

### Some studies on Anisakidae larvae in some marine fish species

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**Abstract:** The present study was designed to investigate the distribution of Anisakis species among some marine fish at Al-Qatef, Eastern province, Saudi Arabia to detect the clinical signs, prevalence, seasonal variation and histopathological alterations in naturally infested fish species. The distribution of parasitic nematode, Anisakis sp. larvae was investigated in 40 host species, only 5 species, *Cephalopholis hemistiktos*, *Epinephelus chlorostigma*, *Epinephelus tauvina*, *Lutjanus ehrenbergi* and *Lutjanus malabaricus* were found susceptible to infestation with Anisakis sp. The distribution of Anisakis sp. in tissues of infested fish species revealed its significant existence in the abdominal muscle, abdominal cavity and liver. The infested fishes showed no any clinical external abnormalities. The total percent of Anisakis sp. larvae infestation among the investigated fish species reached 5.33%. The infestation rates showed seasonal variations, the highest prevalence of infestation among the infested fish occurred during summer (20.45%), followed by spring (19.44%), autumn (10%), winter (5.66%). The histopathological alterations showed the presence of migrating larvae in-between the abdominal muscle fibers and liver tissues of *Cephalopholis hemistiktos*, surrounded with fibrous connective tissues accompanied with of variable inflammatory reactions of the infested tissues.

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**Key Words :** Marine fishes. Anisakis; *C. hemistiktos*; *E. chlorostigma*; *E. tauvina*; *L. ehrenbergi*; *L. malabaricus*; Seasonal variations;. Histo-pathological alterations.

#### Introduction:

*Anisakis* sp. larvae is a worldwide distribution parasite commonly found in the flesh and the body cavity of many species of marine fishes as well as cephalopods that act as paratenic or transport hosts (Eissa, 2002; Choi *et al.*, 2011 and Tantanasi, *et al.* 2012).

Anisakids are ascaridoid nematodes dependent upon aquatic hosts for the completion of their life cycle, which generally involves an array of invertebrates and fish as intermediate or paratenic hosts, and marine mammals or fish-eating birds, reptiles and fishes as definitive hosts (Koinari *et al.*, 2013).

The infective third stage larvae (L3) of *Anisakis* is common in commercially important marine fishes and its presence is of great concern for both public health and economic reasons (Choi *et al.*, 2011 and Tantanasi *et al.*, 2012). The infective larval stage of *Anisakis simplex* is the most important etiologic agent of anisakidosis in humans (Choi *et al.*, 2011).

Human Anisakiasis is a seafood borne parasitic zoonosis caused by larval nematodes of the genus *Anisakis*. Humans are accidental hosts of the nematodes; they become infected by consuming raw or undercooked seafood that harbor the nematode

larvae in their flesh and muscle. The larvae do not further develop in humans; however, they can penetrate the gastrointestinal tract and form eosinophilic granulomas, often with pathologic consequences including sudden epigastric pain, nausea, vomiting, diarrhea and allergic reaction (Audicana and Kennedy, 2008, and Mattiucci, *et al.*, 2013).

Most cases of human infection involve anisakid species belonging to the genus *Anisakis*. There are nine described species of *Anisakis*, which are further subdivided into two types. Type I consists of *Anisakis simplex* sensu stricto (s.s), *A. pegreffii*, *A. simplex* C, *A. typica*, *A. ziphidarum* and *A. nascettii* while Type II consists of *A. paggiae*, *A. physeteris* and *A. brevispiculata* (Mattiucci and Nascetti, 2008; Mattiucci *et al.*, 2009), of these, only *A. simplex* s.s, *A. pegreffii* and *A. physeteris* have been shown to cause infection in humans (Mattiucci *et al.*, 2011; Arizono *et al.*, 2011).

The present study was designed to investigate the distribution of anisakis species among some marine fish at Al-Qatef, Eastern province, Saudi Arabia to detect the clinical signs, prevalence, seasonal variation and histopathological alterations in naturally infested fish species.

**Materials and methods:****Collected Fish:**

At different times between March, 2011 and February, 2012, a total number of 750 marine fish species were collected from Al-Qatef wholesale fish market, Eastern Province of Kingdom of Saudi Arabia. Each sample of fish was weighed and measured. Fish belong to different fish families. Fishes were transported to the laboratory on ice in ice tank, all fishes were measured to the nearest cm for total length, and the weight was recorded to the nearest gm. The whole body cavity and viscera of each sample were carefully dissected and thoroughly examined for anisakids.

