

Occurrence and histopathological effect of *Ligula intestinalis* on Sea bream (*Abramis brama Orientalis*)

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Received: March 2016

Accepted: May 2016

Abstract

Ligula intestinalis originally belongs to Cestoda parasite that infests a range of fish family especially Cyprinidae. It is also a zoonosis parasite that affects fish and probably human health. This study tried to isolate *L. intestinalis* from Sea bream (*Abramis brama Orientalis*), an species classified in family, Cyprinidae, from Caspian Sea. Sixty Sea bream were caught from Caspian Sea, Babolsar Fisheries port. Among that, 26.66% were infected with *L. intestinalis*. The tissues of infected fishes including gonads, liver and kidney were examined for pathological findings. Ovaries changes showed fibrosis, infiltration of inflammatory cells, atrophy of oocytes; testicular hyperemia, necrosis, fibrosis and degeneration; kidneys necrosis, destruction of tubules, vacuolar degeneration; and liver vacuolar degeneration, biliary duct hyperplasia, necrosis, lymphatic vessel dilatation, fibrosis

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and cholestasis. Considering the effect of *L. intestinalis* on fish and human health, the prevention programs to disturb the cycle of parasite and more monitoring studies in fish epidemiology especially food fish are recommended.

Keywords: *Ligula intestinalis*, *Abramis brama*, Histopathology

Introduction

L. intestinalis Linnaeus, 1758 is a cestoda from family Pseudophilidae known to be as three-host parasite, infests a range of fish species and found in the forms of free living and farmed fishes all over the world (Geraldine, Jean-Luc & Sovan 2002; Scholz, Kuchta, Shinn, Snabel & Hanzelova, 2006). *Ligula* has a life cycle containing copepods as first, fish as second and piscivorous birds as the final hosts. Parasite eggs are released via bird feces into the water and develop to ciliated coracidium larva. It survives 1-2 days in the water and develops to proceroid larva in copepod. Fish eats the copepod and subsequently, large plerocercoid larva develops

in the body cavity of fish (Innal, Keskin & Erk'akan 2007) after eating and digesting by fish. Ligulosis is a food-borne zoonosis and there are several reports of human infestations with this parasite (Urdeş & Hangan 2013). Sea bream is a species of fish from family Cyprinidae that live in ponds, lakes, canals, slow-flowing rivers and brackish sea waters with muddy bottoms and plenty of algae. It is a food source for human health and has a special ecological and economical importance. Teleost fish especially Cyprinidae are the second intermediate host of *L. intestinalis* and is the most affected because the parasites occupies the body cavity of the fish for several years and is responsible for pathologic effects. Infection occurred by *L. intestinalis* causes an inflammatory response (Taylor & Hoole 1995). Severe pathological changes of *L. intestinalis* result from compression for replacement of the organs by the parasites that disturb normal function and histological structures (Abowei & Ezekie 2011). This article tries to show the effect *L. intestinalis* on occurrence of the histopathological findings in Sea Bream.

Materials and Methods

Fish Sampling and Parasite Identification

The station of this study was located in southern east of the Caspian Sea, Babolsar. Sixty Sea bream with average weight of 324 ± 79.85 g and total length of 29.4 ± 3.58 cm were prepared by

seine net (Table 1). After biometry, the fish samples were necropsied and the body cavities examined for the presence of plerocercoids of *L. intestinalis*. The parasite samples were then fixed with 70% ethanol and identified using biological keys (Bykhovskaya 1964; Gusseve 1985). Length and weight of each parasite isolated from the infected fish was measured (Table 2). The number of infected fish was recorded; the prevalence of parasites and the mean intensity of infection were calculated (Table 2) according the following formula (Bush, Lafferty, Lotz & Shostak 1997)

Prevalence of parasites (%) = the number of infected fish/ the total number of examined fish $\times 100$

Mean intensity of infection = the number of counted parasites/ the total infected fish

Pathological Examination

The tissues of infected fishes including gonads (ovary and testis), liver and kidney were dissected and fixed in 10% formalin buffer and transferred to the laboratory. The tissue samples were prepared in tissue processor (DS2000/H, Iran). Serial sections of formalin-fixed, paraffin-embedded of tissue samples were cut using rotary microtome (Leitz, 1512, Germany) at 5 μ m, then stained by routine hematoxylin and eosin (H&E) and examined by light microscopy for pathological findings (Bancroft & Gamble 2007).

