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Gonads Tissue Changes of *Chalcalburnus mossulensis* (Heckel, 1843) Infected by *Ligula intestinalis* (Cestoda)

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Abstract: *Chalcalburnus mossulensis* from the *cyprinidae* family is one of the indigenous fish in Gheshlag lake of Kordestan-Iran. *Ligula intestinalis* is one of the infective parasites among various species of fish and causes gonads atrophy. In this study, detectection of species and age of samples, the effects of this parasite on gonads tissues and sexual maturation of *Chalcalburnus mossulensis* are investigated. By seasonal sampling 144 speciemens of these fish were collected. It was clear that a significant difference was between the means of male and female gonads maturation rate in infected and non infected samples ($p< 0.05$). The lack of gonads maturation in infected fish may be due to *Ligula intestinalis*. In addition, the abnormal degenerative changes like absorption follicle, hemorrhage and infiltration of inflammation cells in ovary tissue of infected fish were seen. In testicle tissue dispersed hemorrhage, atrophy and MMC (melano-macrophage center) were seen as a pathological signs. So the spread of this parasite in different water sources is important as the point of the maintenance of indigenous species and cultivated fish.

Key words: *Chalcalburnus mossulensis* · *Ligula intestinalis* · Gonads · Histopatholog

INTRODUCTION

The Queshlaq dam lake, 12 kilometers away from the north of Sanandaj, with 38° North width and 30° East length is located on Queshlaq river [1] and belongs to the ecologic area of Bainol Nahrain [2]. One of the native fish of this region is *chalcalburnus mossulensis* which lives in Queshlaq river and its dam [3, 4]. This fish belongs to *Ciprinidae* family and has a long mandible and body and lives in freshwater and brakishwater. The number of the fish soft rays and its anal fin amounts 10 to 12 and has a long lateral line with 66 to 82 scales on it [5]. Considering the fact that the multi host parasitic diseases are seen among the fish of wild and open areas, their infection with *Ligula intestinalis* parasite in this lake is not impossible and some cases have been reported [2, 6, 7]. The matured parasite is in the ending part of intestine of the fish-eating birds and after their death, the eggs are released in the water, then after a while the free coracidiums from the eggs will be swallowed by the host- medium crustaceans - mostly Cyclops- and then enter the crustacean's abdominal cavity. The host- medium crustaceans are eaten by the fish and the parasite plerocercoids drill the fish intestine septum and enter the abdominal cavity of the fish. The number of plerocercoids in abdominal cavity can also be 13 [2, 8-10]. By the time being, of *Ligula*, the subspecies of *Ligula intestinalis* and *Digrama*, subspecies of *Digrama interrupta*, 10 cases have been reported from the *cyprinidae* family. The difference between these species is the presence of a pair of female sexual system in *Ligula* and two pairs of female sexual system in each proglotid of *Digrama* which can be identified by unequipped eyes. At the growth process, this parasite damages the fish gonads in abdominal cavity in two ways: first, physically, resulted from pressure of the parasites onto viscera and second, infusing chemicals and their effects on brain- hypophises- gonad axis, which causes malfunction in action of the mentioned axis and prevents infusing LH hormone and finally leads to immature gonads and immaturity [11,12]. Infection in Iran

