HVM BIOFLUX
Human & Veterinary Medicine
International Journal of the Bioflux Society

A study of infestation of *Alburnoides bipunctatus* with *Ligula intestinalis* in Latian reservoir Dam Lake, Tehran province, Iran: A histopathological study

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Abstract. Objective: The objective of this study was to report infestation of the fish with *Ligula intestinalis* as a food borne zoonoses and to study its histopathological effects on one *Alburnoides bipunctatus* in Latian reservoir Dam Lake, Tehran province, Iran. Material and methods: In July 2010, 6 fish (*Alburnoides bipunctatus*) from Latian reservoir Dam Lake, Tehran province, Iran were referred to Laboratory. After autopsy, two of them (Mature and Female) were found infested with plerocercoid of *Ligula intestinalis* based on taxonomical features. For histopathological studies samples were stained with Haematoxylin and Eosin (H&E) and observed under light microscopy. Results: Pathological findings showed inflammation of the reservoir adipose tissue (in the loose conjunctive tissue between lipocytes) surrounding intestine. Severe atrophy in follicular cells of ovary was evident. In liver cholangiohepastitis with metaplastic hyperplasia was seen. Conclusion: Considering the importance of *Ligula intestinalis* as a food borne parasite, performance of other comprehensive studies is a necessity.
Key Words: *Ligula intestinalis, Alburnoides bipunctatus,* histopathological effects, food borne zoonoses.

Introduction. Fish meat is a main source of food that has high quality protein for human beings and provides approximately 16% of animal protein of people diet in all over the world (Tidwell & Geoff 2001; FAO 1997). *Alburnus* and *Alburnoides* belong to Cyprinidae family and are inhabitants of Europe and northern part of south-west Asia. Till now, 8 species of them are recorded in Iran (Froese & Pauly 2008). *Alburnoides bipunctatus* is inhabitant of fresh waters of fast running rivers and feed on planktons and insects (Vossoughi & Mostajeer 2004). The area required for spawning of the fish is extremely limited (Mann 1996) and very sensitive to mankind activities, hence, the number of suitable nests for spawning and reproduction of the fish are remarkably limited (Penaz & Jurajda 1993).

Parasitic diseases are the most common infections of fishes that inflict heavy economic loss to meat sources and meat processing industry. Some of them infect human beings through eating of fish meat. *Ligula intestinalis* Linnaeus, 1758 is a common Pseudophilidae cestoda with a three-host complex life cycle, that infests free living and farmed fishes all over the world (Geraldine et al 2002; Scholz et al 2006). Procercoid, a first step in the life cycle, develops in copepods and gain potential to infest fish that is second host of the parasite. The procercoid develops to plerocercoid in abdominal cavity of fish that bulges out the abdomen; hence, the infested fish is easily

preyed. The plerocercoid is matured in intestine of the third host, birds, and laid eggs are released and hatched into the water and again are ingested by copepods (Innal et al 2007). There are few reports on infestation of humans with the parasite. So, *Ligula intestinalis* may be act as a food borne parasite (Eslami 2006).

Alburnoides bipunctatus could be found in suburban areas of Tehran metropolitan city in Haraz, Karaj and Jajrood rivers and aqueducts of southern Tehran, Iran (Vossoughi & Mostajeer 2004). Latian reservoir Dam Lake is constructed in 1967 on Jajrood River 35 km northeast from Tehran (Kashefi-Asl & Zaeem 2009). This water basin with 71000 acres area is located in north area of Tehran in a mountainous area (Ravanbakhsh et al 2010). *Alburnoides bipunctatus* is also found in Latian Dam Lake. The objective of this study was to report infestation of the fish with *Ligula intestinalis* and to study its histopathological effects in an *Alburnoides bipunctatus* in Latian reservoir Dam Lake, Tehran province, Iran.

Material and Methods. In July 2010, 6 fish from Latian reservoir Dam Lake, Tehran province, Iran were transferred to the Laboratory of Department of Aquatic Animal Health of Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. Following primary observations and morphological studies based on key features like presence of a black lateral line, horizontal stomal cleft and two-row pharyngeal teeth, the species of the fish was identified as *Alburnoides bipunctatus* Bloch 1782 (Vossoughi & Mostajeer 2004). The fish were examined on the surface of the body and then were autopsied. Two mature female specimens were infested with plerocercoid of the parasite that were measured in length and studied under invert light microscope. For microscopic studies the plerocerciods were immediately fixated in 70% alcohol, stained with acetocarmine and mounted on glass slide. Identification of the observed parasites was done based on taxonomical features (Chubb et al 1987). Following autopsy, for histopathological studies samples from liver, ovary, intestine and intestine surrounding fat were taken and fixated in 10% formalin. Six µm semithin sections were prepared and stained with Haematoxylin and Eosin (H&E) and observed under light microscopy.

