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Incidence of Intestinal Parasitic Worms of Spotted Grouper (*Epinephelus coioides*) Collected from Kuwait

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***ABSTRACT*: This study dealed with the prevalence of endoparasitic worms that infect the gastrointestinal tract of Spotted groupers (*Epinephelus coioides*) collected from Kuwait. Trematodes were found in *Prosorhynchus pacificus* with the incidence (8.3%), *Prosorhynchus epinepheli* with infection rate (11.6%) and in *Prosorhynchus jexi* with rate (6.6%). Nematodes were found in *Hysterothylacium epinepheli* with rate (5%) and in *Cucullanus epinepheli* with rate (10%). Acanthocephalan parasites *Rhadinorhynchus sp.* with rate of infection (8.3%). The total infection rate was 53.3 % of examined fish sample. *P. epinepheli* represent the highest prevalence percent while the rate of *H. epinepheli* represent the lowest rate of infection. The histopathological examination of the examined fish intestine revealed some pathological symptoms such as degeneration, necrosis and fibrosis in the site of infection.**

KEYWORDS: Grouper, Helminths, Incidence, Kuwait.

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I. INTRODUCTION

The Orange-spotted Grouper *Epinephelus coioides* was considered the most preferred fish species in Kuwait fish markets and is the most valuable grouper species among other species in the Gulf **(Al-Baz *et al.*,2018)**. The Orange-Spotted grouper, *Epinephelus coioides*, is economically significant marine teleost **(Chen *et al.* 2005).** The orange-spotted grouper colouration is produced by different color pigments in skin epidermal and dermal layers which are largely contained within cells called chromatophores. The erythrophores have erythrin pigments (red), xanthophores contain xanthin pigments (yellow) and melanophores have melanin pigments (black) (**Attia *et al.* 2023)**. However, when commercial grouper aquaculture expands, different stressors are increased as a result of high-density breeding, which creates negative changes in the environment and increases the susceptibility of fish to illnesses. Wild and cultured grouper could be infected by different protozoan parasites, trematode and nematode which in turn makes the muscle of fish rejected by the consumer **(Attia *et al.* 2023).**

Although studies on parasitic worms are important in controlling the impact of such parasites on fish health and fish production **(Abdel-Ghaffar *et al,* 2013)**. Fish parasites of groupers from marine waters have been of special interest in recent years as the groupers are of high commercial value **(Kleinertz and Palm,2013)**. **Bray & Cribb, 2012** reported some species of *Prosorhynchus* from *Epinephelus spp*., as *P. jexi*, *P. pacificus*, *P. epinepheli*. There were about 43 valid species of *Rhadinorhynchus* Lühe, 1911, and 20 species which are now known from the Pacific Ocean off Australia, Japan, and Vietnam **(Omar *et al,* 2019b)**. Some endoparasite worms found in the gastrointestine of several species of groupers are different species of *Prosorhynchus, Camallanus carangis*, *Procamallanus variolae* and *Neoechinorhynchus* sp **(Boot and Cribb, 2009; Justine et al., 2010)**. *Prosorhynchus pacificus* was isolated from intestine of Epinephelus *tauvina* collected from Arabian Gulf, fish market Kuwait. The total prevalence of infection was 25% while the intensity of infestation was 6.3 % **(Nahhas et al. ,2006)**. *Prosorhynchus epinepheli* was detected from intestine of *Epinephelus areolatus* collected from Arabian Gulf, fish market Kuwait. *Prosorhynchus jexi* was reported from the *Epinephelus spp*. on the Great Barrier Reef, Australia. **(Bott and Cribb, 2009)**. *Hysterothylacium* sp was isolated from the abdominal cavity and the intestine of *T. draco* collected from the Bay of Bizerte **(Azizi *et al,* 2017**). parasitic nematodes *Cucullanus epinepheli* sp. (Cucullanidae) isolated from the intestine of the brown spotted grouper *Epinephelus chlorostigma* on light and electron microscopically scanning. **(Moravec and Justine, 2017)**. About 35 species of *Rhadinorhynchus* were described from 36 fish species. The characteristic features to distinguish them between other species are Proboscis and trunk spination **(Smales, 2014).**

Little studies had been done on these parasites and poorly investigated So, the present study aims to investigate the prevalence of endoparasitic worms that infect the gastrointestinal tract of Spotted groupers (*Epinephelus coioides*) collected from Kuwait and the pathogenicity of these parasites.