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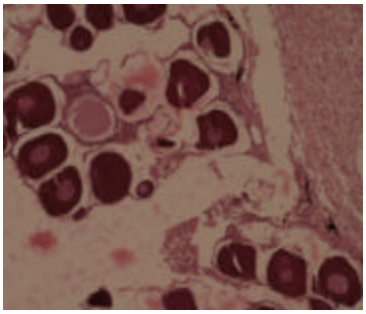
has had an extended spread and has been seen in the three regions of country's ecology [2]. There are numerous reports on Ligulosis outbreak in fish living in freshwater. Ligulosis reported in silver carps in Hamoon marsh [10] such a way that the percentage of infection reached to 100% in summer. Ligulosis reported in Satarkhan dam's fish [13]. In the performed research in the Zerivar lake of Iran Ligulosis outbreak in fish reported [11], it was discovered that the maximum of infection in the autumn was 28/91% and the maximum infection was in a fish with 13 parasites (about 30/7 % of its weight). According to Dubinanna search, 47 species of the fish, which 31 out of them are from *Cyprinidae*, can be the second intermediate host of *Ligula* parasite [14]. In the parasitological studies in Zerivar lake the ligulosis outbreak was reported 15%, 60%, 15% and 2% in summer, fall, winter and spring respectively [7]. This parasite in the growth process in abdominal cavity causes malfunction in the activity and maturation of gonads and the fish maturation, which is called parasitic sterility that leads to the fish reduction [14,15]. Various researches have studied the effects of this parasite on the rate of fish reduction and have shown its negative effect on the growth process and reduction of the fish weight [16-18]. The study of gonads tissue in teleostei fish also can give sufficient information on sex and sexual maturation and reproduction process [19]. In addition, the study of gonads tissues changes during different seasons of the year and the time of reproduction and its comparison to healthy fish is important in identifying reproduction disorders in the year's various seasons, especially in spawning season and the aim of this research is to identify the effect of this parasite on gonads tissues of male and female fish and its pathogenesis and its effects on sexual maturity process of the fish during different seasons, that can guide supporting actions in fish stocks' preservation and reduction of their mortality and can be of benefit with generalization to other water resources and fish species regarding better production of economical fish.

MATERIALS AND METHODS

In one year, since March 86 to March 87, in the second half of the second months of each season, sampling was done with small seine net (0.5 cm) in southern coast of Queshlaq dam and the samples were delivered lively to the laboratory of Veterinary Faculty of Azad University of Sanandaj and soon they were put under biometry and for the fish identification Abdoli (1999) and Saadati's methods (1977) were used. Then after, using a forceps, the scale sample was taken off from the pectoral fin and upper lateral line and in order to purify was washed in xylene and glycerol and was put on slide and was studied under a light-microscope of 40 magnification [3]. The fish of the same age were autopsied separately and in the cases of plerocercoid presence, the parasite of abdominal cavity, sampling was done from anterior part of gonads with dimension (0.5 × 0.5 cm) and in order to gain tissue cross section and to study microscopically was added to buffered formalin 10%. The gained plerocerocoids to be studied were also added to buffered formalin 10% and then marked with Karmen staining and since *ligula*'s reproduction system is seen like a fissure in its ventral surface, is distinguishable by unequipped eyes from its analogous *Digrama* parasite [11]. Simultaneously, the same aged noninfectious fish were autopsied and their gonads were sampled in the same way so that they be compared at the next stages. The mentioned procedure was accomplished for each seasons during the period of project implementation and 1000 fish were caught, which after biometry, identification and age and infection status determination, 144 out of them were picked out. It should be mentioned that in the infectious fish selection in order to consider the same study condition for all of fish, only the fish having plerocerocoids _ with weight over than 1 gram_ in their abdominal cavity were chosen and studied. Then, using a rotative microtome, the tissue section of 6μm was taken from the gonads samples and was stained by method of Pousti and Adibmoradi [20] and later was studied under a light- microscope and at first the fish sex and then the ovary and testicle maturation stages, which were categorized to six stages, were distinguished for each of the fish [19]. At the end, the possible pathological damages in gonads tissue were studied and the results were recorded in special prearranged charts. The gained data from complete random statistical plot of normal type was analyzed by ANOVA method from the statistical software of SAS version 9. To this end, at first the homogeneity of the statistical mean groups were studied by Bartlet Test of this software and then the relation between maturation stages of gonads of the non-infected and infectious fish was determined [21, 22].