Results. The observed parasite was identified as *Ligula intestinalis* cestoda based on taxonomical features. Pseudobands were evident in the middle of ventrum with a clear groove. Figure 1 shows two separated parasites compared with the host.

Figure 1. An *Alburnoides bipunctatus with two* plerocercoids of *Ligula intestinalis.*

Pathological findings showed infiltration of inflammatory polymorphonucleic cells and inflammation of the reservoir adipose tissue (in the loose conjunctive tissue between lipocytes) surrounding intestine (Figure 2).

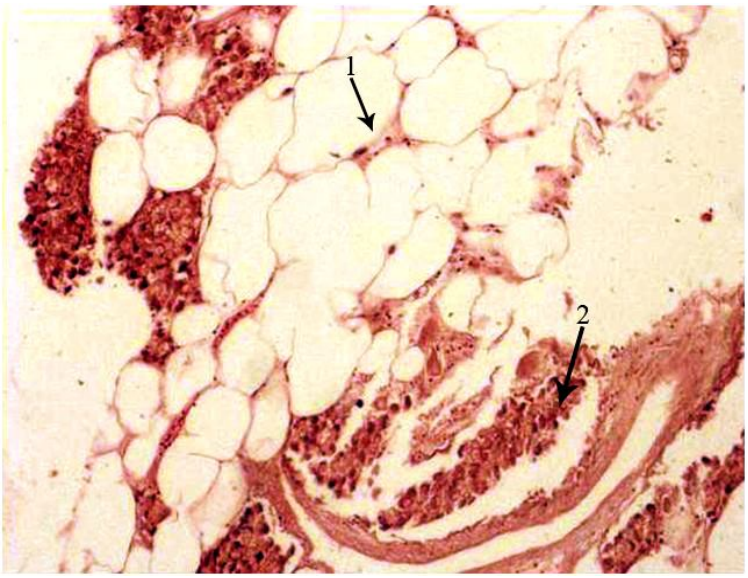

Figure 2. Infiltration of inflammatory polymorphoneucleic cells (1), pancreas (2) (H&E ×100).

Severe atrophy in follicular cells of ovary and degenerative changes along with lymphocytes and erythrocytes in albuginea membrane were evident in ovary. Immature follicules in ovary with melanomacrophages, fibrosis and inflammation in parenchyma were present (Figure 3).

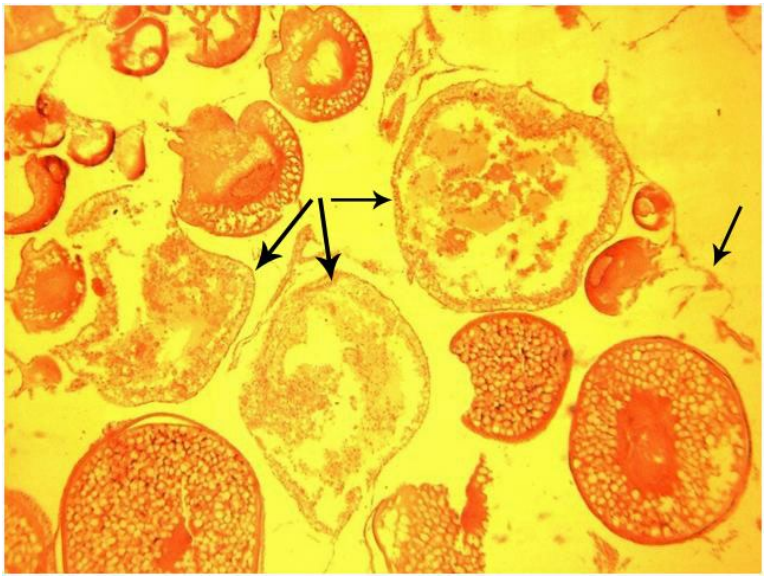
Figure 3. Degenerative changes in ovary with Immature follicules and fibrosis in parenchyma are seen (Arrows) (H&E × 40).

Inflammation of intestinal tissue with severe necrosis and degeneration of goblet cell were seen. Detachment of mucosal epithelium was observed in intestine. In submucosa of intestine congestion and infiltration of inflammatory cells with edema and infiltration of EGCs (Eeosinophilic granular cells) and mononucleic cells were also observed (Figure 4).

