

## HISTOPATHOLOGICAL EFFECTS OF *PARAPLEUROLOPHOCERCOUS CERCARIA* ON THE DIGESTIVE GLANDS OF *MELANOIDES TUBERCULATA*

MANAL ISMAEL KHALEEL AND SHADIA SADDAM ALHAMD

Biology Department, College of Education for Pure Sciences, University of Basrah, Basrah, Iraq

(Received 3 November, 2020; Accepted 7 December, 2020)

**Key words :** *Cercaria*, *Digestive glands*, *Histopathological effects*, *Snails*, *Trematodes*,

**Abstract** – As they can act as intermediate hosts to various parasitic species, snails have a particular veterinary importance. The study was conducted to investigate the histopathological effects of *Parapleuro lophocercous* larval stages on the digestive glands of *M. tuberculata*. Snails infected with *Parapleuro lophocercous cercaria* were collected, and infected digestive glands were isolated and prepared for histological preparation. Histopathological observation in infected snail tissues showed considerable damage to the digestive gland such as lyses necrosis, enlargement of the epithelial layer and separation of connective tissue. The severity of any histopathological damage depends on the type of larval stages and degree of infection.

### INTRODUCTION

Snails have a particular veterinary importance due to acting as intermediate hosts in the transmission of several species of flukes (Lockyer *et al.*, 2011; Taofiq *et al.*, 2017; Chontanarith *et al.*, 2017). As the snails can harbour to larval stages of Trematodes, these larval stages may cause different forms of histopathological damage and structural alteration to the digestive glands and reproductive systems of infected snails when they grow and multiply (Adam *et al.*, 1995; Probst and Kube 1999; Gilardoni *et al.*, 2018a; Gilardoni *et al.*, 2018b; Huffman *et al.*, 2009; Averbuj and Cremonese, 2010; Chousiba *et al.*, 2012; El Sayed *et al.*, 2014; Omoyakhi and Okhale, 2015). A number of investigations have reported that the hepatopancreas or digestive gland in gastropoda suffer attack more than any other organ (Wright, 1966; Crews and Gerald, 1987). The snail *Melanoides tuberculata* naturally harbours several trematodes, and is known as an intermediate host to them (Pinto and Melo, 2010). Various types of *Cercaria* that emerge from *M. tuberculata* have been reported in Iraq (Al-Husseiny, 1999; Mohammad, 2015). Some histopathological and histochemical observations in *M. tuberculata* infected with larval stages of trematodes have indicated a number of abnormalities in the digestive glands of snails (Choubisa, 1988). In general, histopathological studies of infected snails in Iraq are rare. This study

aims to investigate the histopathological effects of *Parapleuro lophocercous cercaria*, redia and sporocysts on the digestive glands of *M. tuberculata*.

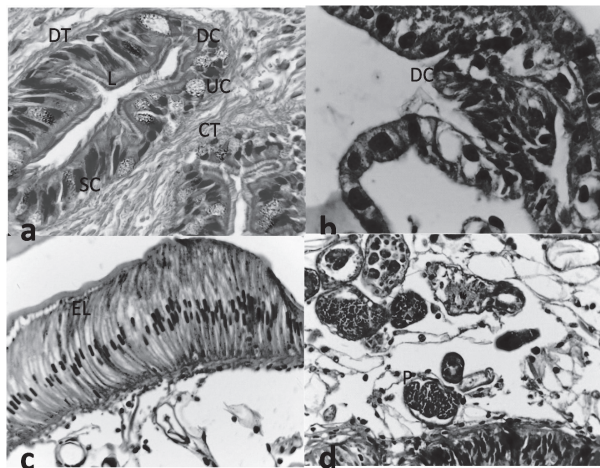
### MATERIALS AND METHODS

About 70 specimens of the snail *Melanoides tuberculata* were collected from the Garmat Ali River in Basrah province in Iraq in the spring of 2018, which were then taken to the laboratory and separated into different aquaria and equipped with fresh water and some aquatic plants from the snails' original habitats. The collected snails were exposed to artificial light for two hours in order to ensure the shading of the cercaria, and the emerging cercaria were fixed and examined under the microscope for identification purposes. The infected snails were separated from their shells then fixed in 10% formalin and prepared for histological examination according to Humason, 1972.