RESULTS

In the performed researches on the samples under study, it was clear that in all samples in one year the mean of gonad maturation stages (stage1 to stage 6) in infected fish (2.57 ± 0.71), compared to the non-infected fish (3.89 ±1.71), have a delay in their gonads maturation



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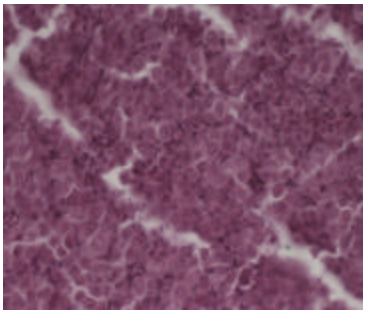
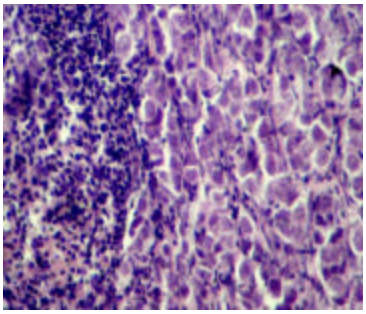


(2)

Fig. 1: Testicle tissue of infected fishes - H&E staining- 400X
Fig. 2: Ovary tissue of infected fishes - H&E staining – 400X



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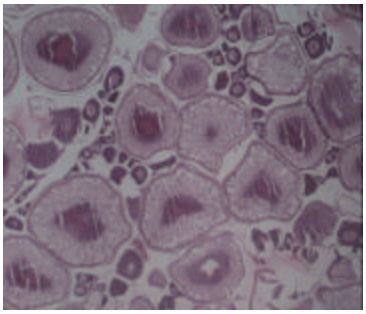

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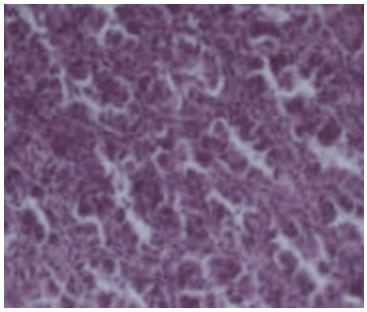
Fig. 3: Testicle tissue of non infected fishes - H&E staining- 400X
Fig. 4: Ovary tissue of non infected fishes - H&E staining – 400X



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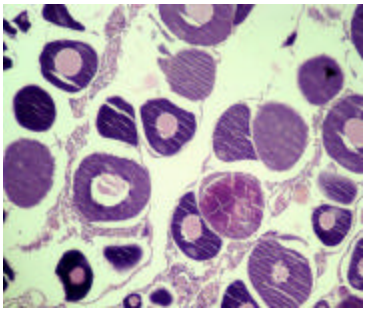
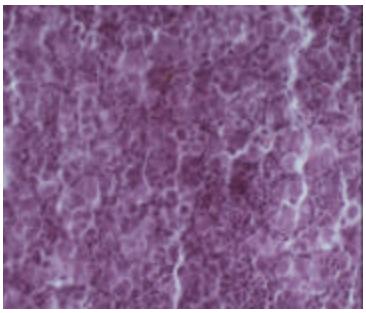


(6)

Fig. 5: Ovary tissue of infected fishes - H&E staining – 400X
Fig. 6: Testicle tissue of infected fishes - H&E staining- 400X



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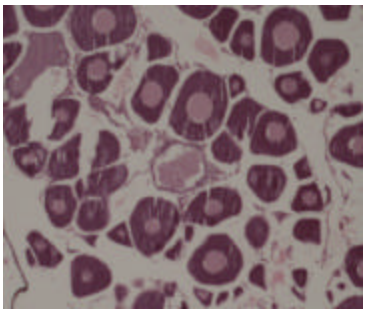
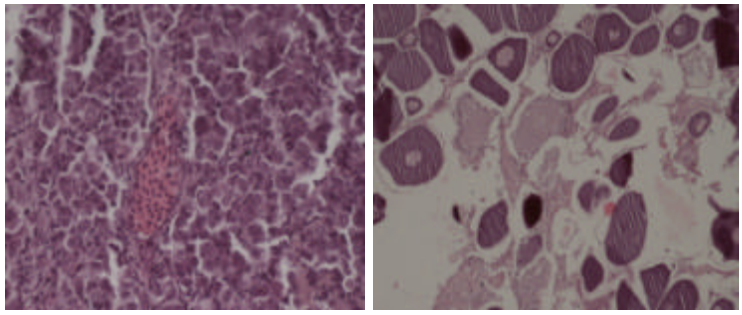


