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Mycobacteriosis in an appearantly healty atlantic mackerel (Scomber Scombrus, L.) and zoonotic potential

[Micobacteriose em uma cavala atlântica aparentemente curada (Scomber Scombrus, L.) e potencial zoonótico]

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ABSTRACT

Mycobacteriosis was detected in seven out of one Atlantic mackerel (*Scomber scombrus*) that was purchased for human consumption from a fish market. The fish was apparently healthy but during cleaning, several granulomatous foci were noticed in the visceral organs. Histopathological examination of the lesions revealed numerous foci characterized by caseous necrosis in the center of the lesion surrounded by epithelioid giant cells. Ziehl–Neelsen staining revealed the presence of rod-shaped, acid-fast bacteria. Furthermore, immunohistochemical examination revealed the presence of a protein of mycobacterial origin in giant cells and macrophages. Based on gross and microscopic findings, mycobacteriosis was diagnosed. This report showed that due to its zoonotic potential, mycobacteriosis should be considered even in healthy-appearing fishes for human consumption.

Keywords: mycobacteriosis, fish, pathology, immunohistochemistry, histopathology, zoonotic potential

RESUMO

A micobacteriose foi detectada em uma de sete cavalas do Atlântico (Scomber scombrus) que foi comprada para consumo humano em um mercado de peixe. O peixe era aparentemente saudável, mas durante a limpeza, vários focos granulomatosos foram notados nos órgãos viscerais. O exame histopatológico das lesões revelou numerosos focos caracterizados por necrose caseosa no centro da lesão circundada por células gigantes epitelioides. A coloração de Ziehl-Neelsen revelou a presença de bactérias em forma de bastonete, com rápida acidez. Além disso, o exame imunohistoquímico revelou a presença de uma proteína de origem micobacteriana em células gigantes e macrófagos. Com base em achados grosseiros e microscópicos, a micobacteriose foi diagnosticada. Este relatório mostrou que devido a seu potencial zoonótico, a micobacteriose deve ser considerada mesmo em peixes saudáveis para consumo humano.

Palavras-chave: micobacteriose, peixes, patologia, imuno-histoquímica, histopatologia, potencial zoonótico

INTRODUCTION

Mycobacteriosis is a contagious and chronically productive fish disease caused by bacteria belonging to the genus *Mycobacterium* (Gauthier, 2015; Austin and Austin, 2016). Mycobacteria are acid-fast, gram-positive, aerobic, pleomorphic rod-shaped bacteria (Ojanen *et al*., 2019). Moreover, *Mycobacterium* spp. cause serious and zoonotic disease (Gauthier, 2015). These bacteria may have an impact on various fish species. Mycobacteriosis may be a significant cause of morbidity and mortality in both aquaculture and wild finfishes.

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Mycobacterium marinum is the most commonly isolated mycobacteria from fishes (Jacobs *et al*., 2009; Gauthier, 2015).

Mycobacterium marinum related granulomatous lesions in human skin and deep tissues previously reported (Petrini, 2006). When the agent contacted the injured skin it may produces skin nodules, cutaneous ulcers, and nodular lymphangitis (Jenigan and Farr, 2000). In severe cases, tenosynovitis, arthritis and osteomyelitis may be seen in humans (Wongworawat *et al*., 2003). People may contract mycobacterial agents with fish spine injury occurring at fishing pond, through contaminated pond water or in the fish processing industry (Tsai *et al*., 2007). Furthermore, the increased frequency of epizootics in wild fisheries as well as the ability of a few species to infect humans has gained considerable interest (Jacobs *et al*., 2009).

Mycobacteriosis does not usually result in any visible clinical signs in fish. Lesions typically affect all tissues of the fish. Majority of the symptoms are atypical and generally nonspecific in fish mycobacteriosis. Commonly reported external signs include ulcers and pigmentation changes in the skin, abnormal behavior, emaciation or cachexia, skeletal abnormality, and ascites (Ojanen *et al*., 2019). Generally, granulomatous foci are localized in the liver, spleen, and kidney. In mycobacteriosis cases, characteristic lesions include gray-to-whitish nodules randomly distributed in visceral organs (Ortega *et al*., 2014; Gauthier, 2015; Austin and Austin, 2016).

Mycobacteriosis in fish was first reported in 1897 by Bataillon *et al*. in a carp (*Cyprinus carpio*) (Gauthier, 2015), then it defined in an Atlantic mackerel in the North Sea but the mycobacterial agent could not be isolated, and then in an aquarium population of an Atlantic horse mackerel, *Trachurus trachurus* L. (Ortega *et al*., 2014). This report aimed to describe a case of mycobacteriosis in an apparently healthy Atlantic mackerel (*Scomber scombrus*) with histopathological and immunohistochemical pathological findings.

