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PATHOLOGICAL AND PARASITOLOGICAL INVESTIGATIONS OF EELS (*Anguilla anguilla*) NATURALLY INFESTED WITH ANGUILLICOLA CRASSUS AND PSEUDERGASILUS ZACCONI IN EGYPT

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SUMMARY

For the first time in Egypt *Anguillicola crassus* and *Pseudergasilus zacconi* were recognized and identified from *Anguilla anguilla*. The naturally diseased fish showed skin hemorrhages, low body gain and occasionally abdominal swelling.

In anguillicolosis, the infected swim bladders showed great thickening of their wall and multiple areas of hemorrhages together with the presence of round worms floating in chocolate like material within their luminae. The prevalence of infection was 30 % and the intensity of parasite ranged from 1- 15 worms per fish. Histopathologically , cross sections of adult worms and numerous ensheathed larvae were present inside the lumen of the examined swim bladders. Severe congestion , submucosal hemorrhages and mononuclear cellular infiltration were recorded. Epithelial hyperplasia and desquamation with fibrosis of the swim bladder wall were also detected.

In pseudergasilosis , the gill lesions were represented by excess mucous secretion with presence of grayish white elongated nodules firmly attached to the gill filaments. Microscopical examination of the gills revealed presence of parasitic elements in-between destructed gill filaments with diffuse hemorrhages , mononuclear cellular infiltration and fusion of gill lamellae.

The morphological description used in identification of recognized parasites is discussed in the text.

INTRODUCTION

Eels could be parasitized with different kinds of

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parasites. The increasing of parasitic diseases of eels continued to grow with the increasing development of eel industry as the disease had an adverse effect on their population.

Round worm (nematodes) Anguillicola , their adult and larval forms were found to parasitize the swim bladder of eels . Previously, *Anguillicola crassus* was known only in Japan (Kuwahara et al., 1974).Then the infection was spread to Europe through introduced Japanese eels from East Asia where the first record of the parasite was in Italy in 1982 , from Germany in 1985 and from Belgium in 1987 (Dupont and Petter , 1988).After that , the parasite was reported from American eels (*Anguilla rostrata*) in USA in 1995 (Barse and Secor 1999). Then the infection reach Taiwan via imported eels from USA causing outbreaks with high mortality in *Anguilla rostrata* (Kean et al., 1996) .

The prevalence of anguillicolosis depend mainly on the water salinity that limits the development of the larval stages of the parasites (Pilcher and Moore , 1993). It was ranged from 10 -29 % among *Anguilla rostrata* in USA (Barse and Secor 1999) to 60 -73 % among *Anguilla anguilla* in Portugal (Cardoso and Saraiva , 1998) and reaching 100 % in *Anguilla rostrata* in Taiwan (Kean et al., 1996) .

Sings of abdominal swelling, external hemorrhages on the abdominal wall as well as off food, weakness and anemia were observed in eels i fected with *Anguillicola crassus* (Egusa , 19 and Kean et al., 1996). Anguillicola crassus infe tion cause the major pathological lesions in swi bladder of European eels that characterized gro ly by great thickening of its wall (Beregi et a 1998). Meanwhile , the microscopic changes i the infected swim bladder was varied according t the stage of infection. In acute cases, hyperem and epithelial hyperplasia were recorded (Moln et al., 1993). While edema, fibrosis and mononu clear inflammatory cellular infiltration of th swim bladder wall were found in chronic case (Wurtz and Taraschowshi 2000).

A considerable gill damage usually followed adul female ergasilids parasitism. In China , *Pseude gasilus undulata* was reported from 70 % of th examined *Silver carp* (Markevitch , 1940) La on the same author (1976) , recorded four spe cies of pseudergasilus including *Pseudergasilus major* , *Pseudergasilus undulatus* , *Pseudergasilus parasiluri* and *Pseudergasilus zacconi* fro the gills of Siniperica , *Carassius carassius* , *Parasilurus asotus* and *Plecoglossus altivelis* re spectively. Moreover , *Pseudergasilus zacco* was also collected from the gills of *Plecoglossus altivelis* in Japan (Nakajima and Egusa , 197 and Nakajima et al., 1974).

The present study was designed to investigate th prevalence of *Anguillicola crassus* and *Pseu dergasilus zacconi* , their clinical signs and th

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associated pathological lesions in eels (*Anguilla anguilla*).