(9)

Fig. 7: Ovary tissue of infected fishes - H&E staining – 400X
Fig. 8: Testicle tissue of infected fishes - H&E staining- 400X



(9) (10)

Fig. 9: Testicle tissue of infected fishes - H&E staining- 400X
Fig. 10: Ovary tissue of infected fishes - H&E staining – 400X

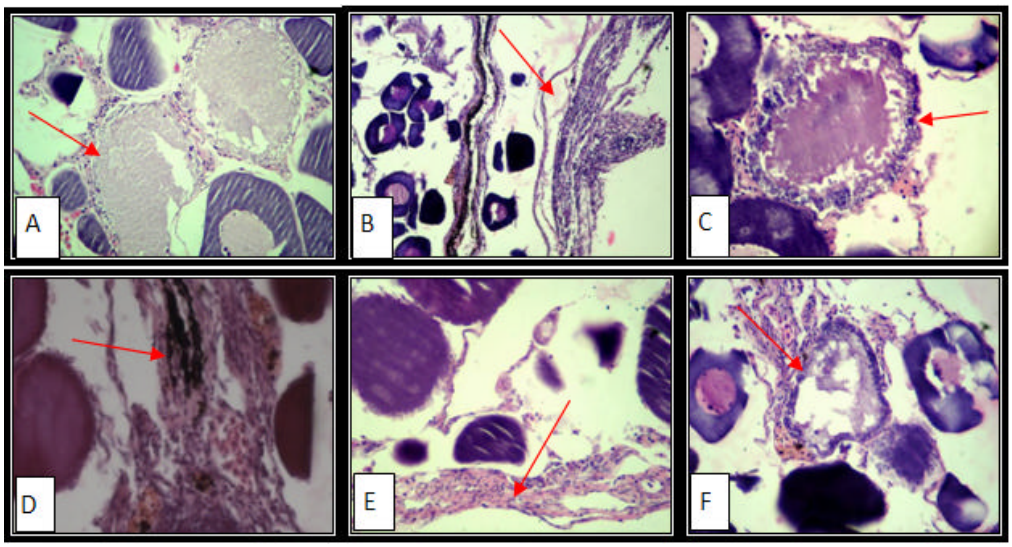


Fig. 11(A-F): Ovary tissue of infected fishes - H&E staining – 400X - Atresia and necrosis were seen in oocyte (A) and(C) – capsule of ovary shrink and separated (B) – melano-macrophage center was seen (D)–inflammation and fibrosis were seen (E)–hemorrhage and necrosis were seen in follicles(F).