II.MATERIALS AND METHODS

I-Fish samples and study area:

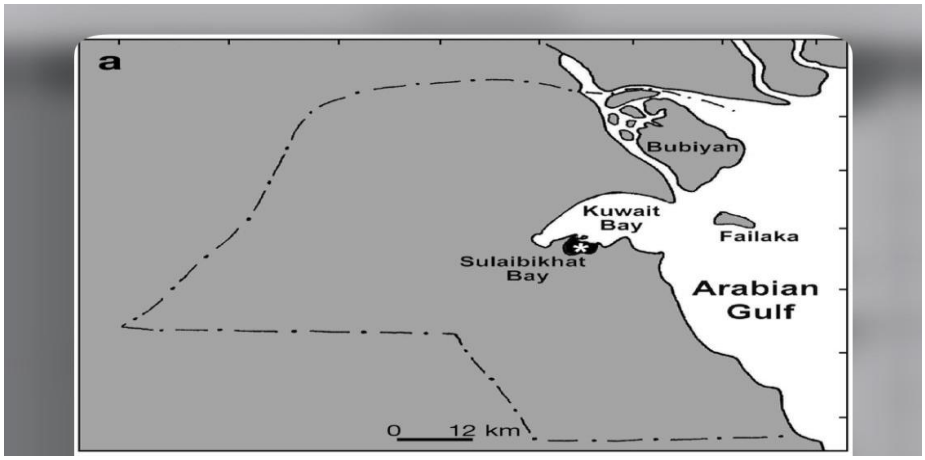


Fig (1): showing a map to Arabian Gulf & Kuwait Bay(Google Map).

About 60 Spotted Grouper fish (Epinephelus sp) were collected freshly dead from Arabian gulf at Kuwait bay during the period from February to November 2019. The post mortem examination and parasitological examination was done at the veterinary and agriculture laboratory center, public Authority for Agriculture Affairs and Fish Resources, Kuwait.

II- Methods:

The stomach and intestine of each sample were removed and placed in petri dish for examination, the contents were washed by physiological saline then search by magnifying lens for helminths The worms were counted and preserved on 70% ethanol for staining and identification. The recovered digenean parasites stained with a simple and rapid technique using lacto phenol cotton blue (LPCB) according to the method of (Henedi and El-Azazy,2013). The adult trematodes were identified according to (Madhavi and Bray,2018), (Manter, 1940) and Hafeezullah & Siddiqi, 1970a). In addition, the recovered nematodes and acanthocephalans were cleared and mounted in polyvinyl Lacto phenol and identified according to (Yamaguti S, 1941) and (Moravec and Justine, 2017,2020).

Tissue specimen's intestine of the examined fishes were taken and fixed in 10% neutral-buffered formalin. The tissue specimens were dehydrated, embedded in paraffin wax and cut in 4-6µm thick sections afterward stained with haematoxylin and eosin according to the method described by Bernet et al. (1999). These sections were examined microscopically and their photos were taken by the microscopic camera. This method was cited by Tayel et al. (2020).

III. RESULTS AND DISCUSSION

1- Clinical signs and postmortem examinations:

The infected fishes with intestinal parasites showed no apparent external lesion, the intestinal wall was distended intestine with yellowish exudates and congested.

Fig (2): showing *Epinephelus coioides* examined fish.

2- Morphological identification of isolated parasites from naturally infested fishes:

Digenetic adult trematode:

subfamily: Prosorhynchinae Nicoll, 1914.

Genus Prosorhynchus Odhner, 1905.

Species: *Prosorhynchus pacificus* Manter, 1940:

Host: spotted grouper (*Epinephelus coioides*)**.**

Description:

The worm has elongated body, blunted at anterior end, its Length reach to 1.5 ml. The mouth located between 1/3 body length from anterior end. Caecum extends anteriorly. Testes large and located near midbody, nearly spherical. The ovary is spherical, at level of the pharynx and overlaps anterior testis. Cirrus sac is short and coiled, Uterus entirely postovarian extend anteriorly from mid-level of caecum to genital opening posteriorly. Vitellarium are in two lateral groups each has 15-20 follicle extend from cecum and rhynchus base. Eggs extend from level of ovary to just anterior to caecum. Excretory pore terminal. As shown in **Fig (3,4,5)**

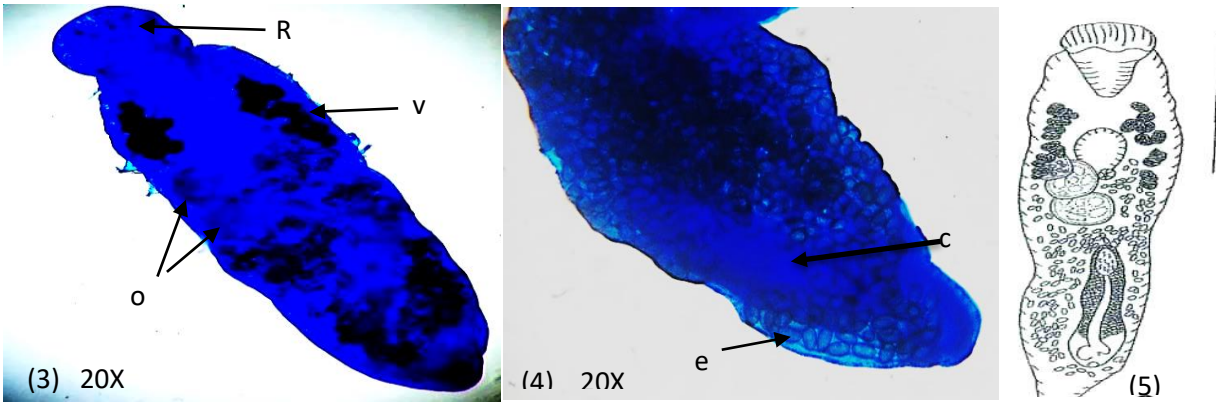


Fig (3,4) showing *Prosorhynchus pacificus* isolated from spotted grouper and stained with cotton blue stain 20X; (R) Rhynchus, (v) vitellarium, (ov) ovaries, (c) cirrus sac, (e)eggs. **Fig** (5) Diagram of *Prosorhynchus pacificus*

I.1.1.2. *Prosorhynchus epinepheli* Yamaguti, 1939.