MATERIALS AND METHODS

The subject of this study was an Atlantic mackerel (*Scomber scombrus* L.) out of seven fish that was purchased for human consumption at a fish market. While cleaning the fish for cooking, numerous granulomatous foci were found in the visceral organs. The fish was brought to the Department of Pathology for histopathological examination and diagnosis. During the necropsy, tissue samples from all visceral organs were collected. Further, these samples were fixed in 10% neutral-buffered formalin for 2 days. Next, samples were placed in processing cassettes and routinely processed by an automatic tissue processing device. Then samples were embedded in paraffin wax for blocking. Tissue sections were cut at 5 μm thickness with a rotary microtome, then stained with hematoxylin and eosin (HE) and Ziehl–Neelsen method for mycobacteria.

Rabbit Polyclonal *Mycobacterium tuberculosis* antibody (NB100-65657; Novus Biological, USA) was used to detect the presence of Mycobacterium spp. proteins in tissue sections using immunohistochemistry. For the immunohistochemical examination, sections were routinely processed according to the manufacturer's instructions. As secondary antibody, the EXPOSE Mouse and Rabbit Specific HRP/DAB detection IHC Kit ((ab80436), Abcam, UK) was used. For the negative control, the antibody dilution solution was replaced with the primary antibody step. Positive controls were also used to ensure that the primary antibody was working properly.

RESULTS

In total, seven fish were bought, and only one exhibited the gross lesions of the disease. The skin of the all fishes was moist, brilliant and in normal color. The affected fish in this study were tiny and small, but not cachectic compared with the healthy ones. There were no obvious pathological findings at the skin or the fish's external organs, such as eyes, gills and fins, before dissection. During the gross examination of the abdominal cavity and visceral organs, significant enlargement of the liver, kidney, and spleen was observed. In addition, numerous, whitish, granulomatous foci were noticed in these organs (Fig. 1A).

Histopathological examination revealed numerous granulomas in the liver, spleen, and kidneys. The granulomas were spherical

and comprised concentric layers of epithelioid cells that formed a distinct lesion (Fig. 1B). In tissue sections stained with the Ziehl–Neelsen method, acid-fast, rod-shaped bacteria were determined (Fig. 1C). During the immunohistochemical examination, mycobacterium-positive immunoreaction was observed in the cytoplasm of epithelioid cells, giant cells, and macrophages (Fig.1D).

