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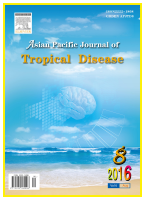

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Lymphocystis in Indian oil sardine, *Sardinella longiceps* (Valenciennes 1847)

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ABSTRACT

Objective: To analyse the prevalence and pathology of lymphocystis disease in *Sardinella longiceps* of three different landing centers in Tamil Nadu of southeast coast of India for a period of three years from January 2013 to December 2015.

Methods: Tumourous sardines were sourced from three different landing centers (Cuddalore, Parangipettai and Nagapattinam) in Tamil Nadu, southeast coast of India. The prevalence of lymphocystis, and its gross pathology, histopathology were investigated.

Results: A total of 369 individuals were affected with lymphocystis. The overall prevalence was 0.11% among the three stations. The uncircumscribed, translucent white, pink and reddish and jelly-like nodules of cell growths scattered on the both sides of eyes and operculum plates. Histopathological studies of the white masses showed abundant hypertrophied cells with cytoplasmic vacuoles and basophilic intra-cytoplasmic inclusion bodies. The hypertrophied cells had pleomorphic, hypertropic and hyperchromatic nuclei and contained basophilic marginated chromatin.

Conclusions: The histological and ultra-structural findings, the lesions were diagnosed as the hypertrophied cells. The lesions were confirmed the lymphocystis disease in *Sardinella longiceps*.

1. Introduction

Lymphocystis disease (LCD) is- a long-term self-limiting disease affecting more than 125 captured and cultured marine species, brackish and fresh water fish worldwide[1]. It is characterized by the external developments of wart-like whitish nodules on the skins, eyes, operculums and fins[1]. In general, the hypertrophied cells named as lymphocystis cells and consisted of three layers of thick collagen and hyaline capsule, have hypertrophic nucleus and prominent basophilic intra-cytoplasmic DNA inclusions bodies[2]. Lymphocystis is a viral disease caused by lymphocystis disease virus (LCDV) which is a large icosahedral DNA virus in the genus *Lymphocystivirus* of the family Iridoviridae[3]. LCD is not mortal, but its hideous appearance leads to severe economic losses. The infected fishes are highly vulnerable to secondary bacterial and mycotic diseases[1,4].

The Indian oil sardine *Sardinella longiceps* (Valenciennes 1847) (*S. longiceps*) belongs to the family Clupeidae. The commercially important sardine is abundantly distributed in the eastern and western coast of Indian waters[5]. It occupies a pelagic-neritic niche at a depth range of 20–200 m, forms

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schools in coastal waters and takes over strongly migratory and strictly marine habitats[6]. In recent days, tumours in sardines reported by Gopalakrishnan *et al.*[7], Sinduja *et al.*[8] and Singaravel *et al.*[9] in Tamil Nadu, southeast coast of India. To our knowledge, this is the first report of lymphocystis in Indian oil sardine (*S. longiceps*). The study was undertaken to analyze the prevalence of lymphocystis, present it gross pathology and provide detailed information on it histology and ultra-structure.

2. Materials and methods

S. longiceps tumour affected specimens were procured from fishermen and merchants of three different landing centres (Cuddalore, Parangipettai and Nagapattinam) on the southeast coast of India from January 2013 to December 2015. Affected fishes were brought to the laboratory for further investigation. Fishes were identified based on an Food and Agriculture Organization fish identification sheet[10]. The clinical signs of distended growths were prospecting on their heads and operculums. Initially physical examination for ecto and endo-parasites and fungal culture test on Sabouraud dextrose agar and potato dextrose agar was prepared. Blood samples and swabs from the internal organs and distended masses were collected for bacteriological assessment. The samples were cultured on Muller Hinton agar, trypticase soy agar, blood agar and brain heart infusion agar in aerobic and anaerobic conditions at 24 ˚C. The extended lesions were excised and fixed in 10% neutral buffer formalin for histopathological assessments. The stained sections were observed under a phase contrast microscope (Nikon Eclipse TS100).

3. Results

The prevalence of lymphocystis in sardines landed at three locations was given in Table 1. Out of 335 784 examined individuals, 369 were affected with lymphocystis and an overall prevalence of 0.11% was recorded. The higher prevalence by location was observed in Parangipettai (0.13%), whereas the lower prevalence (0.09%) was observed in Cuddalore and Nagapattinam. The higher seasonal prevalence (0.24%) was observed during the pre-monsoon, whereas the lower seasonal prevalence (0.01%) was recorded during the monsoon (Table 1). The length and weight of the diseased fishes varied between 153 cm and 187 cm and 56.5 g and 108.5 g, respectively. The sex wise higher prevalence was recorded in female 52.69%, which was higher than that of males (47.31%). Most of them were matured adults and spawners and none of them were juveniles.