**Clinical and post mortem examination:**

Clinical and post mortem examinations were done for all the collected fish species, adopted using the methods described by (Lucky, 1977) for the determination of any lesions or abnormalities on the external body surface and internal of the body.

**Parasitological examination:**

The collected fish was filleted and skinned, thick fillets being sliced lengthwise. Fillets or slices were pressed between two glass plates of 20 x30 cm in size and placed on a candling table over a fluorescent light source. Flesh parasites were easily discernible against the white muscle background. Parasites were removed from the flesh of abdominal muscle, abdominal cavity and liver of the infected fish, counted, relaxed in fresh water or saline solution, flattened, fixed in 70% ethanol and stored in a mixture of 70% ethanol and 5 % glycerol (Lucky,1977).

**Histopathological examination:**

Sections were taken from the affected organs and muscles of diseased fish and fixed in 10% formal saline for twenty four hours. Sections were washed in tap water and passed in serial dilutions of alcohol (ethyl and absolute ethyl) for dehydration. Specimens were cleared in xylene and embedded in paraffin. Paraffin wax tissue blocks were prepared for sectioning at 5-7 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin and examined using light electric microscope (Roberts, 2012).

**Results:****Clinical and post mortem signs:**

Fish infested with anisakis sp. showed no any clinical clear abnormalities and appeared to be normal. The Anisakis larvae were distinguishable by their different shapes, sizes and colours. The Anisakis larvae which were found in the Abdominal cavity of *Cephalopholis hemistiktos*, *Epinephelus tauvina*, *Lutjanus ehrenbergi*, *Lutjanus malabaricus* were free white or yellow in colour, 1 – 2 cm in total length (Fig. 1, A & D). while those which were removed from the abdominal muscle of *Cephalopholis hemistiktos* & *Epinephelus chlorostigma*, were short, brown or black in colour, and 1-1.5 cm in total length, liver highly infected with black or brown coloured larvae of Anisakis sp. which only appeared in *Cephalopholis hemistiktos* (Fig. 1, B), Encapsulated larvae only found in *Epinephelus chlorostigma* & *Lutjanus malabaricus* (Fig. 1, C).

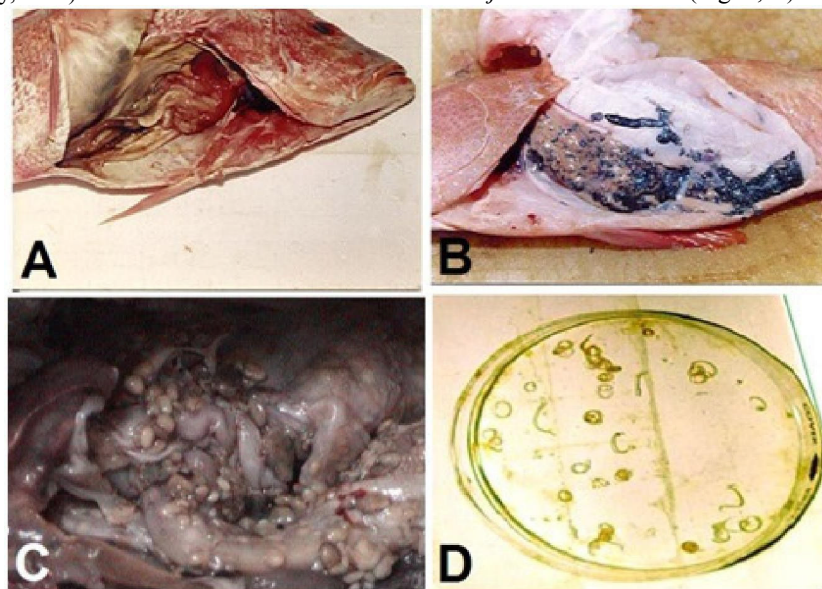


Fig 1. Showing (A) Free Anisakis larvae in the abdominal. Cavity of *Lutjanus malabaricus*. (B) Liver and abdominal cavity of *Cephalopholis hemistiktos* showed heavy infestation with old dead larvae of Anisakis. (C) Viscera of *Epinephelus chlorostigma* heavily infested with Cystic forms of Anisakis. (D) Free Anisakis larvae in petri dish in Saline solution (coiled comma shape larvae).

