Incidence and Pathological Effect of Anisakis Simplex Llarvae among some Egyptian Fish.

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Abstract

This work was conducted to detect the incidence, intensity and tissues distribution of *Anisakis simplex* larvae and study its pathological effect on naturally infested fish and experimentally infested puppies. The survey was performed on Silver side (*Atherina boyeri*), Mediterranean Horse Mackerel (*Trachurus mediterraneus*) and Sardine (*Sardinella spp.*). Parasitological examination was postive for *Anisakis simplex* in both *Atherina boyeri* and *Trachurus mediterraneus* .While the examined *Sardinella spp* samples show no infestation for *Anisakis simplex* larvae. *Atherina boyeri* fish recorded total infestation rate (33.8%) with intensity 1-12 larvae/fish and tissues distribution 88.9%, 22.2 % and 33.3% for abdominal cavity, ovaries and muscles, respectively. *Trachurus mediterraneus* recorded total infestation rate 18.8%, with intensity 1-5 larvae/fish and tissue distribution was 95.7% and 19.1% for abdominal cavity and ovaries, respectively. Experimental Anisakiasis was carried out on four apparently healthy puppies .Three of them were fed one meal once daily from heavy infested fish for two months and the fourth one was kept as control. Experimentally infected puppies showed diarrhea and restlessness during the experimental period. At the end of the experiment the naturally infested fish and all puppies were killed for pathological examination. The histopathological examination of the naturally infested fish revealed fibrous connective tissue capsule in the abdominal cavity around the larvae together with melano macrofied cells. On the same line the histopathological examination of the experimentally infested puppies showed chronic ulcerative gastritis with granuloma formation in the stomach in addition to sloughing of the tips of the intestinal villi together with eosnophilic infiltration in the intestine. Kidneys and liver were also showed pathological lesions. Therefore eating of *Anisakis simplex* infested fish may be preducing pathological lesions and health hazard effects to the consumumers.

Introduction

Anisakiasis is a human zoonotic parasitic disease caused by the ingestion of third stage larvae of ***Anisakis Simplex***, a round worm from the family ***Anisakidae*** of the order ***Ascaridida***. Humans are only accidental hosts and they may be infected by eating raw, improperly salted, cooked or under cooked fish such as **herring, squid, anchovy, cod, mackerel** and specific **salmon (1)**.

Anisakiasis was first recognized 40 years ago from an eosinophilic intestinal lesion in a patient suffering from severe abdominal pain **(2, 3)**.

Anisakis Simplex caused direct tissue damages following invasion of the gut wall caused strong development of an eosinophilic granuloma, and perforation of the gut **(4, 5** and **3)**.

The risk of infestation with ***Anisakis Simplex*** and related parasites of fish was previously recorded for some time, but it is now emerging that ingestion of material from dead parasites in food is also potentially dangerous. The resulting allergic reactions range from rapid onset and potentially lethal anaphylactic reactions to chronic debilitating conditions **(3)**.

On the same line ***Anisakis*** Simplex allergens are highly resistant to heat and freezing therefore cooking which should kill the parasites, might not diminish the potency of their allergens**(6)**.

The ***Anisakis simplex*** larvae mainly penetrated the gastric wall even through man which is an unsuitable host. The larvae penetrating into the submucosa and die in a short time. After death of the larvae, the broken down worms directly caused human tissue reaction, against foreign protein with formation of eosinphilic granuloma. The granuloma was seen surrounding the larva which is already destroyed at the centre of the lesion. This reaction continues for long time till the dead worm is fully absorbed **(1)**.

Anisakiasis errously was diagnosed as acute appendicitis, ileus, intestinal invagination, acute peritonitis, gastric or intestinal perforation and diagnosis of anisakiasis is difficult before laparotomy (3).

In Egypt, few reports about ***Anisakis simplex*** larvae infection, this may be either due to the fact that Anisakiasis is erroneously diagnosed as some other gastro intestinal diseases (7, 8, 9 and 10).

The objective of the present study was throw light on the rate of ***Anisakis simplex*** parasitic infestation among the silverside fish (***Atherina boyeri***), Mediterranean Horse Mackerel (***Trachus Spp.***) and Sardine (***Sardnella Spp.***), study the pathological finding associated with this larvae in naturally infested fish and demonstrate the pathological changes on the experimentally infested puppies eat this infested fish. Discuss the public health importance of Anisakiasis.