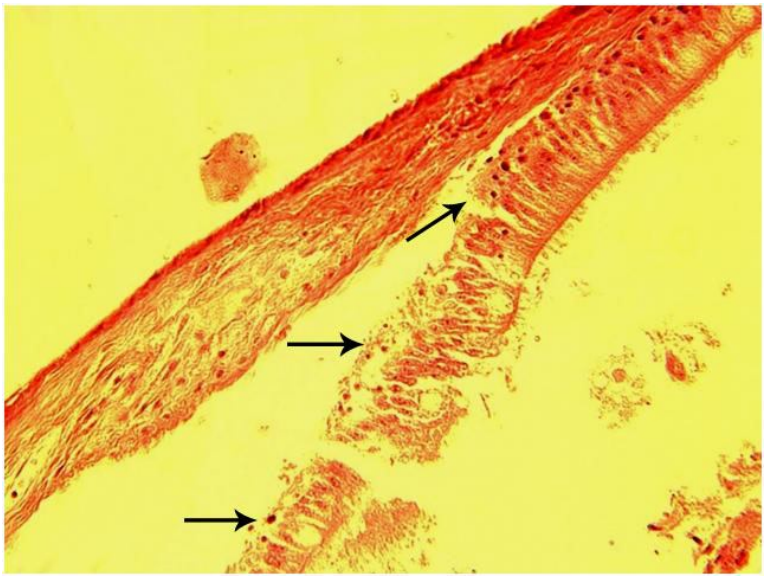

Figure 4. See detachment of mucosal epithelium and infiltration of inflammatory cells with infiltration of EGCs (H&E×250).

In liver cholangiohepatitis with metaplastic hyperplasia and pressure athrophy was seen. Focal necrosis, inflammation and infiltration of granulocytes, atrophy of hepatocytes, haemorrhagia, congestion, vasodilatation, thrombosis, picnotic nucleus and nucleolus were observed in liver (Figure 5).

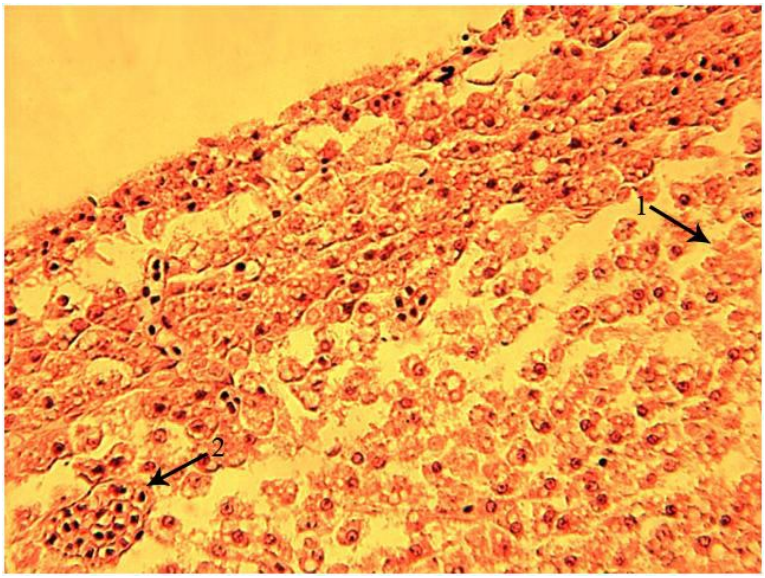

Figure 5. Focal necrosis (1), Haemorrhagia and vasodilatation (2) are seen in liver (H&E ×250).

In both parts of pancreas, hepatopancreas and mesenteric pancreas, congestion, vasodilatation and picnotic necrosis of nucleus with vacuolated cytoplasm and degranulation of EGCs were observed (Figure 6).