### Identification of isolated parasites:

The isolated nematode, parasites was identified as anisakis larvae L3. The identification based on the following morphological characters; the shape and the presence of a prominent boring tooth, three lips and excretory pore on the ventral side at the anterior end (Fig.2, A,B,C&D) the shape of the tail and the presence of small prominent mucron, at the posterior end (Figure 3, A,B,C&D) and the shape of the ventriculus (Chai *et al.*,1989 and Smith, 1983).

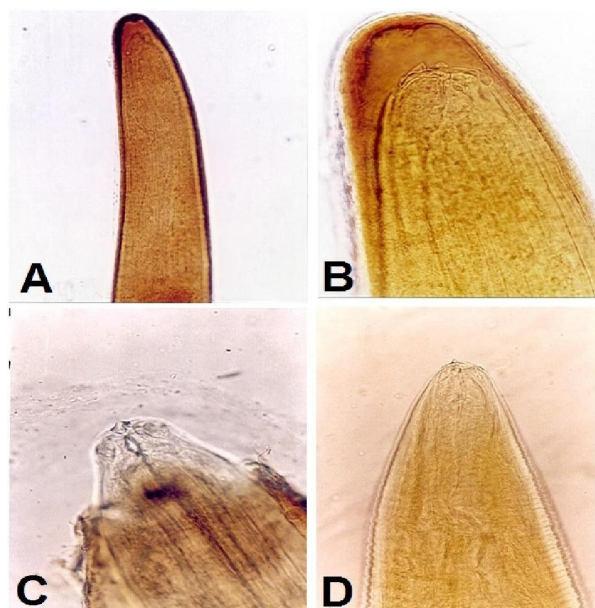


Fig 2. (A&B). Encapsulated larvae of *Anisakis* sp. revealed the characteristic portions in the anterior end, a prominent boring tooth and 1 of 3 lips (C). A prominent Boring tooth and 3 lips in the anterior end of encapsulated larvae of *Anisakis* sp. (D) Free larvae of *Anisakis* sp. revealed the prominent boring tooth at the anterior end.

### Prevalence of *Anisakis* infestation:

The results of the present study demonstrated that the overall percentage of *Anisakis* sp. infestation among examined fish species reached 5.33%. However, the infestation percentages in *Cephalopholis hemistiktos*, *Epinephelus chlorostigma*, *Epinephelus tauvina*, *Lutjanus ehrenbergi* and *Lutjanus malabaricus*, reached 27.77, 10, 12.5, 22.22 and 11.90 respectively, while in the remainder of the examined fish species were 0 % Table (1).

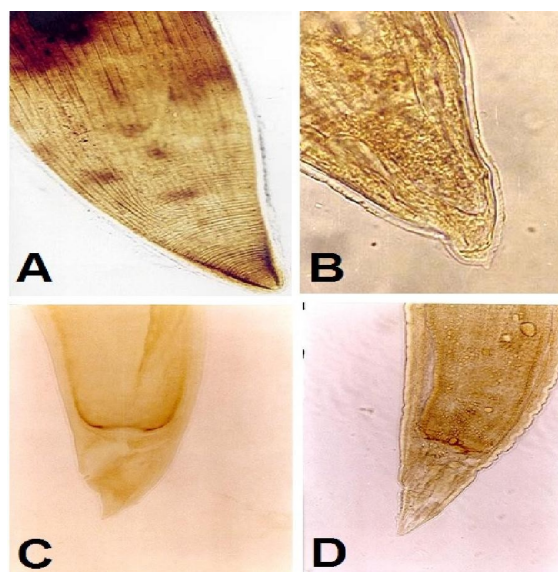


Fig 3. Showing (A&B&C and D) posterior part of encapsulated larvae of *Anisakis* sp. Small prominent mucron at the posterior end of free larvae of *Anisakis* sp.