Table 1

Prevalence of lymphocystis in *S. longiceps* landed at the three locations in Tamil Nadu, southeast coast of India from 2013 to 2015.

Seasons	Cuddalore (%)			Parangipettai (%)			Nagapattinam (%)			Total
	2013	2014	2015	2013	2014	2015	2013	2014	2015	
Post-monsoon	0.03	0.07	0.06	0.07	0.05	0.06	0.05	0.06	0.04	0.05
Summer	0.11	0.13	0.10	0.13	0.11	0.13	0.12	0.10	0.11	0.12
Pre-monsoon	0.24	0.26	0.21	0.31	0.29	0.26	0.20	0.19	0.21	0.24
Monsoon	0.00	0.02	0.00	0.01	0.03	0.02	0.00	0.02	0.02	0.01
Total	0.09	0.12	0.09	0.13	0.12	0.11	0.09	0.09	0.10	0.11

Seasons are based on calendar months. Post-monsoon: Jan–Mar; Summer: Apr–Jun; Pre-monsoon: Jul–Sep; Monsoon: Oct–Dec.

The uncircumscribed, translucent white, pink and reddish and jelly-like nodules were scattered on the both sides of eyes and operculum plates (Figure 1). The size of nodules ranged from 2.0 to 20.3 mm. No lesions were evident in other internal organs as necropsy examination showed no gross evidence of invasion and metastasis into other visceral organs. After 72 h incubation, no bacterial growth was observed on differential culture media. After 10 days, no fungal growth was noted on Sabouraud dextrose agar and potato dextrose agar. Wet mount examination, the large vacuoles and enlarged dermal fibroblasts were observed, and no ecto and endo-parasites were seen.

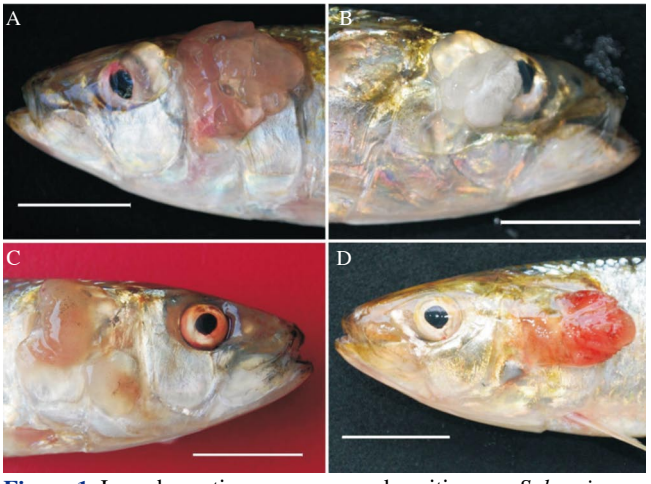

Figure 1. Lymphocystis appearance and positions on *S. longiceps*. A: Pink and translucent masses on operculum and eye; B: White and translucent growth on eye; C: Pink and jelly-like translucent masses on operculum; D: Reddish translucent masses on operculums. Bars: 2 cm.

Histopathologically, the masses showed abundant hypertrophied cells with basophilic intra-cytoplasmic inclusion bodies. Hypertrophic cell surrounded by dense layers were ranged between 60 to 100 μm in diameter. The hypertrophied cells had cytoplasmic vacuoles with basophilic inclusion bodies (Figure 2). The ultrastructure of nodules showed clusters of hypertrophied cells and was covered by a thick smooth hyaline capsule. These cells had pleomorphic, hypertropic and hyperchromatic nuclei, and abundant basophilic marginated chromatins were observed. Numerous basophilic intracytoplasmic DNA inclusion bodies were observed.

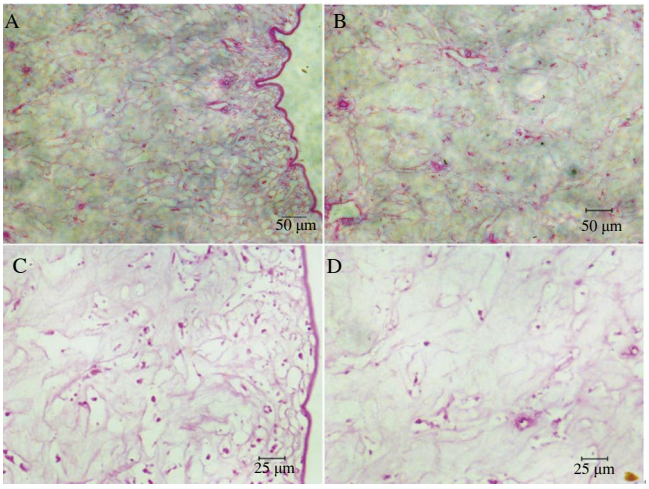

Figure 2. Photomicrograph of lymphocystis masses from the eyes and operculums of the Indian oil sardine.
A and B: Numerous hypertrophied cells with basophilic intracytoplasmic inclusion bodies on peripheral and integral regions of the lesion; C and D: Intracytoplasmic large vacuoles and intravacuolar basophilic inclusion bodies.