MATERIAL AND METHODS

Twenty living *A. anguilla* , weighting 250-400 gm were collected from their natural source (Manzala lake) and sent to laboratory under all accurate methods of transportation according to Brown and Gratzek (1980). The fish were immediately subjected to external and post mortem examination as described by Syme (1966).

The obtained fishes were parasitologically examined according to Hoffman (1970) and Markevitch (1976). The detected nematodes and copepodes were fixed in 70 % alcohol-glycerin , cleared in lactophenol and mounted in gelatin. Drawings and measurements were done and the recorded parasites were identified according to Yamaguti (1963) , Hoffman (1970) , Kuwahara et al. (1974) and Lucky (1977).

For histopathological examination , tissue specimens from swim bladders and gills were taken from sacrificed eels and immediately fixed in 10 % buffered neutral formalin. After proper fixation , the fixed specimens were washed , dehydrated , cleared and embedded in paraffin. Then, thin paraffin sections of about 5 microns in thickness were prepared and stained with hematoxylin and eosin stain. Crossman's trichrome stain and PAS staining techniques were also used for demonstration of connective tissues and parasitic elements according to Drury and Wallington (1980).

RESULTS

Out of twenty clinically diseased *A. anguilla* , twelve fishes were infested with gill copepodes and six harboured nematodes in their swim bladders. These fishes , showed emaciation, abnormal skin coloration, external hemorrhages on the abdominal wall and under the fins and abdominal distention in some examined cases .The prevalence of gills copepodes was 60 % while of swim bladder nematode infection was 30% and the number of nematodes ranged from 1 - 15 worms per fish.

Parasitological identification revealed that, the recorded nematode was *Anguillicola crassus* and the obtained copepode was *Pseudergasilus zacconi* .

Morphological characters of the collected Anguillicola crassus :

Family: Anguillicolidae (Yamaguti , 1935). *Anguillicola crassus* (Kuwahara , Niimi and Itagaki , 1974).

The worms obtained from the swim bladder of A. anguilla, its body is cylindrical, gradually narrowing at both ends and their tips are pointed . Circular curvature of the body is seen in large parasite.

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Female: The body size is 32.30 to 47.95 X 3.7 to 5.2 mm. The mouth is simple with wide buccal capsule. Esophagus is cylindrical with an anterior muscular and posterior glandular part. It measures 1.5-4.3 mm in length and its posterior ends shows glandular appendix projecting into intestine. The intestine is dark and distended with material, which looks like melted chocolates. Anus opens terminally. The vagina is short and surrounded by five giant cells near the tail tip. Vulvular opening is present near the tip of the posterior end of the body .

Male: Its body measures 18.28 40.30 mm in length and 1.9 3.00mm in width. The anterior end of the body is similar to that of female . Testes are ovoid in shape and located near the tail end. Seminal vesicle is well developed. Ejaculatory duct open outside on a prominent papillae projecting backward. Three giant cells appears near the tip of the tail. The posterior end is bifurcate into two parts , one of them bears ejaculatory duct , while the second part carry five pairs of papillae and the anus opened near the tip of the tail (Fig. 1 & Plate I).

Post-mortem findings:

The infected swim bladder wall was thickened , inflamed and had focal hemorrhages. Many round worms (Fig. 2) flow in chocolates like material were seen in their luminae. Moreover, bloody exudate in the lumen of swim bladders was recorded in some cases.

Histopathological findings:

The microscopic examination of the infected swim bladders revealed presence of cross section of adult worms, which contained large number of erythrocytes in their body cavities (Fig. 3). In some cases, numerous ensheathed larvae mixed with destructed erythrocytes within the lumen of swim bladder were detected (Fig. 4). Severe congestion of blood vessels and capillaries with multiple areas of hemorrhages scattered throughout the wall of swim bladder were seen. Hyperplasia of the mucosal epithelium forming finger like projection within the lumen of swim bladder was recorded in some examined cases (Fig. 5). While in other cases , focal desquamation of the lining epithelium, submucosal mononuclear inflammatory cellular infiltration particularly lymphocytes and focal hemorrhages with presence of cyst like structure lined by epithelial cells and filled with pink homogenous material were found (Fig. 6).Thickening of the swim bladder wall due to fibrous connective tissue proliferation that gave positive reaction with Crossman's trichrome stain was prominent in most examined cases (Fig. 7 & 8). Moreover , few number of larvae invaded the wall of swim bladders that gave positive reaction with PAS stain were also seen (Fig. 9 & 10).