Host: spotted grouper (*Epinephelus coioides*) & Silver grunt *Pomadasys*.

Description:

The worm body is fusiform its length is about 1 ml. Rhynchus is triangular. The pharynx presents near junction of testes. Gonads are present near midbody and anterior to cirrussac. Cirrus-sac reaches to posterior testis from genital opening. Testes are slightly diagonal. The ovary presents at level of anterior testis mainly intertesticular. Uterus extends anteriorly under the level of caecum, but not anterior to level of vitellarium. Vitellarium is present in two lateral groups of follicles which extends from level of testis and forming an arch in region between caecum and rhynchus. The excretory vesicle tubular and extends to level of gonads. As in **Fig (6,7,8,9)**

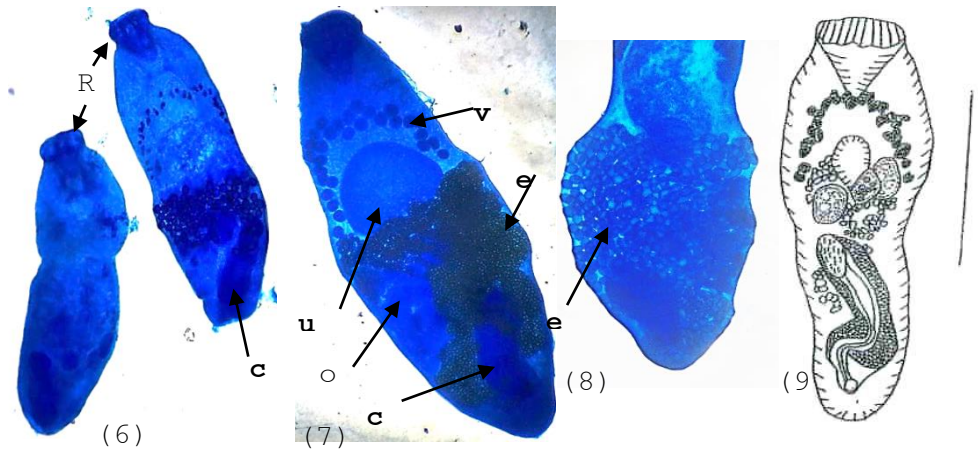


Fig (6,7,8) showing *Prosorhynchus epinepheli* isolated from spotted grouper and stained with cotton blue stain 20X; (R) Rhynchus, (c) cirrus sac, (v) vitellarium, (u) uterus, (ov) ovaries, (e)eggs. **Fig** (9) Diagram of *Prosorhynchus epinepheli*

1.1.1.3. *Prosorhynchus jexi*.

Host: spotted grouper (*Epinephelus coioides*)

Description:

The worm body is fusiform, widest at level of caecum the body length is 0.5 m. The rhynchus is large with well-developed muscles and tapers distally. The pharynx is spherical, muscular and present in anterior half of the worm body. Caecum sac-like, extends anteriorly from pharynx. Two Testes overlapping each other posterior to pharynx they are Ovoid shape. Cirrus-sac is muscular and entirely posterior to testes. Seminal vesicle is elongate in proximal portion of cirrus-sac. The genital pore is subterminal on ventral surface of worm. the ovaries are ovoid, small, at level of pharynx and overlaps each other but anterior to anterior testis. Vitelline follicles present in two lateral fields and beginning at level of mid-caecum along longitudinal mid-line reach to anterior quarter of the worm body. Uterus extends just posteriorly of vitelline follicles reaches beyond posterior margin of cirrussac. Eggs are small and numerous. As shown in **Fig (10,11,12)**