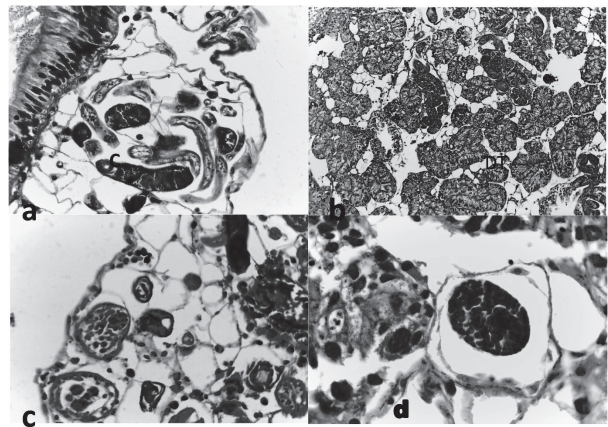
### RESULTS AND DISCUSSION

Out of the 70 collected specimens of *M. tuberculata*, 14 (20%) were infected with *Parapleuro lophocercous cercaria*. Uninfected *M. tuberculata* digestive glands are generally composed of several oral or elongated tubules that are connected to each other by fin loss

connective tissues. Each tubule in the digestive gland has lumen surrounded by a single layer of epithelial cells which can be divided into three types: 1- columnar-ciliated (digestive/absorptive) cells with basal nucleus 2- secretory cells, and 3- undifferentiated (starving) cells, which can be observed in the intestinal component of the digestive gland, with the whole digestive gland enclosed in the outer membrane, the *Tunica propria* (Plate 1a). The infected *M. tuberculata* digestive glands appeared to have undergone considerable damage as caused by *Parapleurolophocercous* sporocysts, redia and cercaria. In general, the histopathological damage in the infected tissue can be categorized as either mechanical or physiological. Mechanical damage includes degeneration of digestive gland cells and their nuclei (Plate 1- b), elongation in the epithelial cells of the intestine (Plate 1- c), a damaged digestive gland architecture (Plate 1- d), and separation of the connective tissue from the epithelial layer of intestine wall (Plate 2- a). The tubules become irregular in shape and there can be an increase in the intertubular and intratubular space of the majority of tubules (Plate 2- b), and hypertrophy and protrusion (Plate 2- c), and infiltration of inflammatory cells and their nuclei (Plates 2- d). Physiological damage has been reported as including lyses in the epithelial tissue of

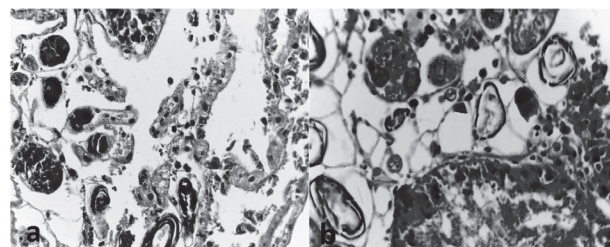


**Plate 1.** a. Histology of uninfected *M. tuberculata* digestive glands x100 (b-d): histology of infected digestive gland b: degeneration of digestive gland cells and their nucleus 100 x, c: elongation in the epithelial cells of the intestine 40 x, d: destroyed digestive gland architecture 40 x. DT: digestive tubule, DC: digestive cells, SC: secretory cells, UC: undifferentiated (starving) cells L: tubule lumen CT: connective tissue, EL: epithelial layer, P: parasite.



**Plate 2.** a. Separation of the connective tissue of the intestine wall 40 x, b: irregular shape of tubules and increase in the intertubular and intratubular space 10 x c: hypertrophy and protrusion 40 x, d: infiltration in inflammatory cells and their nuclei, 100 x. C: cercaria.

intestine (Plates 3- a) and necrosis in the digestive gland epithelial tissue (Plate 3- b). No parasites at the larval stage invade the tubules of digestive gland and some tubules remain unaffected. Damage and alteration to the infected digestive gland are more apparent in heavy infections. Such mechanical alteration and damage to the infected digestive gland are thought to be a result of *Parapleurolophocercous* larval activities such as gaining nutrition, growth, multiplication and larval movement while physiological damage such as necrosis or lyses may be a result of parasitic secretions of enzymes and their toxic effects during metabolic activities or result from digestive enzymes that are released from ruptured cells in the digestive gland (Choubisa, 1988, 2008). Autolysis may occur due to starvation when food is unable to pass into the digestive tubules due to their compression at different sites, as well as lowering in energy and stored nutrients due to developing in the gonads of infected hosts, which could explain the enlargement



**Plate 3.** a. lyses in the intestine epithelial tissue 40 x, b: necrosis in the digestive gland's epithelial tissue, 40x.

of the intertubular and intratubular space of infected glands (Chousiba *et al.*, 2012).

## CONCLUSION

The infection of snails with trematodes cercaria causes various forms of histopathological damage depending on the degree of parasitaemia, and the size and types of larval stages present.

## ACKNOWLEDGMENT

We are thankful to the Department of Biology in the College of Education of Pure Sciences at the University of Basrah for cooperation to complete this research.