Results

Parasite infection

The result of this study showed that 26.66% of total studied fish were infected to *L. intestinalis* (fig.1), of which 81.25% of them were reported as male and 18.75% female. The number of non-

infected and infected fishes, weight and length of plerocercoid of *L. intestinalis* and the prevalence and mean intensity of infection were listed in Tables 1 and 2.

Table 1 Examined fish, non-infected fish, weight and Length of fishes in present study

Examined fish (n=60)		Non infected fish		weight of fish (gr) Mean±SD	Length of fish (cm) Mean±SD
female	male	female	male		
15	45	12	32	324±79.85	29.4±3.58

Table 2 Infected fish, Weight and Length of plerocercoid of *L. intestinalis*, prevalence and mean intensity of infection in present study

Infected fish		Weight (gr) of parasite Mean±SD	Length (cm) of parasite Mean±SD	Prevalence of parasite	Mean intensity of infection
female	male				
3	13	132.25±54.91	60.03±24.68	26.66%	1.75



Figure 1 The *L. intestinalis* parasite isolated from *A. brama*

Pathological finding

The pathologic finding in ovaries showed fibrosis, infiltration of inflammatory cells, atrophy and destruction of oocytes (Fig.2).In testicular tissues, hyperemia, necrosis, fibrosis

and degeneration were observed(Fig.3).In kidneys necrosis, destruction of tubules, vacuolar degeneration, loss of coordination of glomeruli, fibrosis, and infiltration of

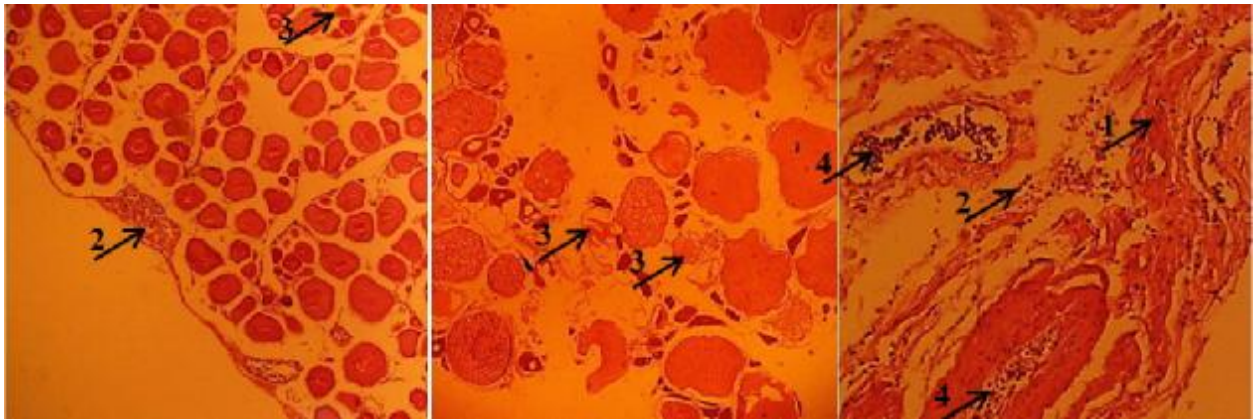


Figure 2 Ovary 1: fibrosis, 2: infiltration of inflammatory cells, 3: atrophy and destruction of oocytes and 4: inflammatory cells in blood vessel, H&E (10, 40, 40X, respectively from the left side).