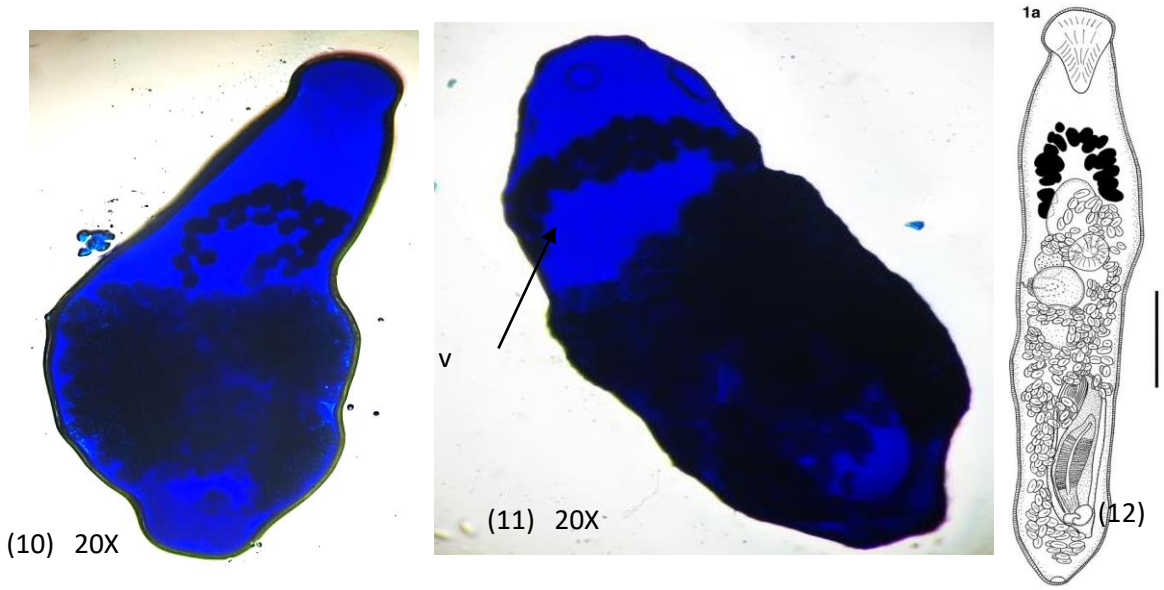


Fig (10,11) showing *Prosorhynchus* jexi isolated from spotted grouper and stained with cotton blue stain; (v) vitellarium. **Fig (12)** Diagram of *Prosorhynchus* jexi

II.1.2. Genus: Hysterothylacium Ward & Magath, 1917

II.1.2.1. Species: *Hysterothylacium epinepheli* (Yamaguti,1941):

Host: spotted grouper (*Epinephelus coioides*).

Synonyms: *Contracaecum tasmaniense* Johnston & Mawson, 1945

Description:

Fourth-stage larvae of *Hysterothylacium* sp. are small worms, with smooth cuticle with prominent cervical rings and distinct lateral alae along each of body side just posterior to lips and pre-cloacal region. fourth larvae have developing3 lips, dorsal lip slightly smaller than subventrals, with prominent lateral flanges but lacking boring tooth. Interlabia is well developed, about 1/2 length of lips. Its esophagus claviform the ventriculus is small and rounded. The tail is long, digitiform, with terminal mucron**.** *H. epinepheli* can be distinguished from other *Hysterothylacium* sp by presence of Spicules 0.6–0.8mm long and having lateral alae and a relative long intestinal caecum. As shown in **Fig (13,14,15,16)**

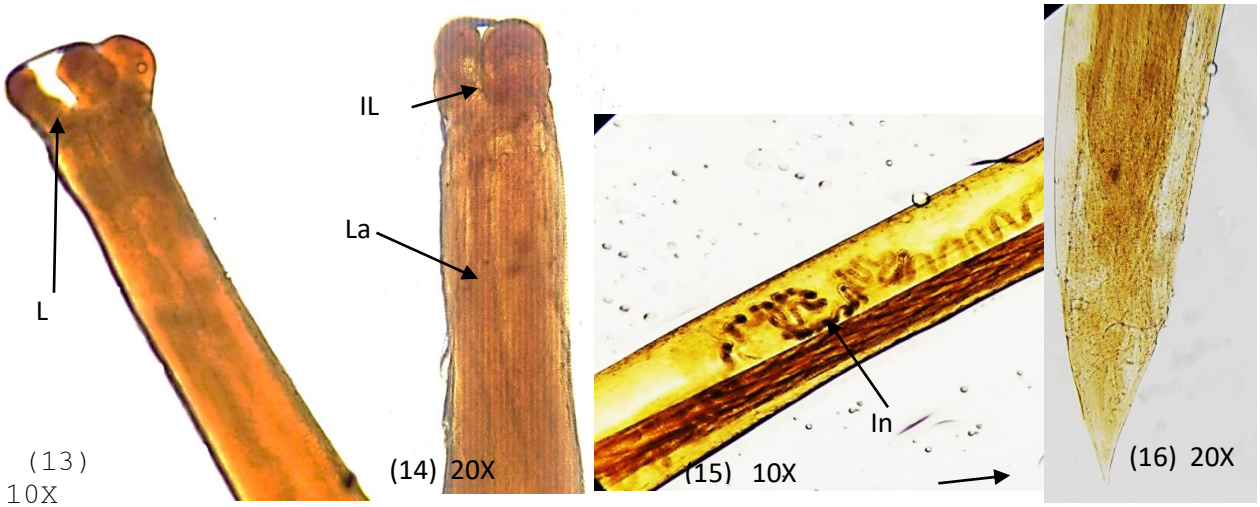


Fig (13,14,15&16): showing *Hysterothylacium epinepheli* isolated from *Epinephelus coioides* intestine;(L) Anterior end with 3 lips, (La)Lateral alae, (IL) Interlabia, (In) long intestine.

