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# Effect of anisakid nematode larvae on *Carangoides bajad* fish liver from the Red Sea, Jeddah, Saudi Arabia

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**Abstract:** *Carangoides bajad* fish are broadly dispersed in tropical and subtropical seas and are economically important in coastal ecosystems. The impact of parasites on their fish populations is well documented. The present study examined *Carangoides bajad* fish liver and clarified the histological response due to *Anisakis simplex* larval infection. One hundred forty-two samples of *C. bajad* were collected monthly from the al-Shoaiba site at Jeddah coast, Saudi Arabia, for one year (from July to 2020 June 2021). Infected and non-infected fish liver tissues were fixed and prepared for histological investigations. The prevalence of anisakid nematode larval infection was (45,77%) ranging from 1 to 60 per one fish. The present results showed that larvae were isolated within a granuloma, and the inflammatory constituents were concluded within the thickness of the granuloma. This encapsulation of the nematodes protects the rest of the liver to maintain its function and keep the fish's life.
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**Key words:** *Carangoides bajad*, Anisakid, Nematoda, Liver, Hepatic granuloma, Histopathology

## 1. Introduction

*Carangoides bajad* (Forsskal, 1775) is the most common jack on inshore reefs in the Arabian Gulf, Oman, the Indian Ocean from Madagascar and the Comoros Islands to the Red Sea (Randall, 1995). They are widely distributed in tropical and subtropical oceans and play a vital economic role in coastal ecosystems (Lin and Shao 1999).

Scientists are interested in fish parasitology because of its relevance to human health. Furthermore, the likelihood of zoonotic disease transmission through the intake of parasitized fish could pose a public health risk (Sakanari, 1990; Williams and Jones, 1994). By eating raw or undercooked fish, humans can become infected with anisakid nematodes. Anisakid nematodes can be found in the fish's body cavity, internal organs, swim bladder, deeper layers of the skin or fins, and exterior muscle layers (Smith & Wooten, 1978; Mattiucci *et al*., 2008). In Saudi waters, there has been very little research on parasites in commercial fish.

The liver of a fish is described as a multipurpose organ that helps with detoxification, vitellogenin production, and carbohydrate and fat deposition and metabolism (Bruslé and Anadon, 1996). The liver of most teleost fish is separated into lobes that are reddish-brown in color and are positioned cranially and ventrally in the body cavity (Bruslé and Anadon, 1996).

The third stage of the nematode anisakid larvae, which is encased in the organs of marine and freshwater fish (Abollo *et al*.,2001; Dezfuli *et al*.,2007; Skov *et al*., 2009; Mehrdana *et al*., 2014; Horbowy *et al*., 2016; Dezfuli *et al*., 2009; Dezfuli *et al*., 2015; Fujimoto *et al*.,2013; Emde *et al*., 2014). As a result of the host tissues' response, they are encapsulated in the colon and visceral organs. Invertebrates, the primary immune response mechanism against helminths is poorly understood (Dezfuli *et al*., 2009; Hoffmann *et al*., 1986: Dezfuli *et al*.,2015; Brinker, Hamers, 2007; Dezfuli *et al*., 2013; Haarder *et al*., 2013 ). As a result of infection, many cells get activated and work together to control and combat the foreign pathogen (Makepeace *et al*., 2012). Mast cells (MCs) ( Dezfuli *et al*., 2013; . Reite and Evensen ,2006; Buchmann ,2014; Dezfuli *et al*., 2016), macrophage aggregates (MAs) or melano-macrophage centers (Dezfuli *et al*.,2015; Dezfuli *et al*., 2016 ; Havixbeck and Barreda,2015), neutrophils (Havixbeck and Barreda ,2015; Havixbeck *et al* 2016 ), and rodlet cells ( Dezfuli *et al*.,2016 ; Manera and Dezfuli 2004) ) are all part of the innate defense response to helminths in fish (RCs). Histopathology can be utilized to determine the influence of parasitism on one's health (Feist and Longshaw, 2008).

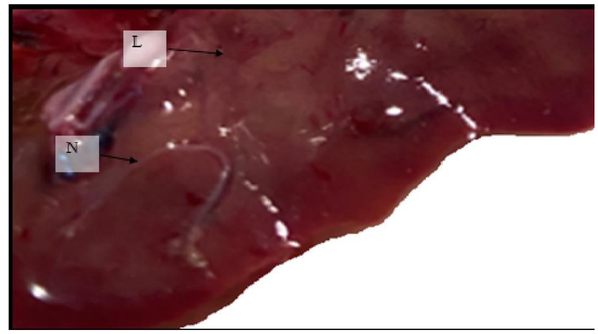
The present study aimed to examine *Carangoides bajad* fish liver and clarify the histological response due to *Anisakis simplex* larval infection.