Materials and Methods

Fish samples:

A total number 1300 (800 silversde(***Atherina boyer***),250 Mediterranean Horse Mackerel(***Trachurus mediterraneus***) and 250 Sardine (***Sardinella spp.***) fresh fishes were collected from local fish markets at Kaliobia and Gharbia governorates and transported to the laboratory after putting in ice box.

Parasitological examination:

All of the collected fish were examined macroscopically for detection of any abnormal changes. Each fish sample dissected and the abdominal cavity was examined for presence of ***Anisakids*** larvae. The larvae were removed, killed and fixed in 70% ethanol and cleared in glycerin (11). Additionally portions from peritoneal cavity, ovaries and musculature were pressed between two glasses and examined by light microscope and microphotographed. Identification of the collected ***Anisakid*** larvae was based on morphologic characteristics as ***Anisakis Simplex***, third stage larvae according to (12).

Experimental infection:

Four apparently healthy puppies were obtained and reared under strict hygienic measures. Faecal examination was carried out along two weeks for detection of any parasitic eggs before the experimental infection to exclude any accidental infection.

Fishes used in the experimental infection were firstly examined for detection the ***Anisakis simplex*** larvae and the un infested fishes were excluded. The infested fish were taken and homogenized. The number of the ***Anisakis simplex*** larvae was calculated in each infested fish. Three puppies were fed one meal from the infested fish daily for two month (about 25 gm. from the infested fish per meal for each puppy) and the forth puppy was left as control. After two months all puppies were killed and the specimens from the internal organs were taken for pathological examination.

Pathological examination:

The tissue spacemen from the naturally infested fish and the internal organs from the experimentally infested puppies including stomach, intestine, liver and kidneys were taken and recorded any macroscopical changes, then fixed in 10% buffered neutral formalin for histopathological examination according to**(13).** The prepared paraffin sections were stained with Hematoxylin and Eosin and examined for pathological abnormalities.

Results

Table (1): Incidence and intensity of *Anisakis simplex* larvae (L3) among naturally infested fish of examined samples

Fish species	Rate of infestation			Intensity Number/fish
	N	N	%	
Atherina boyrei	800	270	33.8	1-12
Trachurus mediterraneus	250	47	18.8	2-5
Sardinella spp.	250	-	-	-
Total	1300	314	24.4	1-12

N: number of examined samples
n: number of infested samples

Table (2): Tissue distribution of *A. Simplex* larvae (L3) among naturally infested fishes of examined samples.

Fish species	No. Of Infected samples	Abd.cavity		Ovaries		Muscles	
		n	%	N	%	n	%
Atherina boyeri	270	240	88.9	60	22.2	90	33.3
Trachurus mediterraneus	47	45	95.7	9	19.1	-	-
Sardinella spp.	-	-	-	-	-	-	-

Pathological findings:-

1-Infested fish:-

The infested fish were apparently normal & showed no characteristic gross lesions, the miroscopic examination of the infested fish demonstrate the ***Anisakis Simplex*** larvae in the abdominal cavity Fig (**1**). The **histopathological** examination of the naturally infested fish revealed the presence of large number of the ***Anisakis Simplex*** larvae in the abdominal cavity in addition these larvae were surrounded by thin layer of fibrous connective tissue capsule together with melanophore cells Fig (**2**).

2- Experimentally infested puppies:-

a- Clinical signs, clinically the experimentally infested puppies were suffered from diarrhea, and restlessness.

b-The gross findings of infested puppies organs were characterized by the presence of variable sized and shaped ulcers in the gastric and intestinal mucosa. Congestion and edematous thickening of the gastric mucosa were also seen. Moreover, the internal organs particularly liver and kidneys were enlarged and pale in color.