II.2. Family Cucullanidae:

II.2.1. Species: *Cucullanus epinepheli* sp.:

Host: spotted grouper (*Epinephelus coioides*)

Description:

Medium-sized nematodes. Body whitish, elongate, 9–9.9 mm (9.5 mm) long, narrow lateral alae present, extending from approximately level of base of pseudo buccal capsule to level of posterior esophagus. The oral aperture surrounded by raised narrow membranous ala supported by row of minute basal teeth. Presence of four sub median cephalic papillae. Esophagus is muscular, expanded at anterior end and form bulbous pseudo buccal capsule (esophastome); posterior part of esophagus also expanded, broader than esophastome, the length of entire esophagus representing 13–14% (14%) of whole body length. Esophagus opens into intestine by a large valve. Presence of nerve ring. Excretory pore just posterior to esophago- intestinal junction. Anterior lip of cloaca elevated and forming large posterior oval outgrowth covering cloacal aperture. Gubernaculum small, rod-like in lateral view. As shown in **Fig (17,18,19).**

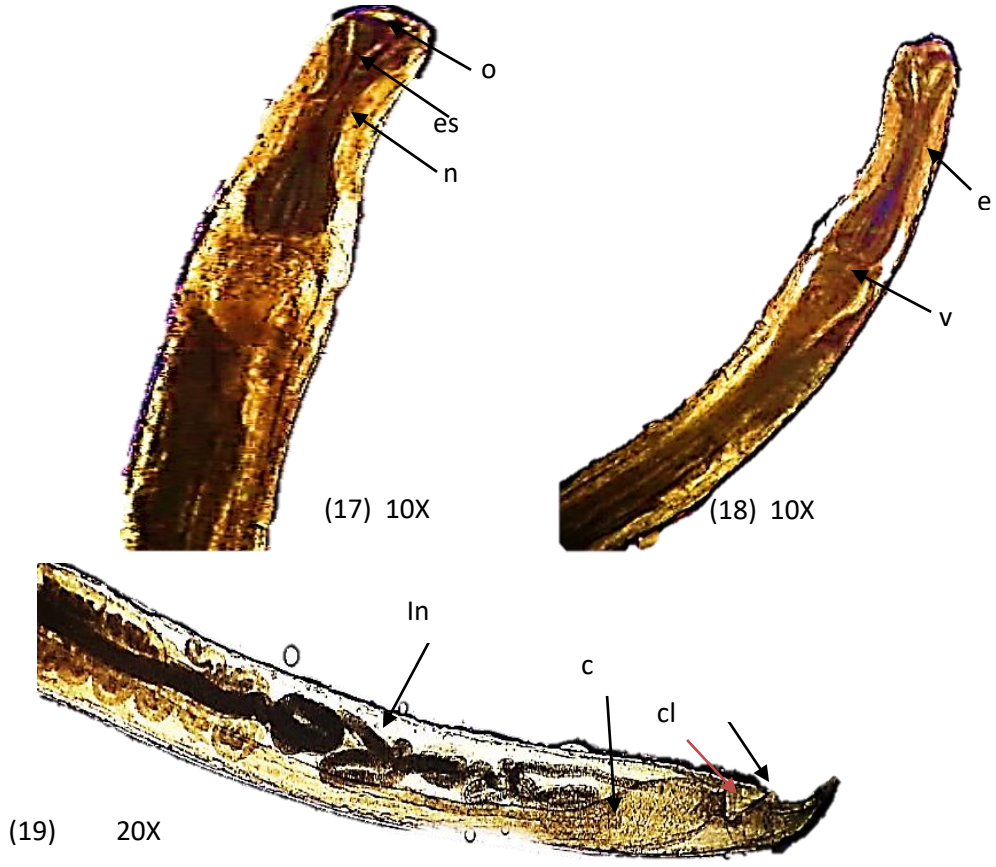


Fig (17,18,19): showing *Cucullanus epinepheli* sp. male isolated from *Epinephelus coioides* intestine, Fig (17,18) anterior part show, (o) oral aperture, (es)esophagus, (e)esophastome, (n) nerve ring, (v)valve. Fig (19) posterior part showing, (In)intestine, (c) cloaca, (cl)cloacal lips, (G) Gubernaculum.

III. Acanthocephalans:

III.1Order: Echinorhynchida

III.1.1 -Genus: Rhadinorhynchus Lühe, 1911,

III.1.1.1. species: Rhadinorhynchus sp.:

Host: spotted grouper (*Epinephelus coioides*)

Description:

The body length is 4-4.5 cm has long cylindrical Trunk which is spines anteriorly. characterized by having trunk spinose with irregular complete spines circles. Proboscis was Cylindrical, slightly wider anteriorly with short neck and It was covered with 12-15 rows of 20-23 hooks each. Hooks of anterior rows were larger than the remaining rows, strongly curved basally. Proboscis receptacle is elongated and double-walled, with two tubulars, elongated Lemnisci on sides of proboscis receptacle. In female gonopore sub-ventral with well-developed vagina Uterus long with elongate and prominent uterine bell with parallel sides. Male Reproductive system situated in posterior half of trunk. Testes elongate, almost equal in size, Spermatozoa carried by sperm duct and collected in a seminal vesicle opens in terminal bursa capped with copulatory plug. As shown in **Fig (20,21,22,23)**.