Morphological characters of the collected *Pseudergasilus zacconi*:

Family : Ergasilidae (Yamaguti , 1936.) Pseudergasilus (Yamaguti , 1936). *Pseudergasilus zacconi* (Yamaguti , 1936 and Nakajima and Egusa ,

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1973)

Adult female copepodes collected from the gills of *A. anguilla*; the body is elongated and gradually constrict toward the posterior end. Cephalon fuses with the body and appears indistinctly set apart from each other. The boundary between the cephalon-body and the reproductive segment is distinct, but the boundary between the reproductive segment and the abdominal segment is unclear. The 5th pair of the legs absent . Body length (from the tip of head to the end of abdominal segment) range from 1.10 to 1.86 mm . The width of head-trunk section measure 0.26-0.30 mm. Genital segment is larger than the abdominal segment. Abdomen composes of three segments. Furcal rami well developed , cylindrical and each furcal ramus ends in two unequal bristles. The first antenna composes of six segments with three terminal bristles. The 2nd antenna consists of four segments and its terminal segment is deformed into a massive, sickle- shaped claw. Four pairs of thoracic legs are well developed and biramus , each ramus compose of three segments , except exopodite of 4th pair had only two segments. Ovisacs are narrow, cigar shaped , measure 2.5-3.25 mm X 0.20- 0.25 mm. The egg have a diameter of 62-79 microns (Fig. 11 & Plate II).

The number of spins and bristles on individual segment of four pairs of thoracic legs is shown in the following table.

Legs	Exopodite						Endopodite					
	1st segment		2nd segment		3rd segment		1st segment		2nd segment		3rd segment	
	Spine No.	Bristle No.	Spine No.	Bristle No.	Spine No.	Bristle No.	Spine No.	Bristle No.	Spine No.	Bristle No.	Spine No.	Bristle No.
1st pair	0	1	1	2	1	5	0	1	1	2	1	5
2nd pair	0	1	1	2	1	5	0	1	1	2	1	5
3rd pair	0	1	1	2	1	6	0	1	1	2	1	5
4th pair	0	1	1	6	-	-	0	1	1	2	1	9

Post-mortem findings:

The gross examination of infested gills showed elongated grayish white nodules firmly attached to the gill filaments and grayish white patches on gills with mass of thick mucous secretion especially in heavy infestation.

Histopathological findings:

The microscopical examination of gills of *A. anguilla* infested with Pseudergasilus zacconi showed presence of some parasitic elements in-between destructed gill filaments and attached to gill arch (Fig. 12) Severe congestion of blood vessels with extravasations of erythrocytes in

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Fig. (1): *Anguillicola crassus* 1- Anterior part of adult worm.
2-Posterior part of adult female
3-Posterior part of adult male

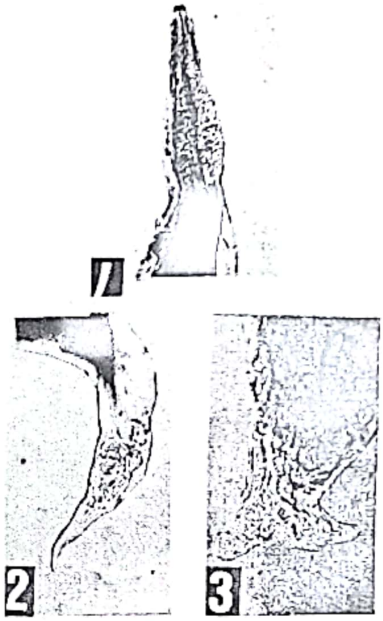

Fig. (2) *Adult Anguillicola* crassus (Fresh specimens from swim bladder).