4. Discussion

Lymphocystis is a common disease reported in more than 125 captured and cultured species of marine brackish and fresh water fishes[1]. However, studies on its prevalence are meager. Bowser *et al.*[11] reported the LCD in walleye *Stizostedion vitreum*. The higher seasonal prevalence was recorded during the early spring and fall season, and the lower was during the summer. In contrast, in the present study the seasonal prevalence of LCD was fluctuated, but a higher prevalence was recorded during pre-monsoon, and a lower prevalence was occurred during monsoon. In culture systems, the LCDV prevalence is very high. The LCDV incidence rate is 70%, which causes significant economic losses in the aquaculture industries and ornamental fish hatcheries[1,3]. In wild, the LCDV incidence rate is 0–2.7%[12]. Similarly, in the present study the LCDV relative incidence is low, because the horizontal transmission of this virus may be attributed to the relative incidence.

In terms of the prevalence of lymphocystis in *S. longiceps,* almost all the stations showed the similar observation, which can be the reason of spatial adjacent locations. However, the seasonal prevalence is fluctuated. The higher lymphocystis prevalence was recorded during the pre-monsoon season (July–September) in Parangipettai. Similarly, on August and November the LCDV incidence and detection rate is 50% and 10%, respectively[13]. The spawned sardines are highly landed in pre-monsoon season[14]. Similarly, sardines are at spawning peaks during August and September[15]. This present investigation revealed that the tumour higher prevalence was observed on adults and spawners on spawning season, and the sex hormones hyperactivities maybe enhance the tumours prevalence in *S. longiceps*. Similarly, high levels of reproductive and stress hormones, hormonal manipulation and sex steroid injections as well as a high level of testosterone induced skin tumours in fishes[16,17]. In this study, the higher prevalence was observed in female fishes. Hence, Zaki *et al.*[18] and Shah *et al.*[19] stated that female proportion is higher than the male in sardine population, which may reflect the higher tumour prevalence in female fishes.

The results confirmed the presence of lymphocystis in Indian oil sardine (*S. longiceps*). Lymphocystis lesions observed in this study were similar to those described by Rahmati-holasoo *et al.*[2]. In fishes, the lymphocystis nodules grow primarily on the body surfaces but can also rarely develop on the internal organs[4,20]. Similarly, the irregular white colour firm lesions develop on heads, operculums, skins, fins, eyes and mouths[2,21]. Rarely, viral infection creates nodular lesions on the spleens and gastrointestinal tracts of the internal organs[22].

The histological results are resembles to the report by Kurkjian *et al.*[22] and Rahmati-holasoo *et al.*[2] which proved that the lymphocystis virus prefers to replicate in dermal fibroblasts resulting in hypertrophied cells. The hypertrophied dermal fibroblasts may aggregate to form nodules[22]. Rahmati-holasoo *et al.*[2] reported that, in infected flowerhorn fishes, cell size and the presence of intracytoplasmic vacuoles and DNA inclusion bodies were different from those reported for lymphocystis. In present study, the results are similar to that reported by Rahmati-holasoo *et al.*[2] in hypertrophic cells and cytoplasmic vacuoles and basophilic intracytoplasmic inclusion bodies were observed.

The size of the hypertrophic cells first reported by Weissenberg[23] was 700 µm in diameter in *Stizostedion vitreum* and *Pleuronectes flesus*. However, Rahmati-holasoo *et al.*[2] reported that the size was ranged between 150 and 350 µm in flowerhorn fish. In present study, the cell size was ranged from 60 to 100 µm in *S. longiceps*. Rahmati-holasoo *et al.*[2] and Yanong[20] described three layers that covered the hypertrophic cells. The external layer was collagen and the remaining middle and internal layers were hyaline capsules[24]. Similarly, the present study also examined the hypertrophic cells that covered three layers.

Pirarat *et al.*[21] reported that in infected false clown anemonefish (*Amphiprion ocellaris*) hypertrophied cells and viral particles were reported for lymphocystis. The size and shape of viral particles reported by Pirarat *et al.*[21] were 200 nm and icosahedral, whilst in this study also the viral particle size is 200 nm and icosahedral. This is the first report demonstrating the presence of LCDV in wild Indian oil sardine *S. longiceps*. The histopathological and ultrastructural findings, the lesions, were diagnosed as typical hypertrophied cells containing iridovirus-like particles. This is the first report demonstrating the presence and prevalence of lymphocystis disease in Indian oil sardine, *S. longiceps*. The gross and histopathological findings, the lesions, were diagnosed as lymphocystis disease.

Conflict of interest statement

We declare that we have no conflict of interest.

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