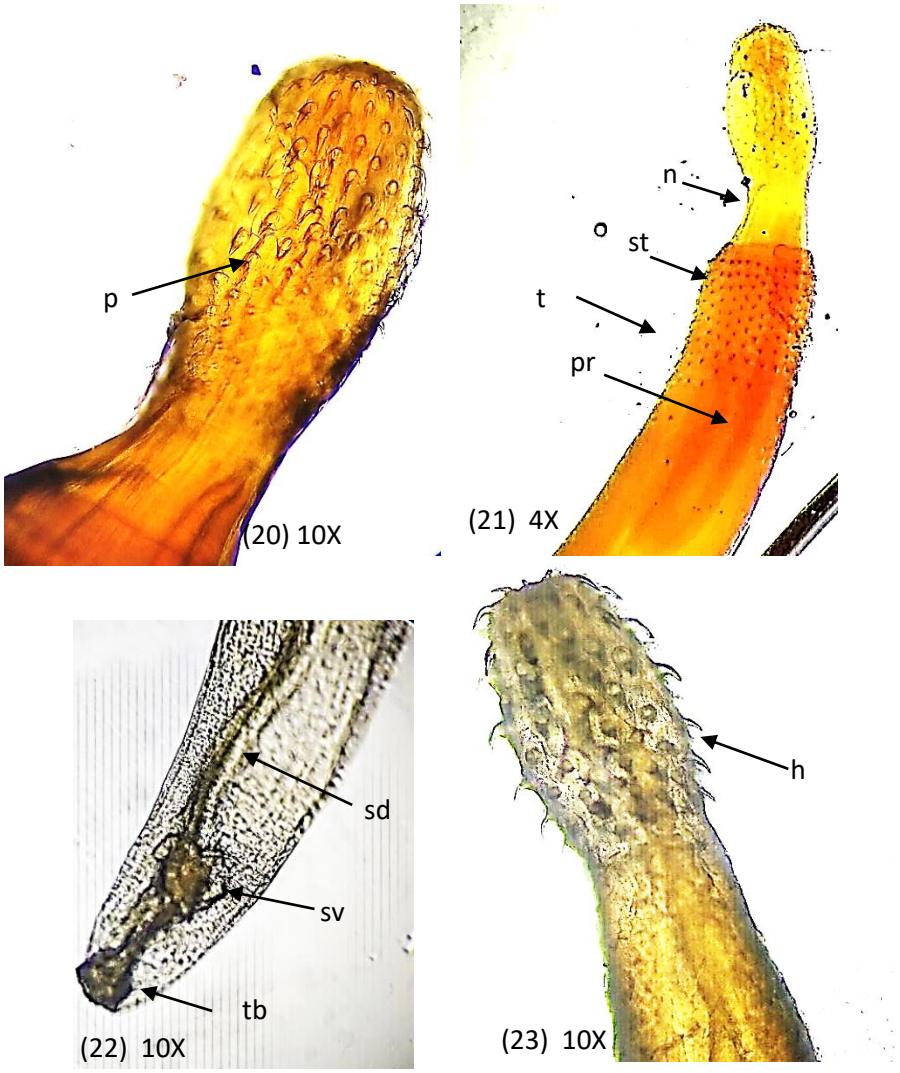
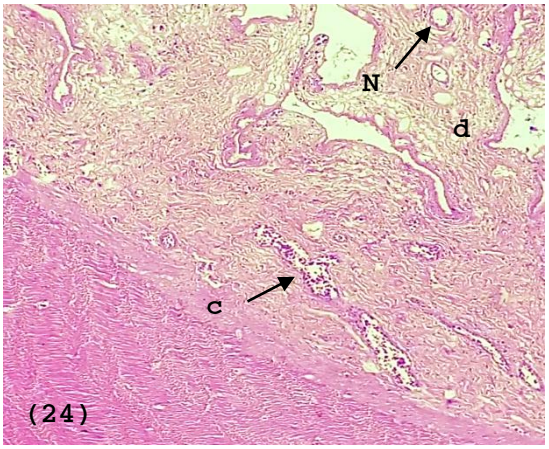
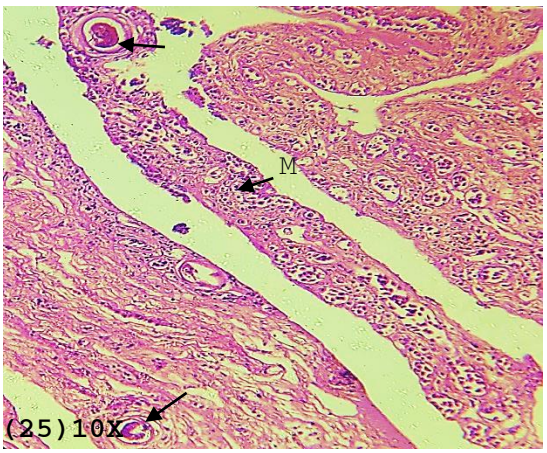