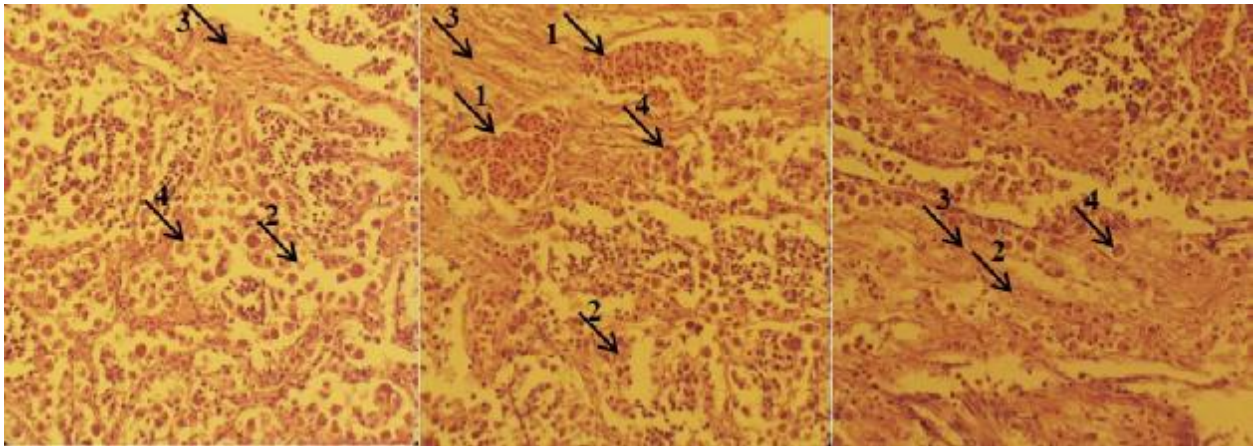


Figure 3 Testicular tissue 1: Hyperemia, 2: Necrosis, 3: Fibrosis and 4: Vacuolar degeneration, H&E, (40, 40,40X, respectively from the left side).

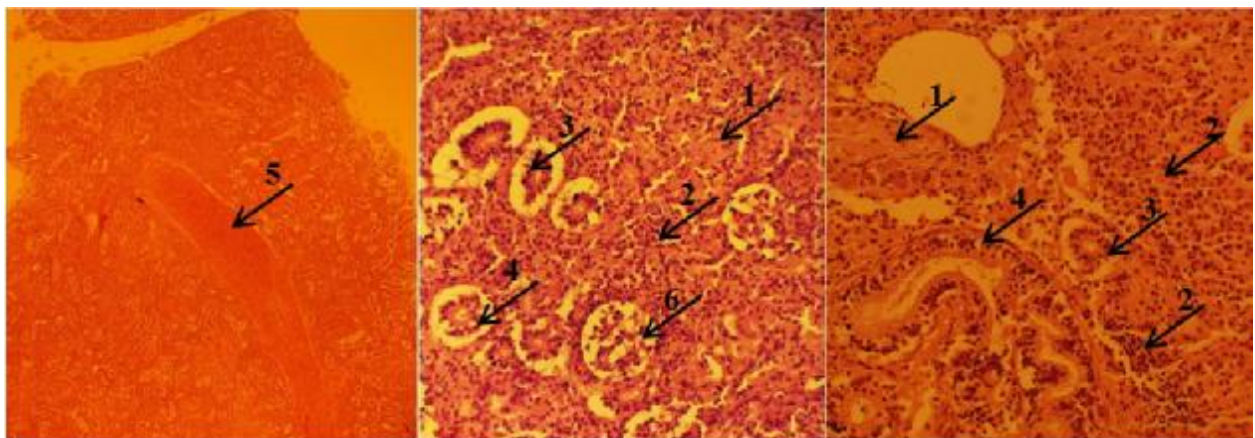


Figure 4 Kidney 1: fibrosis, 2: infiltration of inflammatory cells, 3: necrosis, 4: vacuolar degeneration, 5: hyperemia and 6: loss of coordination of glomeruli, H&E (4, 10, 40X, respectively from the left side).

inflammatory cells (lymphocyte, plasma cell and macrophage) were seen (Fig.4). In liver pathologic finding showed vacuolar degeneration, infiltration of inflammatory cells,

hyperemia, biliary duct hyperplasia, necrosis, cirrhosis, lymphatic vessel dilatation, fibrosis and cholestasis (Fig.5).