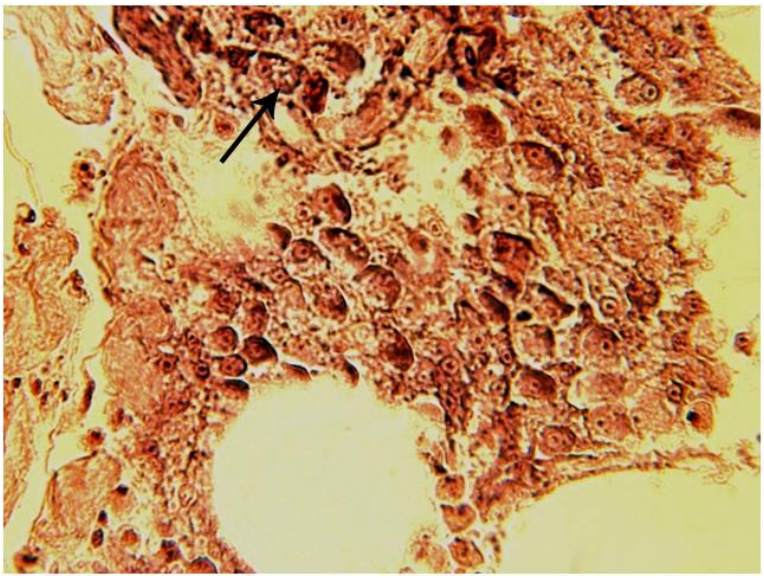

Figure 6. See picnotic necrosis of nucleus in pancreas tissue (arrow) (H&E ×400).

Discussion. Ligulosis due to plerocercoid of *Ligula intestinalis* is one of the most common diseases of freshwater fishes in general, in lakes and water reservoirs in particular (Lott et al 2002). There are many reports on infestation of various kinds of fishes of Cyprinidae with plerocercoid of *Ligula intestinalis* from many countries including Iran (Hool & Arme 1982; Brown et al 2002; Marshall and Barson 2003; Ergonul & Altindag 2005; Jalali & Barzegar 2006; Oyoo-Okoth et al 2010). However, the reports on infestation of *Alburnoides bipunctatus* are limited.

Innal et al (2007) described that the most common fish parasite reported from waters of Turkey was *Ligula intestinalis* and they also reported infestation of *Alburnoides bipunctatus* with the plerocercoid.

Regarding pathological findings, it could be hypothesized that presence of the parasite in abdominal cavity of the infested fish exerts a continuous pressure on abdominal organs and could disturb their normal function and histological structures. Gametogenesis is hampered in ovary, ovules are degenerated and remained immature, and hence, sterility is ensued. On the other hand, 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. Some researchers showed that ligulosis in *Rutilus rutilus* prevented gamatogenesis and resulted in noticeable hypopituitarism and reduced expression of mRNA of gonadotrophins of pituitary gland (Trubiroha et al 2009, 2010). Cholangiohepatitis and metaplastic hyperplasia in liver was in result of compressive stimulations of *Ligula intestinalis.* And it caused to damage structure of hepatocytes and normal function of them. Thrombosis in liver was in result of pressure of *Ligula intestinalis*. Decrements in intestine and pancreas were in cause of compressive effects of this parasite. In this study Infestation of various kinds of fishes to this parasite in fish ponds and rivers has a high prevalence in Iran and has been reported from Hamoon Lake and Caspian Sea (Shargh et al 2008). Pazooki et al (2007) studied metazoan parasites of fresh waters of North West of Iran and separated 11 species of parasites. They showed that wide range of hosts was infested with *Ligula intestinalis* including *Alburnoides bipunctatus*. Hajirostamloo in a 2009 research, studied ligulosis and possible hosts in Sattarkhan reservoir Dam Lake in East Azerbayejan province, Iran and reported infestation of *Alburnoides bipunctatus* with plerocercoid of *Ligula intestinalis.* Mortazavi Tabrize et al (2004) also reported infestation of *Alburnoides bipunctatus* with plerocercoid of *Ligula intestinalis* in Sattarkhan reservoir Dam Lake in East Azerbayejan.

It should be noted that Latian reservoir Dam Lake supplies drinking water of about 2600000 people living in eastern Tehran (Kashefi-Asl & Zaeem 2009). So, considering

the importance of *Ligula intestinalis* as a food borne parasite, performance of other comprehensive studies is a necessity.

Conclusion. *Ligula intestinalis* is very important from standpoint of nutritional health. When a huge number of fishes are infested with the parasite, a main source of high quality protein is jeopardized. Overall regarding economic losses and zoonotic importance of the food borne infection, prevention programs, conducting tough measures and performing comprehensive studies seem necessary.

Acknowledgement. Authors wish to thank specially to Mr. Mahmudi, technician of laboratory of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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Received: 07 January 2011. Accepted: 01 March 2011. Published online: 05 March 2011.
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How to cite this article:
Rahmati-holasoo H., Hajimohammadi B., Ahmadiara E., Ebrahimzadeh Mousavi H., Rostami-bashman M., Haghighi khiabanian asl A., Haghdoost I. S., Shokrpoor S., Ghorbanalipour A., 2011 A study of infestation of *Alburnoides bipunctatus* with *Ligula intestinalis* in Latian reservoir Dam Lake, Tehran province, Iran: A histopathological study. HVM Bioflux **3**(1):18-24.