## 2. Materials and Methods

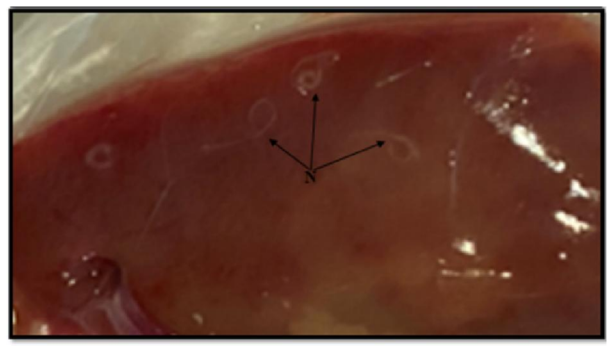
One hundred forty-two fish samples of *Carangoides bajad* were collected monthly from the Al-Shuaiba site at the Jeddah coast for a year between 2020 to 2021. The liver was removed and examined under a stereomicroscope for the presence of parasites. Infected as well as non-infected pieces fixed in 10% neutral buffered formalin, dehydrated in ethanol series, cleared, and embedded in paraffin, Sectioned 4μm thick and stained by Haematoxylin and Eosin (HE), periodic acid Schiff (PAS) and alcian blue (AB), examined and photomicrographed under a light microscope. Examination of stained sections was done by using the Olympus DP72 microscope in King Fahd Medicinal Research Center.

## 3. Results

After the morphological study, the nematodes infecting fish liver were identified as belonging to the Anisakis simplex genus (fig.1&2). The bulk of the fish's viscera had nematode larvae, with the liver being the most polluted. Larvae were visible on the surface of the liver with the naked eye after dissection (fig.1), with an infection prevalence of (45,77 percent) ranging from 1 to 60 per fish. On histological inspection, the normal liver was covered by a bit of capsule (fig.3A). The liver tissue has hepatocyte plates surrounded by sinusoids (fig.3B). Hepatocytes were polyhedral, with a spherical nucleus in the center, a prominent central nucleolus, and eosinophilic cytoplasm (fig.3B). The infected liver shows cytoplasmic vacuolation, nuclear hypertrophy, and polygonal hepatocytes with one or two central nuclei (fig.4F). The localized zone of lymphocytic aggregation is caused by dilated blood vessels (fig.4A), necrosis in the nucleus of some liver cells, and karyolysis of the nucleus, as well as dilatation and sinusoidal congestion. The larvae were surrounded by the localized host tissue reaction, which resulted in cercal granulomas (fig.4B&C). In most granulomas, especially those with larger larvae, exfoliation of the inner layer of epithelioid cells resulted in the formation of amorphous necrotic tissue. PAS-positive necrotic accumulation with a moderately to severely basophilic phenotype (fig.5). To expand the cysts, a slightly eosinophilic, Alcian Blue positive fluid was employed (fig. 4B & C). Granulomas have three layers: an outside layer of fibrous connective tissue and thin elongated fibroblasts (fig.4D); a middle layer of MCs imprisoned in a light fibroblastic-connective mesh, which is frequently spongiotic in appearance; and an interior layer of densely packed epithelioid cells (fig.4D). Between granulomas, the thickness of each layer varied greatly. Melano-macrophage centers (MMC) were discovered along blood arteries and around larval cysts and were distinguished by brown pigmentation of cells (melanization) (fig.4E&F).




A




B

Figure (1) A&B Liver of *C.bajad* infected with larva of *Anisakis simplex* (N); Liver (L).