## REFERENCES

- Adam, R., Pipitgool, V., Sithithaworn, P., Hinz, E. and Storch, V. 1995. Morphology and ultrastructure of the digestive gland of *Bithynia siamensis goniomphalus* (Prosobranchia: Bithyniidae) and alterations induced by infection with the liver fluke *Opisthorchis viverrini* (Trematoda: Digenea). *Parasitology Research*. 81(8) : 684-92. DOI: 10.1007/BF00931847.
- Averbuj, A. and Cremonte, F. 2010. Parasitic castration of *Buccinanops cochlidium* (Gastropoda: Nassariidae) caused by a lepecreadiid .digenean in San Jose Gulf, Argentina. *Journal of Helminthology*. 84 (4): 381-389. DOI: 10.1017/S0022149X10000052.
- Al-Husseiny, R.A. 1999. Study of larval stages of digenetic trematodes parasitizing snails of some Shatt Al-Arab branches and the physiological effects of *Ornithobilharzia turkestanicum* in rabbits and mice. Msc. Thesis, University of Basrah, College of Science, 113 pp.
- Chontanarith, T., Tejangkura, T., Wetchasart, N. and Chimburu, C. 2017. Morphological Characteristics and Phylogenetic Trends of Trematode Cercariae in Freshwater Snails from Nakhon Nayok Province, Thailand. *Korean J Parasitol*. 55 (1) : 47-54. <https://doi.org/10.3347/kjp.2017.55.1.47>.
- Chousiba, S.L., Sheikh, Z. and Jaroli, V.J. 2012. Histopathological effects of larval trematodes on the digestive gland of freshwater snail species *Vivipara beugalensis* and *Lymnaea acuminata*. *Journal of Parasitic Disease*. 6 (2) : 283 – 286.
- Crews, E. and Gerald, W. 1987. Histopathology of larval trematode infections in the fresh pulmonate snail, *Helisoma anceps*. *Journal of Invertebrate Pathology*. 49 (1) : 76-82.
- Choubisa, S.L. 1988. Histochemical observations on the digestive gland of *Melanoides tuberculata* (Gastropoda) infected with certain larval trematodes and focus on their mode of nutrition. *Proceeding Animal Sciences*. 97 (3) : 251 – 262.
- Choubisa, S.L. 2008. Mode of nutrition in pathogenic trematode larvae (redia, and cercaria) which infect hepatopancreas of freshwater snails (Mollusca: Gastropoda). *J. Parasit. Dis*. 32 (1) : 68 – 73.
- El Sayed, T., Rizk, K., Hamada, H., Abdelghany, S., Sherif, R. and Ramez, M. 2014. A many histological and biochemical investigations on the freshwater snail *Pirenella conica* (blainville, 1829) infected with the developmental stages of *Heterophys* sp. *Egypt J. Exp. Biol. Zool*. 10 (2) : 89 – 97.
- Gilardoni, C., Giorgio, G.D., Bagnato, E. and Cremonte, F. 2018a. Survey of trematodes in intertidal snails from Patagonia, Argentina: new larval forms and diversity assessment. *Journal of Helminthology*. 93 (3) : 1-10. DOI:10.1017/S0022149X18000329.
- Gilardoni, C., Giorgio, G.D. Ituarte, C. and Cremonte, F. 2018b. Atypical lesions and infection sites of larval trematodes in marine gastropods from Argentina. *Diseases of Aquatic Organisms*. 130 (3). DOI: 10.3354/dao03273.
- Huffman, J., Klockar, J., Keeler, S.P. and Fried, B. 2009. Histopathological effects of the intramollusca stages of *Zygocotyle lunata*, *Echinotoma trivolvis* and *Ribeiroia odatrae* on *Helisoma trivolvis* and observations on keratin in the trematode larvae. *Parasitology Researches*. 105 (5) : 1385 – 1389.
- Humason, G.L. 1972. *Animal Tissue Techniques*. 3rd. ed. W.H. Freeman and Company, Son fran. 13: 614p.
- Lockyer, A.E., Jones, C.S., Noble, L. and Rollinson, D. 2011. Trematodes and snails: An intimate association. *Canadian Journal of Zoology*. 82 (2) : 251-269. DOI: 10.1139/z03-215.
- Mohammad, M.K. 2015. The parasitic infection of the freshwater snails collected in central Iraq. *Int. Curr. Microbiol. App. Sci*. 4 (3) : 47 – 55.
- Omoyakhi, J.M. and Okhale, O.E. 2015. Histology of male and hermaphroditic R. *Productive Nigerian Journal of Agriculture, Food and Environmental*. 11(1) : 284 – 289.
- Probst, S. and Kube, J. 1999. Histopathological effects of larval trematode infections in mud snails and their impact on host growth: What causes gigantism in *Hydrobia ventrosa* (Gastropoda: Prosobranchia). *Journal of Experimental Marine Biology and Ecology*. 238 (1) : 49 – 68.
- Pinto, A.H. and Melo, L.A. 2010. *Melanoides tuberculata* as intermediate host of *Philophthalmus gralli* in Brazil. *Rev. Inst. Med. Trop. Sao Paulo*. 52 (6) : 323 -327.
- Taofiq, S., Bunza, M.D.A., Majeed, Q., Abubaker, M.B. and Ladan, M.U. 2017. Studies on snail vectors of Helminth Disease agents along Rima river village at Kwalkwalawa Vilage, Wamakko local government Area. Sok. to state, Nigeria. *S.M. Trop. J*. 2 (1): 1011.
- Wright C. 1966. The pathogenesis of helminths in the Mollusca. *Helminthol*. 35 (3) : 207-224.