### Infestation and Size of fish:

Present study revealed that there was a relationship between the size of the fish and its infestation with *Anisakis* larvae. The results recorded that, the infestation detected in *E. chlorostigma* and *E. tauvina* at more than 68 cm, and 70 cm respectively. Infested *Cephalopholis hemistiktos* and *Lutjanus malabaricus* species were more than 35 cm while the smallest infested species was *Lutjanus ehrenbergi* more than 28 cm. It is noticed that medium and large size were only infected table (1).

### Seasonal prevalence:

Results clarifies that the seasonal variations of *Anisakis* sp. infestation rates. Generally, infestation peaked during the Summer season (20.45%), followed by the Spring season (19.44%), Autumn (10 %) and Winter (5.66%) with some variations among the *Cephalopholis hemistiktos* Table (2).

Table 1. Showing occurrence of Anisakis sp. larvae in local and imported marine fishes

Species	Length ( cm )	No. Exam.	No. Infect.	%
<i>Acanthopagrus berda</i>	29 - 69	14	0	0
<i>Acanthopagrus bifasciatus</i>	28 - 39	15	0	0
<i>Arius thalassinus</i>	48 - 51	12	0	0
<i>Caesio sp.</i>	30 - 38	11	0	0
<b><i>Cephalopholis hemistiktos</i></b>	<b>20 - 46</b>	<b>36</b>	<b>10</b>	<b>27.77</b>
<i>Cheimerius nufar</i>	27 - 41	13	0	0
<i>Dussumieria acuta</i>	18 - 21	11	0	0
<b><i>Epinephelus chlorostigma</i></b>	<b>33 - 95</b>	<b>100</b>	<b>10</b>	<b>10</b>
<b><i>Epinephelus tauvina</i></b>	<b>34 - 99</b>	<b>40</b>	<b>5</b>	<b>12.5</b>
<i>Euryglossa orientalis</i>	27 - 44	14	0	0
<i>Halichoeres stigmaticus</i>	30 - 37	15	0	0
<i>Hemiramphus far</i>	25 - 28	12	0	0
<i>Johinus maculatus</i>	25 - 66	18	0	0
<i>Lethrinus nebulosus</i>	25 - 72	18	0	0
<i>Liza alata</i>	34 - 38	14	0	0
<b><i>Lutjanus ehrenbergi</i></b>	<b>14.5 - 38</b>	<b>45</b>	<b>10</b>	<b>22.22</b>
<b><i>Lutjanus malabaricus</i></b>	<b>23 - 75</b>	<b>42</b>	<b>5</b>	<b>11.90</b>
<i>Nematalosa nasus</i>	12 - 15	16	0	0
<i>Nemipterus japonicas</i>	25 - 54	13	0	0
<i>Pampus argenteus</i>	28 - 35	10	0	0
<i>Parastromateus niger</i>	32 - 36	11	0	0
<i>Pardachirus marmoratus</i>	25 - 36	10	0	0
<i>Parupeneus heptacanthus</i>	25 - 35	14	0	0
<i>Plectorhinchus gaterinus</i>	28 - 46	12	0	0
<i>Plectorhinchus pictus</i>	30 - 48	14	0	0
<i>Pomacanthus maculosus</i>	28 - 31	10	0	0
<i>Pomadasys argenteus</i>	30 - 49	12	0	0
<i>Psettodes erumei</i>	38 - 70	20	0	0
<i>Rastrelliger kanagurta</i>	13 - 18	14	0	0
<i>Rhabdosargus haffara</i>	29 - 33	16	0	0
<i>Scolopsis taeniatus</i>	19 - 24	15	0	0
<i>Scomberomorus commerson</i>	60 - 135	17	0	0
<i>Seriolina nigrofasciata</i>	30 - 50	12	0	0
<i>Siganus canaliculatus</i>	21 - 34	14	0	0
<i>Sorsogona tuberculata</i>	49 - 53	17	0	0
<i>Sphyraena obtusata</i>	35 - 52	18	0	0
<i>Squirida tumbil</i>	23 - 26	12	0	0
<i>Trachurus indicus</i>	26 - 39	15	0	0
<i>Trichiurus lepturus</i>	59 - 75	10	0	0
<i>Tylosurus crocodilus</i>	65 - 88	18	0	0
<b>Total</b>		<b>750</b>	<b>40</b>	<b>5.33</b>