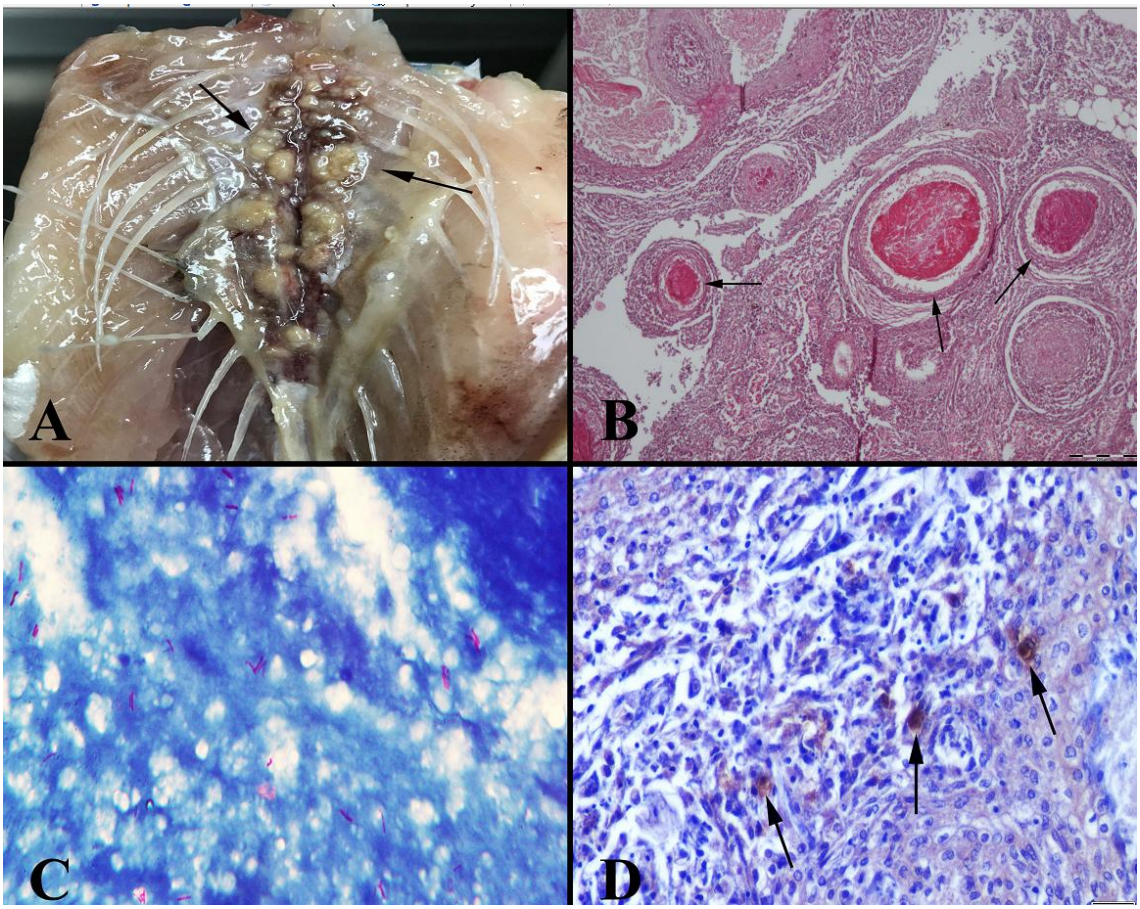

Figure 1. (A) Numerous nodules in the kidney of the fish. (B) Granulomatous foci (arrows) with caseous necrosis, HE, scale bar = 200 µm. (C) Presence of red rod-shaped *Mycobacteria* (arrows) in the tissue section of the kidney, Ziehl–Neelsen staining method, scale bar = 50 µm. (D) *Mycobacterium* spp. immunopositive reaction in epithelioid cells in a granuloma, Streptavidin biotin peroxidase method, scale bar = 20 µm.

DISCUSSION

In this study, mycobacterial infection in an Atlantic mackerel purchased for cooking was described using gross, histopathological, and immunohistochemical findings. At the histopathological examination, granulomatous foci were found in visceral organs. Histopathological examination revealed typical caseous necrosis covered by epithelioid giant cells at the parenchyma of the visceral organs. Most of the granulomas were encapsulated by connective tissue. Similar granuloma morphology was observed in all organs. Acid-fast bacteria were commonly localized inside the granulomas. These findings were in accordance with the findings of previous studies (Gjurčević *et al*., 2020).

Mycobacterial infections have been occasionally observed in the skin and muscle tissues of tropical aquarium fishes. In this disease, agents cause numerous distinctive chronic granulomatous foci. Moreover, skin lesions

resemble those found in other organs (Gjurčević *et al*., 2020). In this study, there were no lesions in skin and the fish was apparently healthy. The gross and microscopic examinations revealed distinct and characteristic findings in the visceral organs. The diagnosis was supported by Ziehl–Neelsen staining, which revealed numerous acid-fast rod-shaped bacteria in the granulomas.

Fish that are severely affected may exhibited darker in color, with cachexia and swelling of the abdomen. At necropsy, miliary caseous nodules can be found in all the visceral organs, but they are most common in the liver, kidney, and spleen. Histopathological findings range from mild to severe lesions, but ZN-positive bacilli are commonly seen in lesions (Ortega *et al*., 2014). Mycobacterial cell wall resists the acid–alcohol decolorization after being stained with carbolfuchsin. For that reason, Ziehl–Neelsen acid-fast stain method commonly used for identification of mycobacteria in tissue sections. In marine or aquarium fishes infected with mycobacteria, typical caseation, giant cell production, and cell-mediated immune response are common (Ojanen *et al*., 2019). In this case, the fish showed no signs of cachexia, skin discoloration, or abdominal swelling. However, typical and characteristic granulomatous foci in the visceral organs were discovered accidentally during the cleaning of fish for cooking.

Mycobacteriosis continues to play an important role in infectious diseases of wild and cultured fishes worldwide. Because several mycobacteria that infect fishes cause infections in humans, the potential for zoonotic infection poses an additional challenge (Gauther, 2015). Mycobacteria originated from fishes may penetrate the human skin via minor lesions of hands (Ang *et al*., 2000). In human, mycobacterial infections may be either painful or painless, but it may be life threatening in severe cases (Wu *et al*., 2002).

In the current study, Mycobacterium spp.-positive immune reaction was observed in the cells of the granulomatous foci. This case demonstrated that mycobacteriosis in fish for cooking may result in zoonotic infection when preparing or eating uncooked fishes.

Mycobacteriosis has been diagnosed in various fish species; however, to the best of the author's knowledge, there has been no previous report on the identification of mycobacteriosis in Atlantic mackerels. Because of its contagious and zoonotic potential, mycobacteriosis may have an impact on hunting fishes and humans according to this report.

Mycobacterial diseases are zoonotic, which means that they can affect humans who come in contact with infected fish or environments. Mycobacterium causes a chronic disease, usually characterized by wasting. It should be suspected when fish were in poor condition or had granulomas in visceral organs. Even the risk of disease transmission to healthy adults not very high, employees in fish industry must be informed and educated about the risk so they can take appropriate precautions. But this study indicated that mycobacteriosis can be diagnosed apparently normal fishes, this results showed that this disease should be taken in to consideration especially fishes consuming raw fish.

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