c- The microscopic findings:-

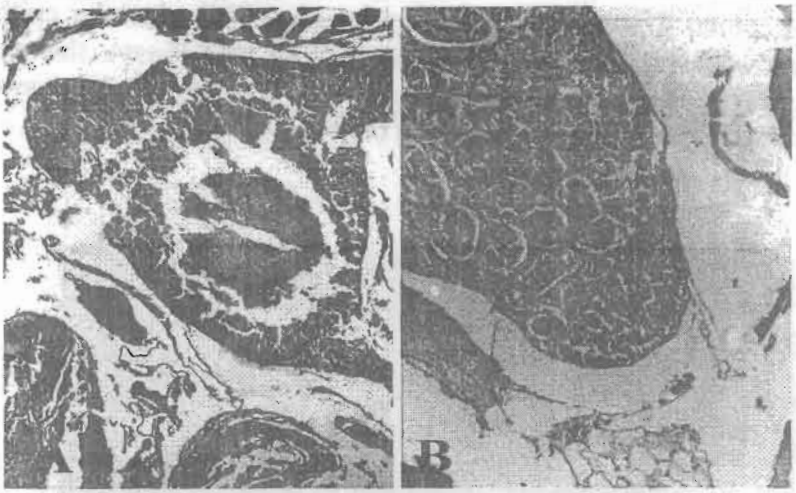
1- Stomach revealed chronic ulcerative gastritis with granuloma formation in addition to the presence of cross sections of the parasite. A discrete focal aggregation of mononuclear inflammatory cells mainly lymphocytes, plasma cells and macrophages were scattered under the gastric mucosa (Fig. **3 and 4**). Focal sloughing of the gastric mucosa with leucocytic cellular infiltration of the submucosal layer was found (Fig. **5**). Epithelial mucinous metaplasia of the gastric mucosa with fibrous connective tissue proliferation replaced degenerated and necrotic mucosal glands were seen (Fig .**6**). In few cases, the granulomatous reaction was formed mainly from eosinophils and giant cells around the dead parasites.

2- Intestine showed congestion of intestinal blood vessels with sloughing of the tips of the intestinal villi (Fig.**7**). Although no parasitic cross section were found in the examined intestine however, eosinophilic cellular infiltration of the intestinal walls particularly the mucosal layer was prevalent (Fig .**8**).

3- Liver revealed remnants from cross section of the parasites with extensive vacuolar and hydropic degeneration of the hepatocytes Fig (**9 and**

10). Periductular fibrosis and slight hyperplasia of bile ductular epithelium were also recorded. The **microscopical** finding of **Kidneys** showed only degeneration of the renal tubular epithelium in the form of cloudyswelling, hydropic and vacuolar degeneration Fig **(11).**

Fig (1): *Anisakis Simplex* larva in (*Atherena boyeri)* fish tissue (High power).



Fig(2): The abdominal cavity of the infested fish showing the anterior and posterior end of the *Anisakis Simplex* larvae surrounded by thin layer of fibrous connective tissue capsule together with melanomicrofied cells. H&E stain X 200.

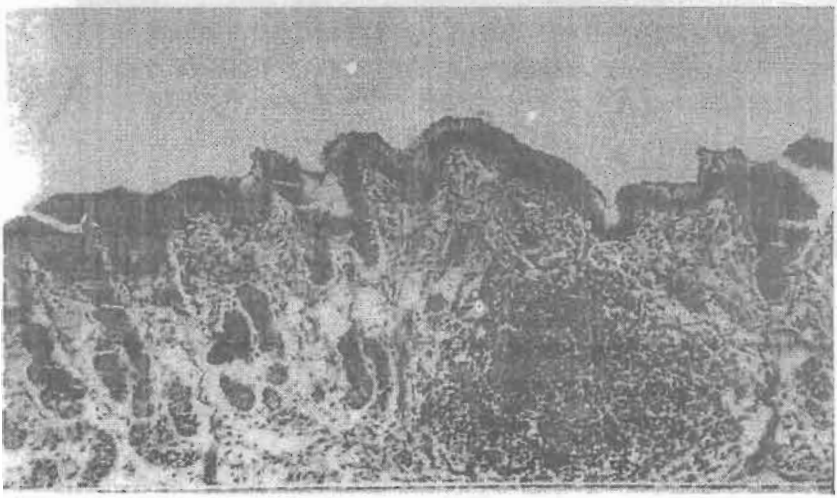


Fig. (3): Stomach of the puppies experimentally infcted with infested fish showing focal aggregation of mononuclear inflammatory cells. H&E. stain. X200