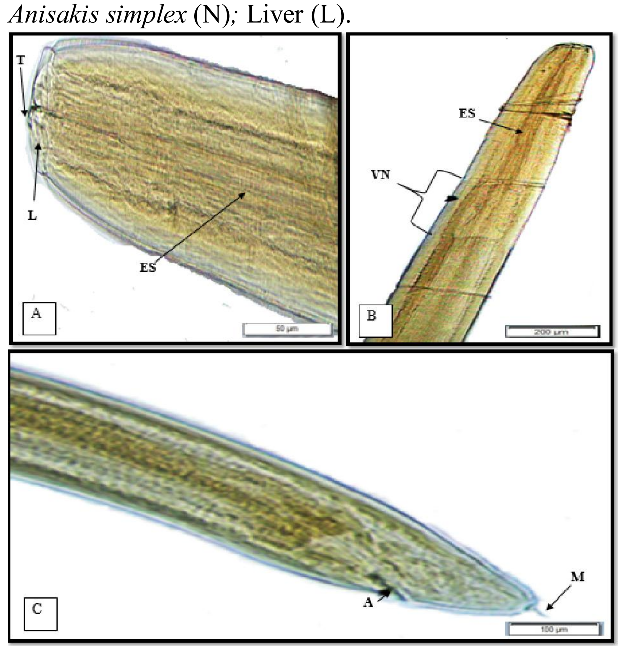
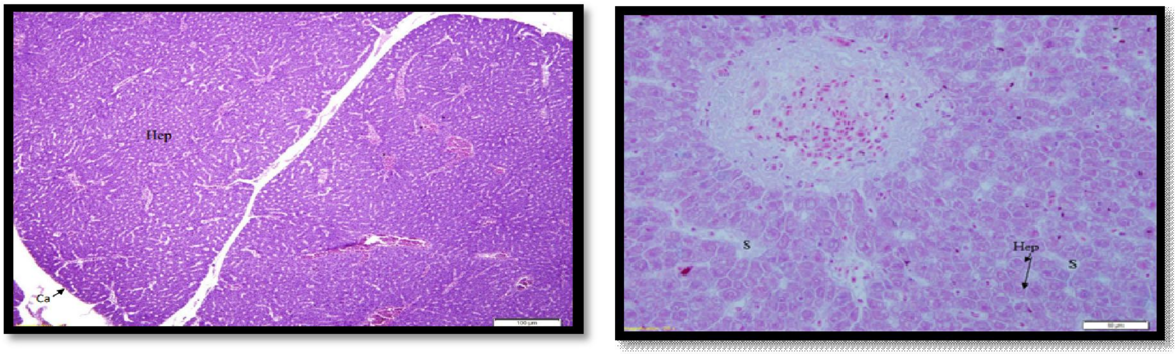



Figure (2) Third stage larva of male of *Anisakis simplex in C. bajad* A&B: The anterior end showing tooth (T) , lips (L) and esophagus (ES) ,Ventricular region(VN); C: The posterior end showing mucron (M) ,anus (A).(A:20x,50µm ,B:4x,200µm ;C:10x,100 µm).




**A** **B**

Figure (3) A: Histological sections of normal liver *C.bajad* stained with hematoxylin-eosin showing Capsule (Ca); hepatocytes (Hep) (10x, 100µm); B: Histological sections of normal liver *C.bajad* stained with periodic acid Schiff (PAS) showing hepatocytes (Hep) spherical nucleus with a clear, dark nucleolus; hepatic sinusoids (S)(20x,50µm).