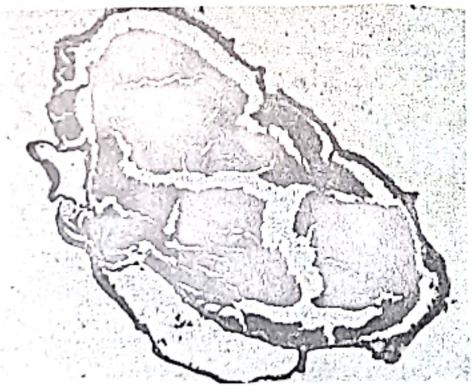

Fig. (3) Swim bladder of *Anguilla anguilla* infected with *Anguillicola crassus* showing presence of cross section of adult worm contained large number of erythrocytes in their body cavities. H & E stain X 100.

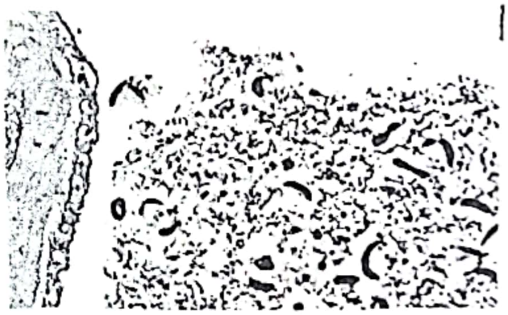

Fig. (4) Swim bladder of *Anguilla anguilla* infected with *Anguillicola crassus* showing numerous ensheathed larvae mixed with destructed erythrocytes within the lumen of swim bladder. H & E stain X 200.

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Fig (5): Swim bladder of *Anguilla anguilla* infected with *Anguillicola crassus* showing hyperplasia of the mucosal epithelial cells forming finger like projection within the lumen . H & E stain X 200.

Fig.(6): Swim bladder of *Anguilla anguilla* infected with *Anguillicola crassus* showing desquamation of the lining epithelium , hemorrhage and mononuclear inflammatory cellular infiltration with presence of cyst like structure filled with homogenous pink material . H & E stain X 200.

Fig. (7): Swim bladder of *Anguilla anguilla* infected with *Anguillicola crassus* showing thickening of the swim bladder wall due to fibrous connective tissue proliferation. H & E stain X 100.

Fig. (8): Swimbladder of *Anguilla anguilla* infected with *Anguillicola crassus* showing thickening of the swim bladder wall due to fibrous connective tissue proliferation. Crossman's trichrome stain X 100.

Fig. (9) Swim bladder of *Anguilla anguilla* infected with *Anguillicola crassus* showing some larvae invaded the wall of swim bladder. H & E stain X 200.

Fig. (10) Swim bladder of *Anguilla anguilla* infected with *Anguillicola crassus* showing larval invasion of the wall of swim bladder. PAS stain X 400.

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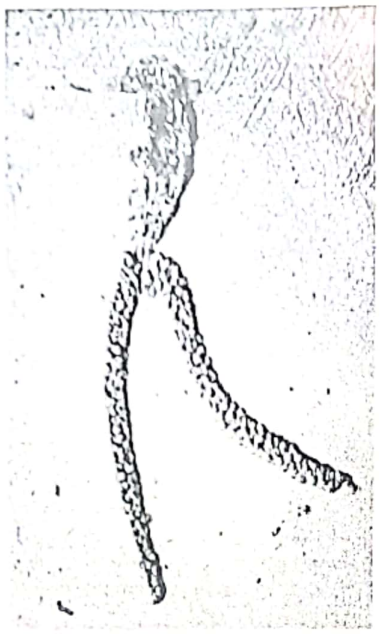

Fig. (11): Adult female of *Pseudergasilus zacconi*

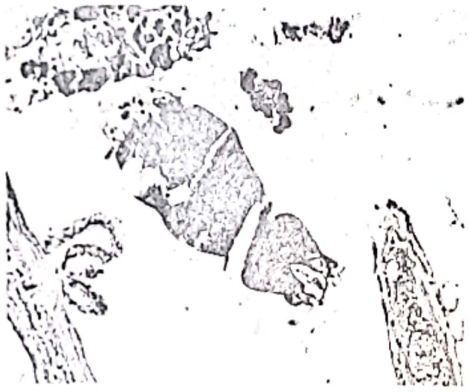

Fig. (12): Gill of *Anguilla anguilla* infested with *Pseudergasilus zacconi* showing presence of some parasitic elements inbetween gill filaments. H & E stain X 100.