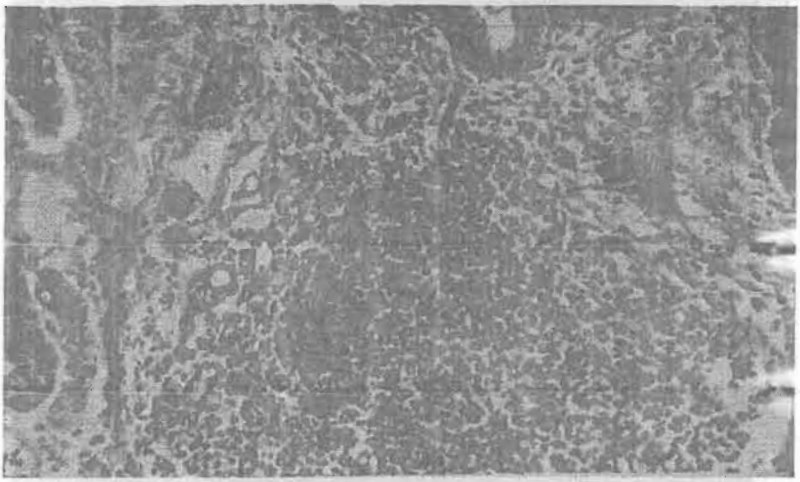


Fig. (4): High power of the previous figure showing aggregation of lymphocytes and macrophages in the gastric mucosa. H&E. stainX400.

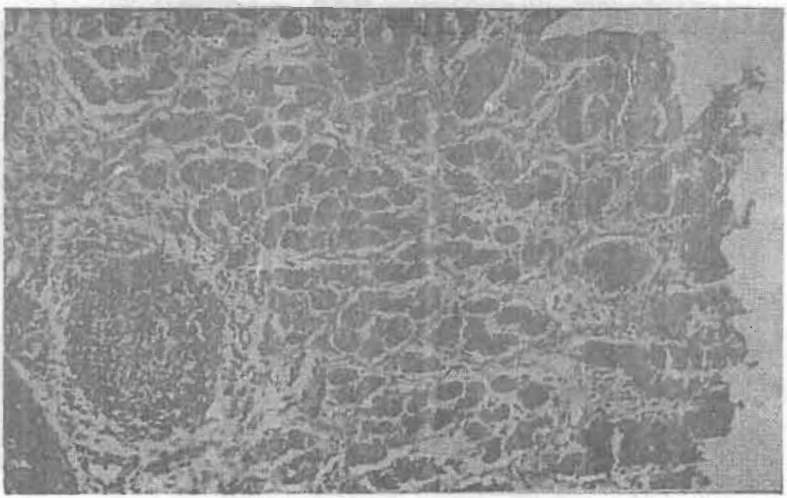


Fig (5): Stomach of the puppies experimentally infected with infested fish sloughing of the gastric mucosa with granuloma formation under the mucosa. H&E. stain. X 200.

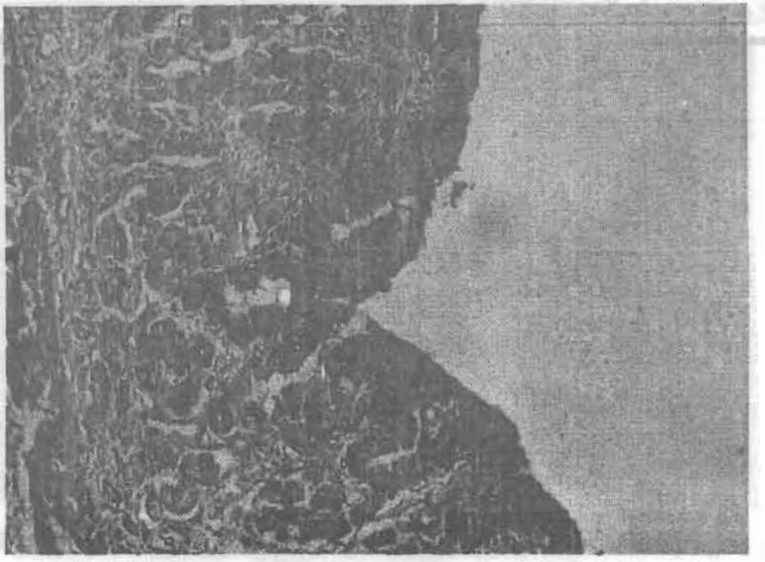
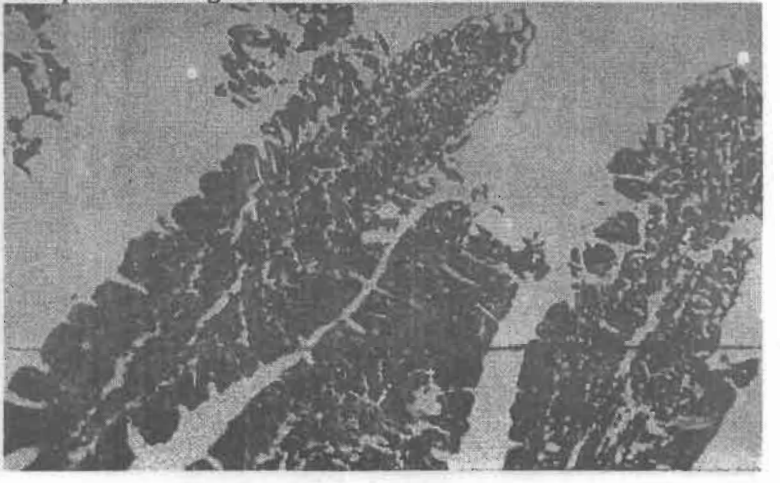
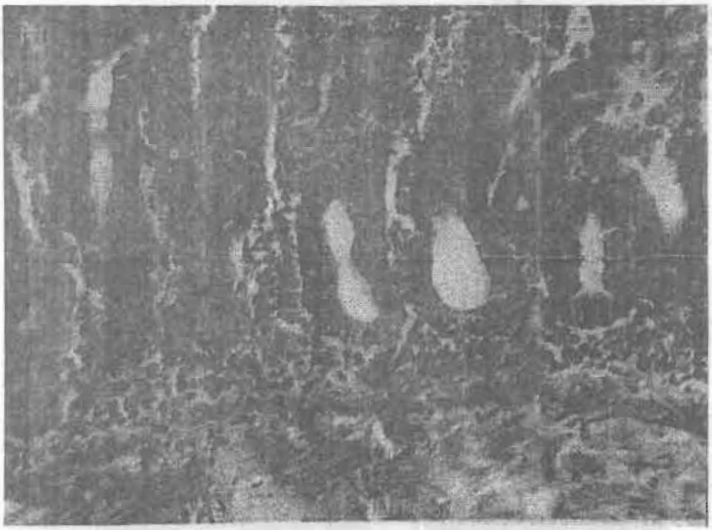