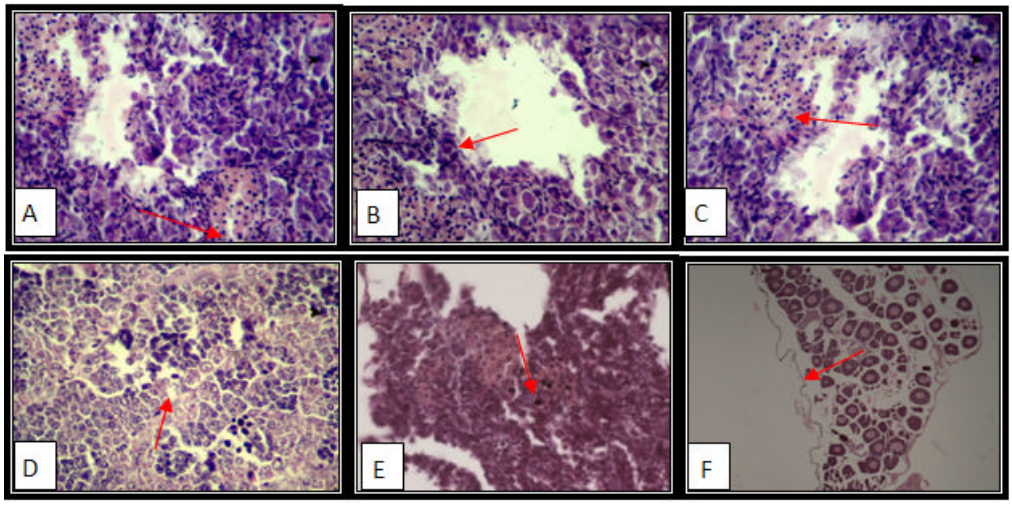


Fig. 12(A-F): Testicle tissue of infected fishes - H&E staining- 400X – hemorrhage and inflammation (A) and (C) – necrosis and abnormal tissue were seen (D) - melano-macrophage center was seen (E) - capsule of testicle shrink and separated (F).

stages, especially in the spawning season and there has been a meaningful relationship among the gonads maturation stages of male and female fish infected by *Ligula intestinalis* and the non-infected fish ($p<0.05$).

The gonads maturation stages and the comparison of their status in infectious and noninfectious fish marked by the seasons are as follows:

Spring: The taken samples in the spring (the second half of May) showed non-maturation in most of the gonads in the male fish infected by ligulosis that only primary and secondary spermatogonium could be seen in them (Fig.1). The non-maturation trend of follicles could also be seen on ovary tissue of most of the infected fish (Fig.2), while this could not be seen in gonads and ovary tissues of noninfectious fish and in their gonads and ovary tissues spermatozoid and matured follicles could be seen respectively, that is completely natural according to their positioning in spawning season (Fig. 3 and 4).

Summer: In the taken samples in the summer (the second half of August), although the spawning season was finished and the gonads were returning to their primary status and inactive stage, on the most of the infected female fish the non-maturation of follicles and not reach to maturation stage and becoming atrophy was clear (Fig.5) and most of the male infected fish did not have spermatozoid and testicular cell tissues were at the first stages of sexual cell maturation (Fig.6) contrary to the fact that noninfectious fish caught in this season contain matured follicles and in the gonads some spermatozoids and secondary spermatocytes could be seen.

Fall: The taken samples in the fall (the second half of November), considering the entry of the gonads into the stage after ovulation and their inactive phase, distinguishing the noninfectious and infectious fish gonads tissues would be more difficult because of resemblance between sexual cells in the first stage of gonads maturation, but with more care the gonads could be distinguished from the follicles' left coverage, non-released eggs and oocytes having different growth stages from other gonads that have not entered their maturation phase and only have primary follicles and spermatogonium cells (Fig.7).on the gonads tissue, observing the secondary spermatocyte cells having nucleus with diffuse chromosomes without nucleolus and spermatozoid being phagocytosis could be a reason on the gonads complete maturation and spermatozoid production by them, with this case could not be seen in the infected fish's gonads and only the spermatogonium cells and primary spermatocyte cells were seen (Fig.8).

Winter: The taken samples (the second half of February), considering the cold season and inactive phase of gonads, were mostly like the samples of autumn and distinguishing the infected and non-infected fish only from maturation of the gonads tissues because of the sexual cells such as spermatogonium and primary spermatocyte in the first stage of maturation would be so difficult in both groups of the fish (Fig. 9) and also in both groups of fish the immature and primary follicles could not be much distinguished in the ovary (Fig. 10). After this stage the gonads enter the new activity cycle and on the case of fish non-infection, the gonads maturation reaches to the final stage and leads to spermatozoid and egg production in the reproductive season.

In the microscopic section studies, in addition to the gonads' maturation stages divided by the seasons, various pathological changes were seen in most of the fish gonads as following:

Ovaries: abnormal degenerative changes shaped like atretic oocytes and necroses of the sexual cells were seen. The infiltration of inflammation cells into ovary capsule and existence of melano-macrophage centers could be observed. In addition, in the infectious fish the ovary capsule did not have its natural tallness and resistance and has been wrinkled (Fig. 11).