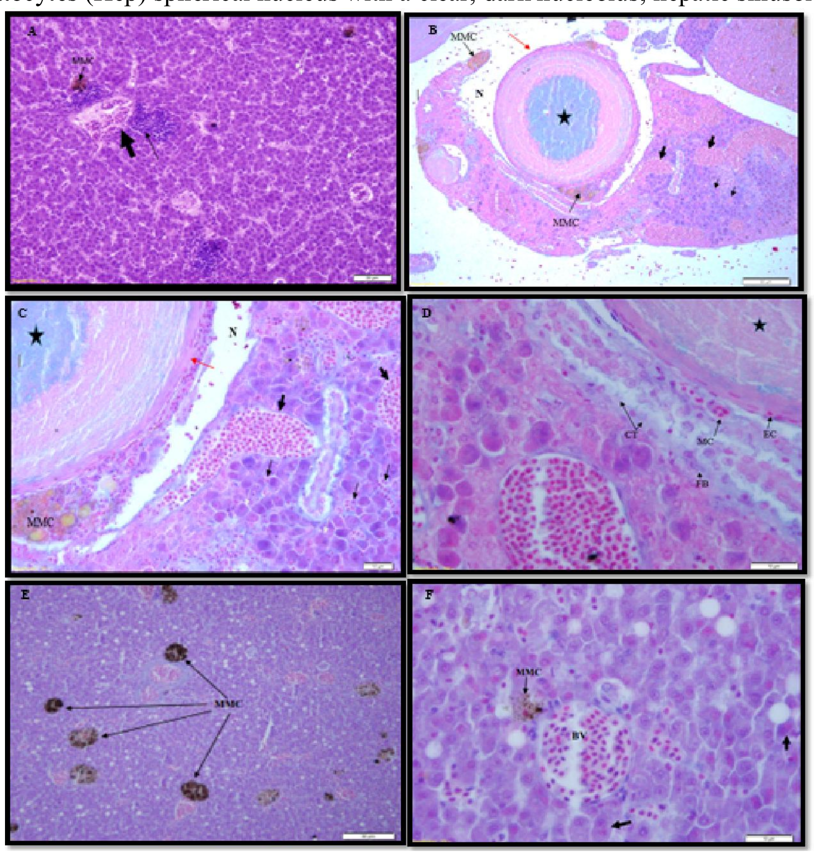



Figure (4) Histological sections of infected liver *C.bajad* A) showing the focal area of lymphocytic aggregation (thin arrow) migrate from dilated blood vessels (thick arrow)and hepatic cytoplasmic cacuolation and nuclear hypertrophy (White Arrow) melanomacrophage centers (MMC), Stained with hematoxylin-eosin (H&E) (40x,20µm). B&C) showing focal area of lymphocytic aggregation and degeneration of blood vessels (thin arrow) migrate from dilated blood vessels (thick arrow) and hepatic cytoplasmic cacuolation and nuclear hypertrophy (White Arrow); Inside the cyst (red arrows), the parasite is immersed in an alcian blue positive fluid (asterisk), necrosis (N) melanomacrophage centers (MMC), stained with Alcian Blue (AB) B(20x,50µm) C (60x,10µm). D) showing three layers that formed the granuloma; inner layer formed by epithelioid cells (EC), mast cells (MC) of the middle layer, fibroblasts (FB), and connective fibres (CF) of the outer layers. b. The epithelioid cells (EC) surrounding the nematode larva (asterisk) were stained with Alcian Blue (AB) (60x,10µm). E&F) showing melanomacrophage centers (MMC) and hepatocytes polygonal in shape with one or two central nuclei (arrows) blood vessels (BV) stained with Alcian Blue (AB)(60x,10µm).

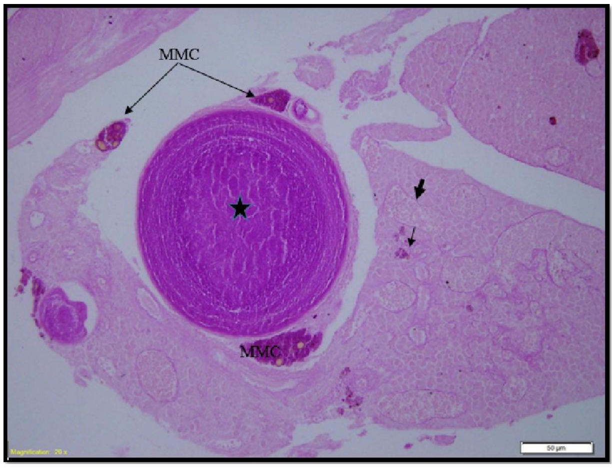



Figure (5) Histological sections of infected liver *C.bajad* stained with PAS showing the larva (asterisk) surrounded with granulomatous infiltrates which was positive for PAS reaction and focal area of lymphocytic aggregation and degeneration of blood vessels (thin arrow) migrate from dilated blood vessels (thick arrow); melanomacrophage centres (MMC)(50µm).