Fig (6): Stomach of the puppies experimentally infested with infested fish showing mucinous metaplasia of the gastric mucosa .H&E. stainX200.



Fig(7): Intestine of the puppies experimentally infested with infested fish showing sloughing of the tips of the intestinal villi. H&E. stain X200



Fig(8): Intestine of the puppies experimentally infested with infested fish showing eosinophilic cellular infiltration. H&E. stain X400.

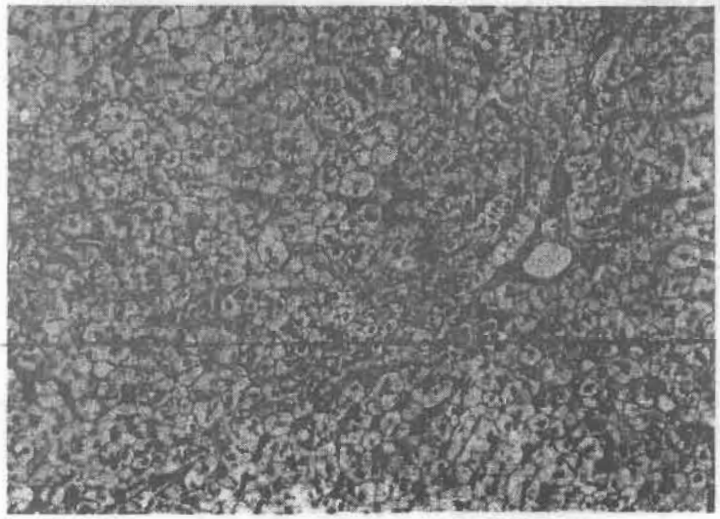


Fig (9): Liver of the puppies experimentally infested with infested fish showing extensive hydropic degeneration of hepatocytes. H&E. stain. X200

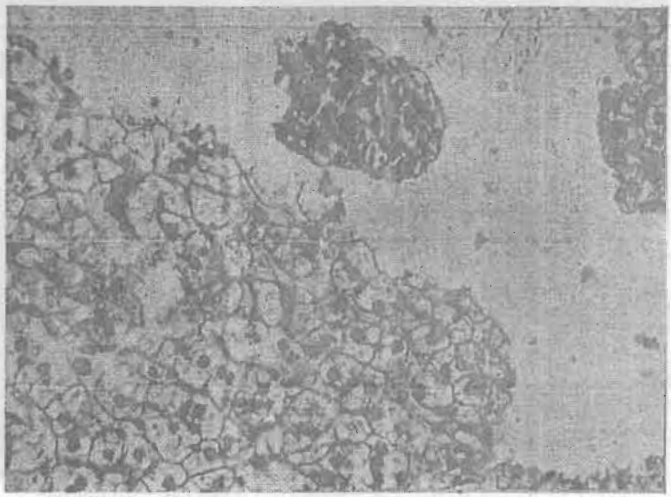


Fig (10): Liver of the puppies experimentally infested with infested fish showing remnants of the parasites with hydropic degeneration of the adjacent hepatocytes. H&E. stainX400

Fig (11): Kidney of the puppies experimentally infested with infested fish showing cloudy swelling and hydropic degeneration of tubular epithelium. H&E. stain. X400.