Fig (13): Gill of *Anguilla anguilla* infested with *Pseudergasilus zacconi* showing diffuse extravasation of erythrocytes in the gill filaments. H & E stain X 200.

Fig. (14): Gill of *Anguilla anguilla* infested with *Pseudergasilus zacconi* showing hyperplasia of the epithelium of gill filaments and fusion of lamellae. H & E stain X 200.

Fig. (15): Gill of *Anguilla anguilla* infested with *Pseudergasilus zacconi* showing aggregation of melanin carrying cells. H & E stain X 200.

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gill filaments were noticed (Fig. 13). Hyperplasia of mucous cells and epithelium of gill filaments with focal fusion of lamellae were seen (Fig. 14). Moreover, inflammatory cellular infiltration of gill arch mostly lymphocytes and melanin carrying cells were also detected (Fig. 15).

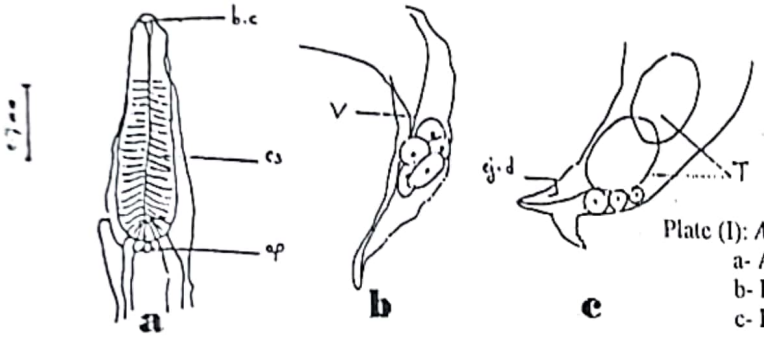

Plate (I): *Anguillicola crassus*
a- Anterior end
b- Posteror and of female
c- Poster and of male
ap- PAppendix
bc- Buccalcapsule
bj- Ejaculating duct
ej.- Ejaculating duct
es- Esophagus
t- Testes
v- Vagina

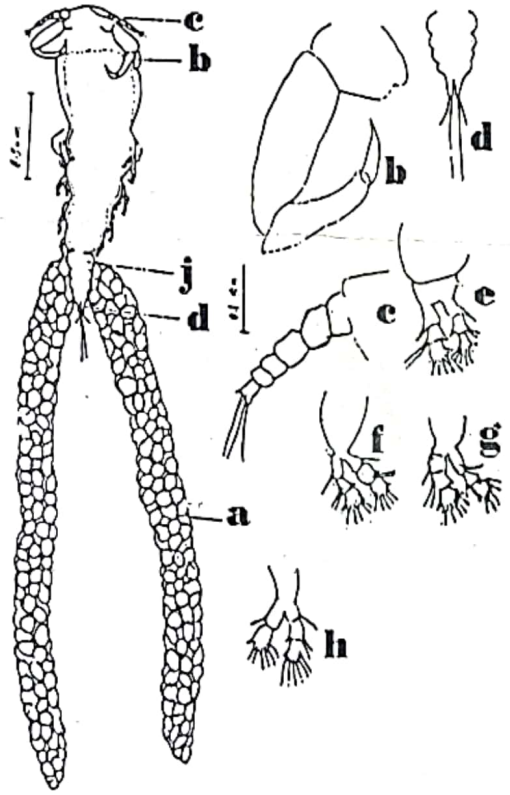

Plate (II): Adult female of *Pseudergasilus zacconi*
a- Egg sac b- Antenule c- Antena d- Furcal rami
e- 1st thoracic leg f- 2nd thoracic leg g- 3rd thoracic leg h- 4th thoracic leg
i- Abdominal segment

DISCUSSION

It is necessary to mention that, all the previous works on parasites among eels in Egypt were not enough and dealt with one or two parasites and according to the available literature there is no investigation of spreading of *Anguillicola crassus* infection among *Anguilla anguilla* in Egypt. So this study could be the first work dealing with anguilliclosis in Egypt with reference to the prevalence of infection and the associated pathological changes in the swim bladder of infected *A. anguilla*.