**4. Discussion**

The anisakid nematode's third larval stage infiltrated all *C.bajad's* internal organs. The high severity of nematode infection in the liver, on the other hand, motivated us to focus on this organ to precisely evaluate hepatic tissue damage. Mechanical injury (Abdelmonem *et al*., 2010; Santoro *et al*., 2013 ), tissue atrophy, host fecundity loss (Chavez and Oliva,2011), and liver pathogenicity (Dezfuli *et al*.,2007 ) (*Dezfuli et al*.,2009) (Ventura *et al*., 2016) are all effects of nematode parasites on their hosts. Mast cells (MCs) in all vertebrates have a similar shape and function (Mulero *et al*., 2007). MCs are mobile ( Reite and Evensen, 2006; Dezfuli and Giari, 2008) and are typically found near blood vessels to control inflammation, identify invading pathogens, and orchestrate a response ( Dezfuli *et al*., 2016; Galindo-Villegas *et al*.,2016; Dezfuli and Giari, 2008; John and Abraham, 2013; Dezfuli *et al*., 2015; Reber *et al.,*2015). The anisakid nematode's third larval stage infiltrated all *C. bajad's* internal organs. The high severity of nematode pathogens that cause mast cells to degranulate in response to a range of infections (Dezfuli *et al*., 2015; Ellis,2001) are known as degranulating agents (Galindo-Villegas *et al*.,2016; Manera *et al*., 2011). Degranulation of fish MCs around the helminth tegument in the colon and other organs were described by Dezfuli *et al*.2016; Dezfuli *et al*., 2015; Dezfuli *et al*., 2011). Multiple granulomas in the current study showed degranulation of MCs along the cuticle of Anisakid nematodes larvae. MCs are influential in the inflammatory response (Brugman, 2016). Due to helminth infection, their numbers increase in fish with parasitized livers (Dezfuli *et al*., 2009; Dezfuli *et al*.,2015; Dezfuli *et al*., 2014). The fibrotic and tissue remodeling processes are aided by MCs (Dezfuli *et al*., 2015; Dezfuli *et al.,* 2008 ;Rocha and Chiarini-Garcia, 2007 ). Anisakid nematodes larvae in the C.bajad liver preferentially attract MCs, fibroblasts, and other immune cells (such as macrophages and epithelioid cells) to infection sites, according to the current findings. Following severe tissue injury produced by nematode larvae in their study, Dezfuli *et al*. (2016) believe that MCs' link with fibroblasts and macrophages may promote liver remodeling/repair.

Phagocytosis is a natural defense mechanism in which phagocytes participate in pro-and anti-inflammatory responses in infected areas (Ellis,2001; Rieger *et a*l.,2012; Grayfer *et al*., 2014). Granulocytes (particularly neutrophils) and mononuclear phagocytes (circulating monocytes and tissue macrophages) have both been recognized as important professional phagocyte types in fish (Secombes and Ellis, 2012; Esteban *et al*., 2015). Neutrophils are important players in the inflammatory response, particularly during the early stages of a pathogen challenge, when they move from the bloodstream to a site of injury or parasite infection (Dezfuli *et al*.,2013; Havixbeck and Barreda, 2015; Havixbeck et al., 2016; Secombes and Ellis, 2012; Dezfuli *et al*., 2011). In infected *C. bajad* livers, neutrophils were found in the parenchyma, hepatic sinusoid lumen, and/or interstitium around capillaries in various studies. These studies comfort with the study of Dezfuli *et al*. (2016). Fish macrophages (MAs) are called macrophage aggregates (MAs) or melano-macrophage centers (Agius and Roberts,2003; Ferguson, 2006) because they contain pigments such melanin, chromolipoids, and hemosiderin (Secombes and Ellis, 2012; Wolke, 1992). MAs encapsulate many foreign chemicals and parasites in tissue (Dezfuli *et al*., 2013; Ferguson,2006; Gregori *et al*., 2014). MAs functions have been identified as targeted destruction, detoxification, and recycling of endogenous and exogenous materials. As a result, MAs are implicated in the helminth infection battle (Dezfuli *et al*.,2013; Dezfuli *et al*.,2015; Agius and Roberts,2003; Wolke, 1992). MAs were discovered in the sick liver of *C.bajad*, particularly near the larvae and blood vessels. The inner layer of the capsule that protects Anisakid nematodes larvae is made up of epithelioid cells. These cells resemble epithelial cells that emerge in response to prolonged inflammatory activation in terms of morphology (Noga *et al*.,1989; Gauthier *et al*., 2004). The formation of fish epithelioid cells from macrophages has been demonstrated in vitro (Secombes, 1996).

**Conclusions**

This study aimed to investigate the liver of *Carangoides bajad* fish and determine the histological response to anisakid larval infection. After dissection, larvae were visible on the surface of the liver with the naked eye, with infection prevalence ranging from 1 to 60 per fish. These parasites do not appear to be a severe public health hazard among the population of Jeddah city at the moment, likely because local dietary habits do not favor raw fish consumption. On the other hand, many foreign people have a tendency to eat undercooked fish and may become infected with anisakid nematodes.

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