LE recorded in this fish species compared to freshwater fish.

From a histopathological view, LE observed in fish are similar to the ones observed in humans (Lambl, 1856), horses (Else & Holmes 1972), and dolphins (Scaglione et al. 2013). Moreover, the variation in number, shape and volume recorded for LE in fish is in accordance with previous findings in humans (Davogustto et al. 2015).

In the present study, all the valves with LE had concurrent valvular endocardiosis. In veterinary medicine, LE were frequently reported associated with endocardiosis in horses, (Passantino et al., 2018) dogs and pigs (Guarda et al., 2013), suggesting a potential relationship between the two pathologies. Previous reports have described endocardiosis as a common valve pathology in fish (Capucchio et al., 2018; Cooper and Spitsbergen, 2016b; LaDouceur et al., 2019) but there was no evidence of LE in these reports. The cause of endocardiosis is unknown. In zebrafish, there may be a correlation with genetic mutation, water circulation and/or diet (Cooper & Spitsbergen, 2016). In domestic mammals, there is a clear genetic and age-related association. To date, in this preliminary study age was not taken into consideration and further studies are needed to clarify the role of age in the onset of LE and endocardiosis in fish. However, it could be hypothesized that endocardiosis could lead to valvular degeneration and insufficiency, producing increased vascular turbulence with consequent valvular tearing and development of LE.

The analogies found for LE among human and different animal species represent a noteworthy finding. Despite the evolutionary and structural differences between species, it seems that valvular tissue can react to different stressors (e.g., age, metabolism, environment) in a similar way. It has also been shown that the intensity and time of exposure to stressors can influence the onset and severity of LE in both humans and animals (Capucchio et al., 2020).

In conclusion, this is the first study reporting LE in teleost fish. Naturally occurring LE were described in 29% of the studied fish with histological features comparable to those reported in humans. Based on the results of the present work, teleost fish - specifically swordfish- might be considered a novel spontaneous model for the study of the pathogenesis of LE.

ETHICS APPROVAL

The experimental protocol was designed according to the guidelines of the current European Directive (2010/63/EU) on the care and protection of animals.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets analyzed in the present study are available from the corresponding author on reasonable request.

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Table 1: Freshwater and marine fish species with the distribution of macroscopic lesions and LE observed in the present study

FISH	N = 185	Macroscopic lesions	LE
Freshwater fish	**103**	0/103	6/103 (5.8 %)
European perch (*Perca fluviatilis)*	25	0/25	
European whitefish (*Coregonus lavaretus)*	19	0/19	
Tench (*Tinca tinca)*	13	0/13	1/13 (7.7%)
Common carp (*Cyprinus carpio)*	11	0/11	1/11 (9.1%)
Pike-perch (*Sander lucioperca)*	10	0/10	
Crucian carp (*Carassius carassius)*	9	0/9	
Roach (*Rutilus rutilus)*	5	0/5	1/5 (20%)
Black bullhead (*Ameiurus melas)*	4	0/4	2/4 (50%)
Rudd (*Scardinius erythrophthalmus)*	3	0/3	
Northern pike (*Esox lucius)*	2	0/2	
Atlantic salmon (*Salmo salar)*	1	0/1	
Wels catfish (*Silurus glanis)*	1	0/1	1/1 (100%)
Marine fish	**82**	54/82 (65.9%)	19/82 (23.2%)
Swordfish (*Xiphias gladius*)	82	54/82	19/82 (23.2%)

FIGURE LEGENDS

Figure 1. a) Gardon (*Rutilus rutilus*), Lambl excrescences in atrioventricular valve (black arrows), 5x, Haematoxylin and eosin (H-e). The inset shows a higher magnification, 20x, H-e. b) Wels catfish (*Silurus glanis*), Lambl excrescences in sinoatrial valve (black arrows), 10x, H-e. The inset shows a higher magnification, 40x, H-e. c) Wels catfish (*Silurus glanis*), Lambl excrescences (black arrows) in atrioventricular valve with an increase of collagen fibers (asterisk), 10x, Wieger Van Gieson. d) Wels catfish (*Silurus glanis*), Lambl

excrescences (black arrows) in atrioventricular valve with increased glycosaminoglycans in the matrix (asterisk-endocardiosis), 10x, Alcian-PAS.

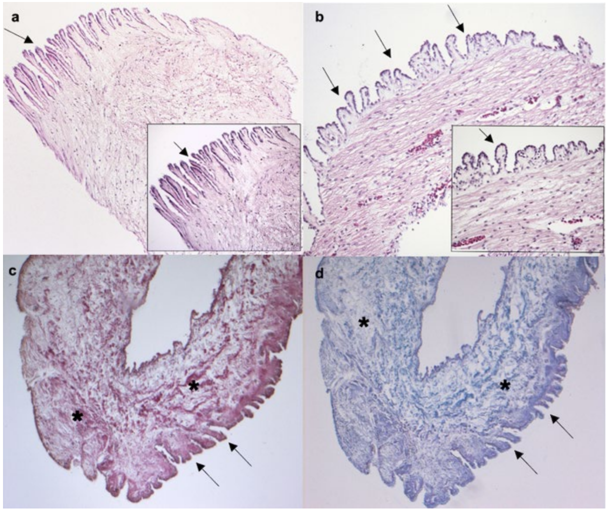

Figure 2. a) Swordfish (*Xiphias gladius*), Lambl excrescences in atrioventricular valve (balck arrows), 10x, Haematoxylin and eosin (H-e). b) Swordfish (*Xiphias gladius*), Lambl excrescences in bulboventricular valve (black arrows), 5x, H-e. c) Swordfish (*Xiphias gladius*), Lambl excrescences (black arrows) in atrioventricular valve with an increase of collagen fibres (asterisk), 10x, Wieger Van Gieson. d) Swordfish (*Xiphias gladius*), Lambl excrescences (black arrows) in atrioventricular valve with increased glycosaminoglycans in the matrix (asterisk-endocardiosis), 10x, Alcian-PAS.