Table 2. Showing seasonal prevalence of *Anisakis* sp. Larvae

Fish species	winter			spring			summer			Autumn		
	No. Exam.	No. Infect.	%	No. Exam.	No. Infect.	%	No. Exam.	No. Infect.	%	No. Exam.	No. Infect.	%
<i>Cephalopholis hemistiktos</i>	8	0	0	10	5	50	12	4	33.33	6	1	16.66
<i>Epinephelus chlorostigma</i>	20	1	5	28	3	10.71	34	5	14.70	18	1	5.5
<i>Epinephelus tauvina</i>	8	0	0	12	2	16.66	14	3	21.42	6	0	0
<i>Lutjanus ehrenbergi</i>	10	2	20	8	2	25	16	4	25	11	2	18.18
<i>Lutjanus malabaricus</i>	7	0	0	14	2	14.28	12	2	16.66	9	1	11.11
<b>Total</b>	<b>53</b>	<b>3</b>	<b>5.66</b>	<b>72</b>	<b>14</b>	<b>19.44</b>	<b>88</b>	<b>18</b>	<b>20.45</b>	<b>50</b>	<b>5</b>	<b>10</b>

**Infestation site of *Anisakis* sp. larvae:**

Five fish species infested with anisakis were *Cephalopholis hemistiktos*, *Epinephelus chlorostigma*, *Epinephelus tauvina*, *Lutjanus ehrenbergi*, *Lutjanus malabaricus*. The site of infestation of larval stage of anisakis differ according to species of fish. Present results recorded that larval stage of *Anisakis* in *Cephalopholis hemistiktos* located in liver with high density (+++), within the abdominal muscle (+) and in the abdominal cavity (++). In *Epinephelus chlorostigma* the anisakis larva encapsulated in the abdominal cavity (+++) and also located in the abdominal muscle (+) while, in *Epinephelus tauvina* larval stage of anisakis located only in the abdominal cavity (++) on the otherhand in *Lutjanus ehrenbergi* & *Lutjanus malabaricus* the larval stage of anisakis located only in the abdominal cavity (+++) table 3.

**Histopathological Studies:**

Histopathological alterations were represented as necrosis and degenerative changes in

the hepatocytes and inflammatory cells proliferation were generally seen and distributed in-between the cells. The infected liver showed an embedded parasite with variable degrees of tissue reaction (encapsulation) depending upon the number and condition of the nematodes as well as the duration of infestation (Fig 4, A). Spleen of *Cephalopholis hemistiktos* showing mild depletion of white pulp with the presence of lodged *Anisakis* larvae cysts H&E. X 100 (Fig4, B). Abdominal musculature of *Epinephelus chlorostigma* fish encapsulated anisakis larvae and embedded in-between the muscle fibers surrounded with fibrous connective tissues associated with edema and inflammatory cells infiltration (fig 4, C). Intestinal wall of *Cephalopholis hemistiktos* revealing the presence of lodged *Anisakis* larvae surrounded with connective tissue capsule with abundance of mononuclear Eosinophilic granular cell infiltration. H&E. X100 (Fig 4, D).

Table 3. Showing distribution and intensity of *Anisakis* larvae in the infested fish species

Fish species	Free in the Abd. Cavity	Encapsulated In the Abd. Cavity	Within the Abd. Musclature	Within The Liver
<i>Cephalopholis hemistiktos</i>	++	-	+	+++
<i>Epinephelus chlorostigma</i>	-	+++	+	-
<i>Epinephelus tauvina</i>	++	-	-	-
<i>Lutjanus ehrenbergi</i>	+++	+++	-	-
<i>Lutjanus malabaricus</i>	+++	-	-	-