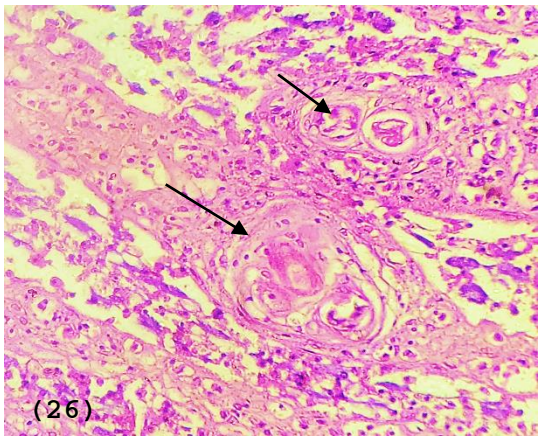
Fig (20,21,22,23): showing *Rhadinorhynchus* sp isolated from *Epinephelus coioides* intestine; Fig (20,21) showing female *Rhadinorhynchus* sp.; Fig (22,23) Male *Rhadinorhynchus sp*.;(p) Proboscis, (t) trunk, (n) neck, (pr) Proboscis receptacle, (st) spinose trunk, (sd) sperm duct, (sv) seminal vesicle, (tb)terminal bursa, (h) hooks.

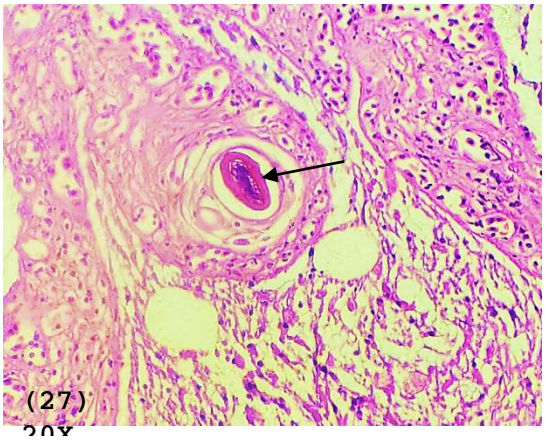
3- Histopathological examination:

histopathological section of *Epinephelus coioides* intestine revealed degeneration, areas of necrosis, mononuclear cell infiltration, congestion of blood vessels and haemorrhage . Inaddition revealed presence of trematodes larvae and nematodes larvae surrounded by area of granulomatous reaction and fibroses tissue.









photomicrograph section of *Epinephelus coioides* intestine, slide (24) revealed congestion of blood vessels (c), degeneration(d) and areas of necrosis. Slide (25&26) revealed presence of trematodes larvae surrounded by area of fibroses tissue and mononuclear cell infiltration(M). Slide (27) revealed nematodes larvae.

4- total Prevalence rate and seasonal prevalence of recovered Parasitis from examined fish species:
60 Spotted grouper *Epinephelus coioides* the total infection rate percent was 53.3 %**Table (1).**
Table (1):- Prevalence rate of recovered Parasitis from examined fish species collected from Kuwait markets:

Fish species	Examined	Infested	%
(*Epinephelus coioides*).	60	32	53.3%

5-The types of detected parasitic species in collected fish species, total prevalence and intensity of infestation:
The total prevalence of each isolated parasite from *Epinephelus coioides*, it was recorded that the *Prosorhynchus epinepheli trematode* recorded highest infection rate 11.6% with intensity of infestation 3 for each sample followed by *Cucullanus epinepheli* nematode10 % with intensity 6 per sample, *Prosorhynchus pacificus* 8.3% and intenisty2 per fish , *Rhadinorhynchus* sp 8.3% and intensity 5 worm, *Prosorhynchus jexi* 6.6% with intensity 3 and the lowest prevalence rate is *Hysterothylacium epinepheli* 5% with intensity 5. **Table (3)**

Table (3): - The detected parasitic species, their prevalence and intensity of infestation in the examined fish species.

Fish species	Type of parasite	Total incidence %	Intensity of infestation per fish
Spotted grouper (Epinephelus coioides).	*Prosorhynchus pacificus*	8.3	2
	Prosorhynchus epinepheli	11.6	3
	Prosorhynchus jexi	6.6	3
	Hysterothylacium epinepheli	5	5
	Cucullanus epinepheli	10	6
	Rhadinorhynchus sp	8.3	5

Discussion:

Groupers scientifically known as *Epinephelus spp*., are one of the most important commercial marine fish species which have high market value and demands. Parasites diseases of fish is one of the major problems tackling aquaculture industry. Pathological conditions arising from parasites infection might cause severely harm to fishes especially under stress conditions as it feed on its host nutrients leading to physiological, immunological or behavioral changes **(Mohammed *et al*.,2017)**.