Testicles: necrosis and the natural gonads destruction plus diffuse hemorrhage and creation of empty space under the gonads capsule, which was the evidence of gonads atrophy, observing melano-macrophage centers and the aggregation of inflammation cells draw attention as pathological changes in the infected fish (Fig.12).

DISCUSSION

According to the pervious studies, the presence of *ligula* parasite in the fish flesh causes changed in their gonads and would have hormone disorders [23-25]. Ligulosis prevalence in Germany's water resources has been reported about 80% of Bream fish, which showed the high infection possibility in cultivated and wild aquatic animals [24]. According to Jalali and Barzegar [7] in Iran the maximum of infection has also been reported 28.91% in Zerivar lake of Kurdistan, which showed the importance of this parasite outbreak and since in the country's water resources behind the dams the cultivated fish were kept, it warns the necessity of fighting approaches against this parasite more than ever. Based on the results drawn by this research it was revealed that in all of the taken samples during a year, the infected fish compared to non-infected same aged fish delayed in their gonads

maturation stages, especially in the spawning season and there was a meaningful relationship between the male and female fish gonads maturation stages of both groups. If other conditions such as age, nutrition, temperature, genetics and so on be the same for all of the studied fish, the reason of non-maturation of the gonads tissues could be their infection to *Ligula intestinalis*, which postpones the gonads maturation through different ways. According to Arme [26], this delay is caused by the parasite's diffusions and anti-gonadotropin substances, which were made by the parasite. The taken samples of the spring showed non-maturation in most of the male fish gonads tissues, which primary and secondary spermatogonium cells could be seen and in the ovary tissues, most of the infected fish follicles non-maturation could be observed. Contrarily, this was not seen in the non-infected fish gonads tissues and because of reaching to reproduction season, spermatozoid in their gonads tissue and complete matured follicles in their ovary tissues could be seen typically, which other studies acknowledge this completely [25, 26]. In the summer, although the spawning season has finished and the gonads return to primary regeneration status, yet remains of the matured sexual cells in most of the non-infected fish could be seen, while in the infected fish the non-maturation of the gonads was observed. In the fall and winter, although the gonads have entered the stage after spawning and their inactive phase, distinguishing between the infected and non-infected fish gonads tissues becomes more difficult and sometimes impossible because of the various sexual cells' resemblance in the first stages of gonads maturation. In all of teleost fish, observing atretic follicles and sexual cells' necrosis could be the natural result of environmental conditions and could be a pathological change [27], which was unlikely in this research by comparing the infected and non-infected fish and the homogeneity of the environmental and optional conditions. The atrophy of oocytes, infiltration of inflammation cells, hemorrhage in the infected fish gonads, melano-macrophage centers and wrinkling of gonads capsule were some significant points of this research, which have not been considered in any of the pervious reports. This status could be an indicator of parasite's various effects from the perspective of chemical infusions and the effect of these infusions on different organs of the fish due to the genetic, environmental and nutritional conditions and the parasite metabolic activity and the difference of fish immunity levels. Observing the melano-macrophage centers, which naturally should not be in the gonads tissues, showed an acute or a chronic inflammation process, that has not been mentioned by Achim [25] and Arme [26] which were the only pathological studies in this field. Presence of these damages could be the reason of pathogenesis of widespread parasite that emerge in various conditions and could be affected by the host's environmental, genetic and nutritional characteristics. Considering the above mentioned cases about Queshlaq dam and the native fish we can say that *Chalcalburnus Mossulensis* fish is the specific host for this parasite and some of other non-economical fish, added accidentally to the dam, also are infected by this parasite, but the possibility of infection outbreak is high about the fish such as common carp and silver carp, which are too sensitive to this parasite and now are the economical fish of this dam and the results of this studies can be useful in the decision makings and actions which have been introduced as reducing factors of losing the local fishery stock.

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