In the present study, the morphological description of the detected *Anguillicola crassus* in the present work coincided with the description given by Kuwahara et al., (1974) , Barus et al., (1999) and Borgsteede et al., (1999). The incidence of anguillicolosis among the examined *A. anguilla* in the present work was 30 %. This result was in accordance with that mentioned by Pilcher and Moore (1993) and Barse and Secor (1999). Meanwhile our incidence was lower than those recorded by Barus et al., (1996) , Cardoso and Saraiva (1998) and Wurtz et al., (1998). The intensity of the recorded parasites ranged from 1- 15 worms per fish. These findings were partially in agreement with that reported by Egusa (1992) and Cardoso and Saraiva (1998) , and were higher that that mentioned by Pilcher and Moore (1993) and Wurtz et al., (1998) and were lower than that recorded by Schabuss et al., (1997).

The infected *A. anguilla* in this study showed sings of abdominal swelling and multiple hemorrhagic areas on the abdominal wall. Emaciation and anemia were observed and could be attributed to intensive blood suckling activity of the worm and the sequllae of hemorrhage due to parasitosis. These findings were also reported by Egusa, (1992) and Chung et al., (1996) . Grossly the wall of the swim bladders of infected eels was thickened, sometimes hemorrhagic and their luminae filled with chocolate like material. This finding was nearly similar with those reported by Chung et al., (1996) , Kean et al., (1996) and Wutz and Taraschewski (2000).

Regarding the pathological changes in swim bladders of infected *A. anguilla*, cross sections of adult worms and numerous ensheathed larvae mixed with digested erythrocytes were seen in their luminae. The presence of these larvae may be due to rupture of uterus of adult female inside the lumen. This opinion was supported by those reported by Hirose et al., (1976) , Egusa (1992) and Chung et al., (1996). Alternative areas of mucosal epithelial hyperplasia and desquamation with congestion of blood vessels and focal areas of hemorrhage within the wall of swim bladder were seen. These pathological changes could attributed to the direct effect of the parasite on the swim bladder tissues. These findings came in accordance with the results of Hirose et al ., (1976) and Molnar et al., (1991 and 1993) . Thickening of the swim bladder wall due to fibrous

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connective tissue proliferation and inflammatory cellular infiltration particularly lymphocytes were noticed in the present work. Similar findings were also recorded by Chung et al., (1996) and Wurtz and Taraschewski (2000). Moreover, larval invasion of the wall of swim bladders which associated with connective tissue proliferation was also recorded in our study. This finding was in complete accordance with Wurtz and Taraschewski (2000) and in a partial agreement with Molnar (1994) and Molnar et al., (1995) who recorded granulomatous reaction surrounding the larvae within the wall of swim bladder of eels infected with Anguilicola crassus. These changes considered to be an advanced lesions which not recorded in our work.

Again , the literature concerning *Pseudergasilus zacconi* infestation among eels in Egypt are almost lacked. So the adult female of *Pseudergasilus zacconi* was detected for the first time from the Egyptian locality.

The morphological characters of the recorded *Pseudergasilus zacconi* were similar to that reported by Yamaguti (1936) and Nakajima and Eugsa (1973).

Gross examination of the infested gills in this study showed presence of parasites in the form of grayish white elongated nodules firmly attached to the gills, which accompanied with increase the mucous secretion. Nearly similar results were also observed by Nakajima et al., (1974) in *Plecoglossus altivelis* infested with *Pseudergasilus zacconi.* Concerning the histopathological changes in the infested gills numerous parasitic elements in-between destructed gill filaments with severe congestion of blood vessels and diffuse extravasations of erythrocytes in both gill lamellae and arch were detected. These changes cleared the destructive effect of the copepodes on the gill structures. Moreover, proliferation of mucous and epithelial cells with focal lamellar fusion and inflammatory cellular infiltration of gills that reflected the mechanical irritant effect of the parasites at its point of attachment were observed. These observations were nearly in agreement with the results of Nakajima et al., (1974) in *Plecoglossus altivelis* infested with similar copepdes.

In conclusion, both *Anguillicola crassus* and *Pseudergasilus zacconi* infection had adverse effect on health of *A. anguilla* leading to death in severe cases. This effect appears as great thickening of swim bladder wall due to mucosal epithelial cells hyperplasia , fibrous connective tissue proliferation and inflammatory cellular infiltration in anguilliclosis and severe damage of gill tissues that cause disturbance in the respiratory function of the gills in pseudergasilosis.

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