- Negative; + low density; ++ medium density; +++ high density

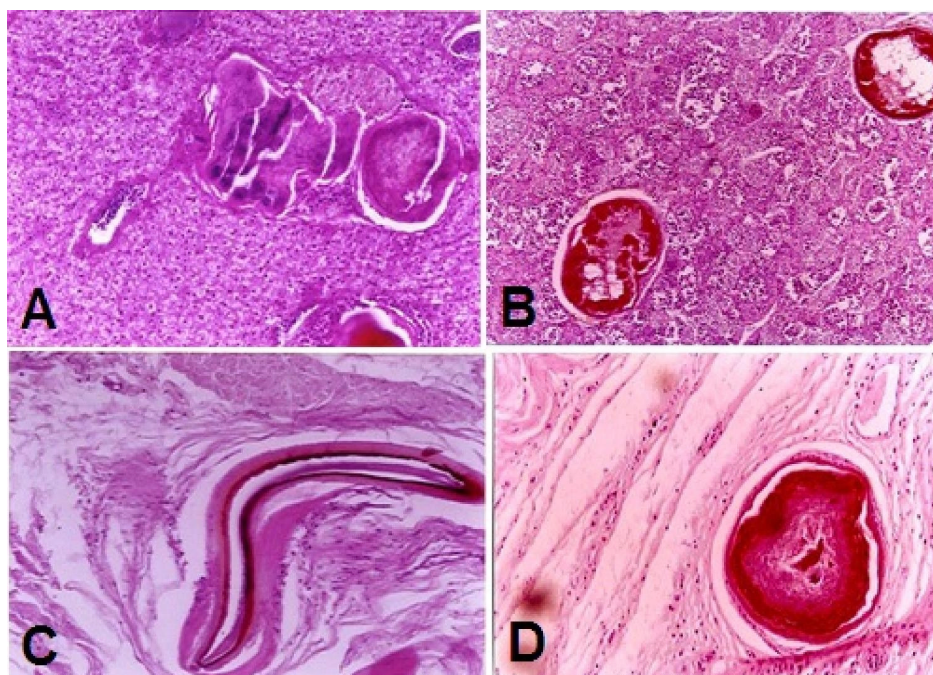


Fig (4) Showing (A) liver of *Cephalopholis hemistiktos* fish suffered from encapsulation of anisakis larvae in the portal region associated with degenerative changes of hepatocytes and inflammatory cells infiltration (B) Spleen of *Cephalopholis hemistiktos* showing mild depletion of white pulp with the presence of lodged *Anisakis* larvae. H&E. X 100 (C) Abdominal musculature of *Epinephelus chlorostigma* fish anisakis larvae encapsulated and embedded in-between the muscle fibers surrounded with fibrous connective tissues associated with edema and inflammatory cells infiltrations (D) Intestinal wall of *Cephalopholis hemistiktos* revealing the presence of lodged *Anisakis* larvae cyst surrounded with connective tissue capsule with abundance of mononuclear Eosinophilic granular cell infiltration (H&E. X100).

#### Discussion:

Nematodes from the super family Ascarididea (families: Anisakidea and Raphidascarididea) commonly named anisakids, are parasites of many water organisms. Low specificity in the choice of hosts, both intermediate and definitive, causes that their geographical distribution is wide. Anisakids of biological and economical importance in aquatic environment (Szostakawska *et al.*, 2005). The present study was designed to investigate the distribution of anisakis species among some commercially exploited imported and local marine fish at Al- Qatuf, Eastern province, Saudi Arabia to detect the clinical signs, prevalence, seasonal variation of infestation and histopathological alterations of naturally infested fish species.

Concerning the clinical signs and post mortem lesions, present study revealed that fish infested with anisakis sp. showed no any clear clinical abnormalities and fish appeared to be normal. The anisakis larvae which were isolated from the abdominal muscle of *Cephalopholis hemistiktos* and *Epinephelus*

*chlorostigma* were short, brown or black in colour and of 1 - 1.5 cm in total length while those which isolated from the abdominal cavity of *Cephalopholis hemistiktos*, *Epinephelus tauvina*, *Lutjanus ehrenbergi* and *Lutjanus malabaricus* were white or yellow in colour and of 1 - 2 cm in total length. On the otherhand cystic form or encapsulated larvae only present in *Epinephelus chlorostigma* and *Lutjanus malabaricus*. Liver highly infected with black or brown coloured larvae of *Anisakis* sp. only observed in *Cephalopholis hemistiktos*. The results nearly agree with that obtained by Eissa (2002) and (Al-Zubaidy, 2010) who reported that Ninety four marine fish specimens were identified as harboring L3 larval nematodes in the Red Sea Fishes, Yemen Coast. Morphological examination revealed that all larvae specimens isolated belonged to the family Anisakidae. Larvae were found free in the intestine and encapsulated (coiled in a thin walled cyst) on the wall of stomach, liver, and muscles. All the examined fishes showed no external visible signs of disease.