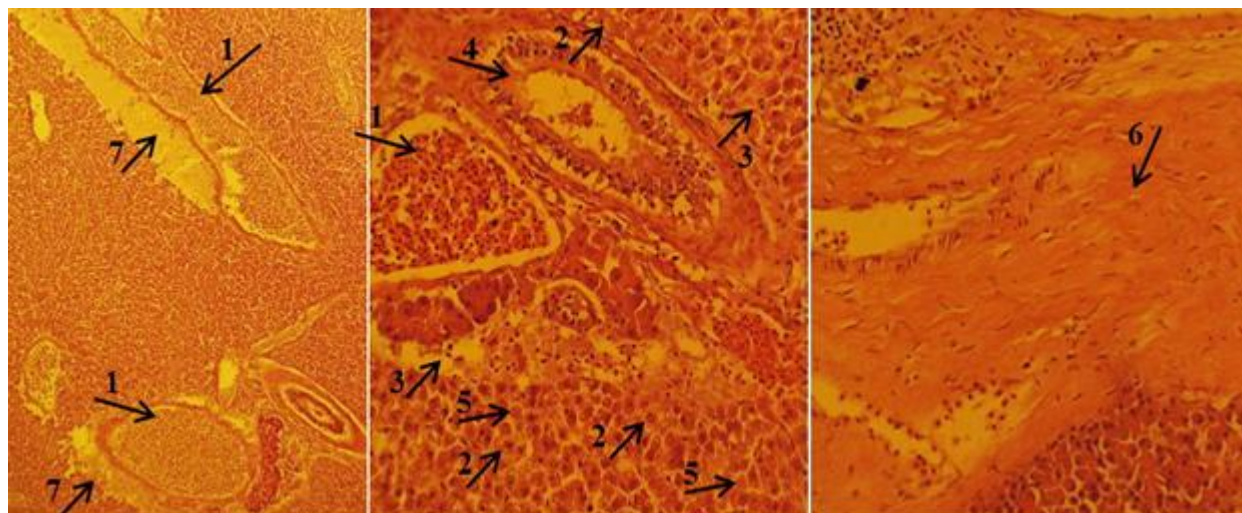


Figure 5 Liver 1: hyperemia, 2: vacuolar degeneration, 3: infiltration of inflammatory cells, 4: biliary duct hyperplasia, 5: necrosis, 6: cirrhosis, 7: exudation. H&E (10, 40, 40 X, respectively, from the left).

Discussion

Fish infected to *L. intestinalis* has been reported in some studies in Iran. Considering the effect of *L. intestinalis* on fish and human health, continuous study in different fishes especially in food fish is required. Ahmadiara, Hosseini, Jalousian, Ebrahimzadeh Mousavi, Sakhaifar & Gerami Sadeghian (2013) reported 16.02% *L. intestinalis* infection in *Alburnoides bipunctatus* and *Abramis brama* from north and northwest of Iran. Pazouki, Masoumian, Yahyazadeh & Abbasi (2007) reported *L. intestinalis* from Sea Bream of Aras dam with prevalence rate of 38.46%. Jalali and barzegar (2006) reported *L. intestinalis* in *Chalcalburnus sp.* from Zarivar

Lake with prevalence of 43%. In this study 27% of a total 60 Sea Bream from Caspian Sea were infected to *L. intestinalis*.

Fishes are the second host that affected with the most pathogenic lesions of *L. intestinalis*, because it invades the body cavity of the fish and can stay there for several years (Barber & Wright, 2005). The rapid growth of the *L. intestinalis* in the fish's body cavity shows the first pathological effect, because of swelling of the abdominal region (Taylor & Hoole 1995; Geraudie, Boulange, Gerbron, Hinfray, Brion & Minier 2009). The parasite can contain up to 10% of the host weight, press on the visceral

organs and the abdominal wall results in loss of fish growth, particularly in juveniles that reduce the ability of ligulosed fish to escape predation (Abowei & Ezekie 2011). Parasite made pressure to abdominal cavity caused peritonitis and chronic granulomatous hepatitis. Pathologic lesions including tubular fibrosis and necrosis were observed near the hematopoietic tissue or kidney portal. In present study, kidneys showed necrosis, destruction of tubules, vacuolar degeneration, loss of coordination of glomeruli, fibrosis, and infiltration of inflammatory cells and in liver vacuolar degeneration, infiltration of inflammatory cells, hyperemia, biliary duct hyperplasia, necrosis, cirrhosis, lymphatic vessel dilatation, fibrosis and cholestasis. Rahmati holasoo, Hajimohammadi, Ahmadiara, Ebrahimzadeh Mousavi, Rostami-bashman, Haghighi khiabani asl, Sohrabi Haghdoost, Shokrpour & Ghorbanalipour (2011) reported cholangiohepatitis and metaplastic hyperplasia in liver, deformity of structure and function of hepatocytes, thrombosis in liver, decrements in intestine and pancreas as the result of compressive stimulations of *L. intestinalis* in *Alburnoides bipunctatus*. Ligula infections are also associated with reduction the muscle mass of fish with atrophy the mass of the body wall musculature (Barber & Wright 2005). Infection by *L. intestinalis* induces pathological lesion such as inflammatory response, atrophy of the viscera specifically, impaired reproduction (Geraudie *et al.* 2009).