The morphological description of *Prosorhynchus pacificus* trematode species isolated from spotted grouper (*Epinephelus coioides*) revealed that the worm has elongated body which is blunted at anterior end, it reaches1.5 mm in Length, the present morphological identification was in full agreement with (**Madhavi and Bray, 2018)**

Prosorhynchus epinepheli species isolated from the spotted grouper (*Epinephelus coioides*) revealed that it has fusiform body. Its length is about 1 mm this description go hand with hand with **(Nahhas et al., 2006)**.

The *Prosorhynchus jexi* trematode species which were collected from the intestines of spotted grouper (*Epinephelus coioides*) has fusiform body, widest at level of caecum the body length is 0.5 ml, Similar to findings were discussed by **(Bott and Cribb, 2009)**.

Hysterothylacium epinepheli nematode species collected from spotted grouper (*Epinephelus coioides*) revealed that it was small worms, with smooth cuticle with prominent cervical rings and distinct lateral alae. It has developing three lips, but lacking boring tooth. *H. epinepheli* can be distinguished from other *Hysterothylacium* sp by presence of Spicules, lateral alae and a relative long intestinal caecum. This description is Similar to findings were discussed by **(Borges et al, 2012)**.

The *Cucullanus epinepheli* nematode was collected from intestine of spotted grouper (*Epinephelus coioides*) it was characterized by Medium-sized whitish Body, the oral aperture surrounded by raised narrow membranous ala supported by row of minute basal teeth, this description was in agreement with **(Moravec and Justine, 2017).** *Cucullanus sp.* was isolated from Scatophagus argus fish intestine and Characteristic features are a long, slender body, a thick cuticle and the dorsal-ventrally elongated oral opening surrounded by a row of numerous minute teeth **(Rueckert *et al*.,2008)**

Acanthocephalan parasite *Rhadinorhynchus sp.* were collected from the intestines of spotted grouper (*Epinephelus coioides*) its body was long cylindrical Trunk which is spines anteriorly. characterized by having trunk spinose with irregular complete spines circles. Proboscis was Cylindrical, slightly wider anteriorly with short neck, Similar findings were discussed by **(Omar and Richard, 2017), (Omar *et al*, 2019a)** and **(Omar *et al*, 2019b).**

Concerning the total prevalence of the detected parasites among the examined fishes; the total infestation rate percent in the Spotted grouper *Epinephelus coioides*, is about 53.3 %. This was nearly similar to the finding recorded by **(Azizi *et al*, 2017)** while results are more than what recorded by **(Agustina *et al*., 2018)**. the relatively high rate of infestation could be attributed to the feeding habits of groupers as it is carnivorous so that it was more likely to be infected by endoparasite worms than herbivores and omnivorous fish this was nearly similar to what revealed by **(Sarjito dan, 2005)**. In the other hand, the presence of invertebrates around the floating net cages acts as intermediate hosts of some endoparasite as discussed by **(Ruckert *et al*., 2009),**

Concerning with the total prevalence and intenisity of infection of each parasite isolated from *Epinephelus coioides*, it was recorded that the *Prosorhynchus pacificus* prevalence rate was 8.3% and intensity 2 per fish this rate was lower than that recorded by **(Nahhas et al. ,2006)** who record (25%) *P. pacificus* from intestine of Epinephelus *tauvina* from Arabian Gulf, fish market Kuwait and 6.3 % intensity of infestation. While *Prosorhynchus epinepheli* recorded highest infection rate of examined grouper (11.6%) with intensity of

infestation 3 for each sample, while **(Nahhas et al., 2006)** recorded 43 % *P. epinepheli* from *Epinephelus areolatus* and 8% intensity of infestation. The prevelance rate recorded of Cucullanus *epinepheli* nematode was 10 % with intensity 6 per sample nearly similar to that recorded by **(Moravec and Justine, 2017)**, In addition, the prevelance rate of isolated acanthocephalan species *Rhadinorhynchus* sp 8.3% and intensity 5 worm, nearly similar to results discussed by **(Morsey *et al.* ,2017)** who isolate *Rhadinorhynchus bicircumspinis* from intestine of *B. bajad* with prevalence (14.28 %) ,4 gravid females and six adult males. The *Prosorhynchus jexi* total prevalence rate was 6.6% with intensity 3. The recorded prevalence rate of *Hysterothylacium epinepheli* was 5% with intensity 5 per fish, this was disagreed with **(Azizi *et al*, 2017)** who record50.5 % prevelance rate of T. *draco* with *H. epinepheli*.

Histopathological sections of *Epinephelus coioides* intestine revealed degeneration, areas of necrosis, congestion of blood vessels, hemorrhage and the presence of trematodes larvae and nematodes larvae surrounded by area of fibroses tissue. This present finding was also reinforced with (**Attia et al., 2021a)** and **(Attia *et al.* 2023)** who recorded granulomatous reaction and mononuclear inflammatory cells infiltration, caused by nematodes infection.

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