Regarding the identification of the isolated nematodes, present study revealed that the isolated nematode, parasites was identified as anisakis larvae L3. The identification based on the following morphological characters; the shape and the presence of a prominent boring tooth, three lips and excretory pore on the ventral side at the anterior end, the shape of the tail and the presence of small prominent mucron, at the posterior end and the shape of the ventriculus. Present results agree with that obtained by Chai *et al.*,(1989), Smith (1983) and Al-Zubaidy, (2010) who reported that the third stage larvae (L3) of *Anisakis simplex* are characterized by: Small, (9- 36 mm in length), with a straight anterior gut structure consisting of esophagus, ventriculus, and intestine, cuticle obviously striated transversely, irregularities, lips inconspicuous but with prominent boring tooth on anterior end, the live larvae white or cream in color and encysted in capsules of host origin, coiled like a watch-spring. Furthermore Choi *et al.*,(2011) reported that *Anisakis simplex* L3 which isolated from Korean marine fish species were identified basing on the following morphological characters: the shape and the presence of the boring tooth, the shape of the tail and the presence of the mucron, and the shape of the ventriculus.

Regarding the prevalence of *Anisakis* larvae infestation, present study demonstrated that the overall percentage of *Anisakis* sp. infestation among examined fish species reached 5.33%. However, the infestation percentages in *Cephalopholis hemistiktos*, *Epinephelus chlorostigma*, *Epinephelus tauvina*, *Lutjanus ehrenbergi* and *Lutjanus malabaricus*, reached 27.77, 10, 12.5, 22.22 and 11.90 respectively, while in the remainder of the examined fish species were 0 % Results nearly agree with that obtained by Al-Zubaidy, (2010) who reported that his study indicate the occurrence of *Anisakis simplex* larvae in five different commercial marine fish species belonging to four families Lethrinidae, Carangidae, Scombridae and Serranidae from the Red Sea coast of Hodeidah city, Yemen Republic also in the Red Sea, Egyptian coast, (Abdou, 2005) recorded anisakid nematodes, *Terranova* larvae from marine fishes belonging to families Carangidae, Scombridae, Lethrinidae, Scaridae and Serranidae and she mentioned that 19 % were found harboring *Anisakis* nematodes larvae, occurrence were within the intestinal lumens of the hosts. In Mediterranean Sea fishes, Nascetti *et al.*,(1986) and Orecchia *et al.*,(1989) reported the *Anisakis* larvae from the Carangidae, Scombridae and Serranidae. Moreover, the three fish species belonging to Carangidae and Scombridae were found harboring the *Anisakis* larvae (Varjabedian, 2000). Parukhin (1988) recorded the *Anisakis* larvae

from Indian Ocean fishes belonging to family Carangidae which was the highest infected fish. Out of 22 fish samples investigated samples 9 (40.9%) were infected with nematodes, 6 of which (27.3%) belonged to the family Anisakidae (Pereira, 2000). From all previous literatures it was noticed that there are differences in prevalence rate and intensity of *Anisakis* larvae infestation among wide range of marine fish species this may be due to different habits in feeding and geographical distribution and the depth living benthic or pelagic.

Regarding infestation rate and Size of fish, present study revealed that there was a relationship between the size of the fish and its infestation with *Anisakis* larvae. The results records that, the infestation detected in *E. chlorostigma* and *E. tauvina* at more than 68 cm, and 70cm respectively. Infested *Cephalopholis hemistiktos* and *Lutjanus malabaricus* species were more than 35 cm while the smallest infested species was *Lutjanus ehrenbergi* more than 28 cm. It is noticed that medium and large size were only infected, the results nearly agree with the results of Szostakawska *et al.*, 2005; Choi *et al.*,2011 Chou *et al.* 2011) and Al-Zubaidy, (2010) who reported that the highest prevalence of infection were shown on the fish group (length >56 cm). In all five different host species, the prevalence of infection showed an increasing tendency with host length, these means that larger fish appear as more susceptible to the infection rather than the smaller one. This may be attributed to the available niches for parasite are more diverse in large hosts, also larger hosts can sustain a higher number of parasites, hence the time it takes for the species to go extinct in an individual host is reduced. Furthermore, longer fishes have lived longer and therefore, have a higher probability of encountering parasites during their life span than smaller and shorter lived fish species in addition of Eissa (2002) who reported that, the large and old fish tend to be more heavily infested by anisakiasis than small fish of the same species. This is because large fish eat more and therefore, ingest greater numbers of parasites and also because the larval worms, although inactive, can survive for a long time in fish, and therefore their numbers accumulate.