Ligula plerocercoids can inhibit gonadal development of fish hosts' population (Ergonul & Altindag 2005). Inhibition of gonadal maturation result to incompletely immature reproductive tissues (Hecker & Karbe 2005). *L. intestinalis* restricts gonadotropin-releasing hormone secretion by interfering with the pituitary-gonadal axis at the level of the host's hypothalamus as result of pressure (Martin-Skilton, Lavado, Thibaut, Minier & Porte 2006). Endocrine disrupting compounds of parasite have been reported to be responsible for impaired gonadal growth, reduced steroid synthesis and inhibition of aromatase activity (Jobling, Beresford, Nolan, Rodgers-Gray, Brighty, Sumpter & Tyler 2002). Ligulosis prevented gametogenesis and result in noticeable hypopituitarism, poor gonad development, inhibition of gametogenesis of the reproductive cells and impaired spawning behavior (Barber & Wright 2005). In this study, the ovaries showed fibrosis, infiltration of inflammatory cells, atrophy and destruction of oocytes and in testicular tissues hyperemia, necrosis, fibrosis and degeneration were observed. Parsa Khanghah, Mojazi Amiri, Sharifpour, Jalali Jafari & Motalebi (2011) reported significant difference between the means of male and female gonads maturation rate in infected and non-infected *Chalcalburnus mossulensis*. They mentioned infection by *L. intestinalis* can be the reason for lack of gonads maturation, abnormal degenerative changes like, absorption follicle, hemorrhage and infiltration of inflammation

cells in ovary tissues of infected fish and in testicle tissue, dispersed hemorrhage, atrophy and melano-macrophage center were reported. Carter, Pierce, Dufour, Arme & Hoole (2005) indicated that the pituitary glands of roach *Rutilus rutilus* infected to *L. intestinalis* contained approximately 50% less luteinizing hormone than non-infected fishes that indicates a significant and measurable effect of parasitism on the pituitary gland. Fibrosis, failure in ovulation, inflammation and presence of melanomacrophages along with infiltration of lymphocytes into parenchyma of ovary indicate atrophy of ovary that is due to exertion of pressure on the tissue and immaturity of ovules (Rahmati-holasoo et al. 2011). The pathological effects mostly involve the inhibition of gonadal development in the fish and may play a role in the regulation of their fish hosts' population (Barber & Wright 2005).

Considering the results of this study and the other researches on *Ligula* and because of this parasite infects a range of fish species including fresh water, brackish water, free living and farmed fishes all over the world and also it is a zoonotic parasite, more monitoring studies on fishes are needed and the prevention programs to disturb the cycle of infection.

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بررسی وقوع و اثرات آسیب شناسی لیگولا اینستینالیس (*Ligula intestinalis*)
Abramis brama orientalis Berg, 1949 در ماهی سیم (Linnaeus, 1758)

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چکیده

لیگولا اینستینالیس *Ligula intestinalis* یک انگل سستد است که طیف وسیعی از ماهی‌ها به ویژه خانواده کپورماهیان را درگیر می‌کند. همچنین یک انگل زئونوز است که می‌تواند سلامت انسانها را نیز تحت تاثیر قرار دهد. این مطالعه سعی داشته است انگل لیگولا اینستینالیس را در ماهی سیم دریایی *Abramis brama* که یکی از ماهی‌های خانواده کپورماهیان است مورد بررسی قرار دهد. تعداد 60 قطعه ماهی سیم از دریای خزر، بابل، صید شد و 26/66% ماهی‌ها آلوده به لیگولا اینستینالیس بودند. بافتهای ماهیهای آلوده شامل گنادها، کبد و کلیه مورد بررسی آسیب شناسی قرار گرفتند. در بافت تخمدان فیبروز، نفوذ سلولهای آماسی و آتروفی اووسیتها، در بافت بیضه پرخونی، نکروز، فیبروز و دژنراسیون، در بافت کلیه نکروز، تخریب توبولها و دژنراسیون واکوئولی و در بافت کبد هیپرپلازی مجاری صفراوی، نکروز، دژنراسیون واکوئولی، اتساع عروق لنفاوی و فیبروز مشاهده شد. با توجه به تاثیر لیگولا اینستینالیس در سلامتی ماهی و انسان، برنامه‌های پیشگیری برای تخریب چرخه زندگی این انگل و تحقیقات برای پایش آلودگی به این انگل به ویژه در ماهی‌های خوراکی توصیه می‌شود.

کلمات کلیدی: لیگولا اینستینالیس، ماهی سیم، آسیب شناسی،

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