Concerning the seasonal prevalence, present study revealed that, the seasonal variations of *Anisakis* sp. infestation rates peaked during the Summer season (20.45%), followed by the Spring season (19.44%), Autumn (10 %) and Winter (5.66%). The result nearly agree with that obtained by Eissa (2002) who reported that The thin-shelled eggs are laid; they are passed out into seawater through the feces of infected final ichthyophagous hosts such as dolphins or whales (*Anisakis*), The first stage larva (L1) undergoes the first molt while still within the egg capsule where its



development is strongly influenced by water temperature. This means that water temperature has the great role on enhancing the life cycle increasing the prevalence during summer and spring seasons where in the present study there is no significant difference in prevalence of *Anisakis* larvae in investigated fish. The results supported by Andersen *et al.* (1993), Choi *et al.* (2011) and Li *et al.* (2011).

Regarding the of infestation site of larvae of *Anisakis*, present study displayed that larval stage of *Anisakis* in *Cephalopholis hemistiktos* located in liver with high density (+++), within the abdominal muscle (+) and in the abdominal cavity (++). In *Epinephelus chlorostigma* the *Anisakis* larva encapsulated in the abdominal cavity (+++) and located in the abdominal muscle (+) while, in *Epinephelus tauvina* larval stage of *Anisakis* located only in the abdominal cavity (++) ,on the otherhand in *Lutjanus ehrenbergi* & *Lutjanus malabaricus* the larval stage of *Anisakis* located only in the abdominal cavity (+++). This may be attributed to the feeding habits of fish the results concined with Al-Zubaidy, (2010) who mentioned that the distribution of *Anisakis simplex* larvae within the fish host may be related to the feeding habits of the fish, in addition of Smith (1983) confirm the results mentioned that the most encapsulated larvae occur in the body cavity of zooplankton feeding fishes, but are more widely distributed throughout the tissues of piscivorous fishes and he suggested that the zooplankton feeders are probably on the, mainstream, of the *Anisakis* life cycle involving a transfer of worms upward from euphausiid to euphausiid-feeding fish to cetaceans.

Concerning the histopathological alterations, present study revealed that *Anisakis* sp. was detected macroscopically in the liver tissue of infested fish as short brown or black threads; however, microscopically, the infected liver showed an embedded parasite cysts with variable degrees of tissue reaction (encapsulation) depending upon the number and condition of the nematodes as well as the duration of infestation. Histopathological alterations were represented as degenerative changes and inflammatory cell proliferation were generally seen fused and distributed in-between the hepatocytes. Spleen of *Cephalopholis hemistiktos* showing mild depletion of white pulp with the presence of lodged *Anisakis* larvae, While abdominal musculature of *Epinephelus chlorostigma* fish showed encapsulated *Anisakis* larvae embedded in-between the muscle fibers, surrounded with fibrous connective tissues associated with edema and inflammatory cells infiltration, Intestinal wall of *Cephalopholis hemistiktos* revealing the presence of lodged *Anisakis* larvae surrounded with connective tissue capsule with abundance of mononuclear Eosinophilic granular cell

Infiltration. The results nearly agree with Ebrahim (2011) and Roberts (2012).

From present study, it was concluded that Ascaridoid larvae have not previously been recorded from Saudi Arabian fish from the Arabian Gulf and thus these findings are new locality records. Out of 40 species of marine fishes 5 species only infested with *Anisakis simplex* larvae *Cephalopholis hemistiktos*, *Epinephelus chlorostigma*, *Epinephelus tauvina*, *Lutjanus ehrenbergi* and *Lutjanus*. Fortunately, since most of inhabitants in the Arabian Gulf countries are not accustomed to eat raw or under-cooked fish, human *Anisakiasis* has not yet been recorded in these countries.

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