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Discussion

Fish are considered the cheapest source of animal protein in the developing countries.Parasitic diseases constitute one of the major factors of limiting fishes production **(14).** .There is no doubt that the parasites found in fishery products is a hazard to human health and reduced their value **(14, 15).** A large number of fish species, can serve as a source of food borne parasitic infection.

The results presented in Table (1) showed the incidence, intensity of ***Anisakis simplex*** larvae from the examined fish species. The total infestation rate was 24.4%, the results were higher than **(10, 16)** who recorded total infestation rate 16.5% and 12.3%, respectively in some fish sp. caught from Alexandria.

The infestation rate among ***Atherina boyeri*** fish was 33.8% with intensity 1-12 larvae/fish (Table 1).This records were higher than that showed by **(15)**, who examined

Atherina boyeri fish for ***Anisakis simplex*** larvae and presented infestation rate 20%. The tissue distribution of ***A.simplex*** larvae in the infected fish (were concentrated in abdominal cavity 88.9% ,then in muscles 33.3% and ovaries 22.2% (Table 2) . ***Atherina boyeri*** is one of the most common and abundant caught fish occurring worldwide in tropical and temperate water in certain time of the year **(17)**. It is popular and the cheapest source of animal protien in Egypt and regarded as food fish which eaten as a whole fish by a large number of people in Egypt, so the risk of human infection by ***A.simplex*** from eating undercooked or low solted ***Atherina boyeri*** fish,

expected to be causing clinical symptoms that mimic other gastrointestinal disturbances such as gastric ulcer or intestinal motality disorders **(15),** in addition to the ingetion of dead parasites can cause food allergy **(3).**

The infestation rate of ***Trachurus mediterraneus*** with ***A.simplex*** was 18.8% with intensity 2-5/ fish. The results came in accordance to that reported in previous studies **(18, 19, 20, 21, 22 and 23).**

Tissue distribution of the parasite in this study was 95.7% and 19.1% in abdominal cavity and ovaries, respectively and no larvae were observed in the muscles. These results partially agree with that reported by **(20) (24)** who examined Horse mackerel fish and found the infestation in abdominal cavity (18.7%) and not found in muscles .On the other hand **(19)** found a high percentage (38%) of these larvae in muscles tissues. Also ***Anisakis simplex*** and ***Anisakis sp.*** has been reported in Horse mackerel muscles, but the percentage of larvae found in this tissue varied from very low (1.8%) to high (41.8%) values **(19, 20 , 22 and 23).** It must be stressed that very low percentages of larvae in muscle may result in a non detection of the parasite in muscles tissue, or may be the absence of larvae in muscles has been suggested that the occurrence of migration of larvae into muscles which depend on the amount of lipids in this tissues**(24)**

Horse mackerel are pelagic fish that eat planketonic crustaceans,squids and small fish **(25)** and may therefore become infested with *Anisakis larvae* by feeding on squids or small fish **(19,24).**

In the present investigation , ***Anisakis simplex*** larvae was not detected in the examined samples of Sardine .This results differ than that recorded by **(17)** who found ***Anisakidae*** larvae in imported Sardine.This defferences could be attributed to geographical differences ,size and feeding habitat of the fish **(24)**

Our results were cleared by the pathological examination of the naturally infested fish and the experimentally infested puppies.The histopathological examination of the naturally infested fish showed the ***Anisakis Simplex*** larvae surrounded by fibrous connective tissue capsule together with melano

macrophages cells. Our results were agreement with the results obtained by **(10).**

In the present study, the experimentally infested puppies were suffered from diarrhea and restlessness. These results were nearly in agreement with the results obtained by **(26)** and **(27)** on human case report suffered from gingivostomatits and other human case presenting with partial intestinal obstruction after ingestion of fish contaminated with ***Anisakis Simplex*** parasite, they reported that the larvae may attach to the digestive mucosa and producing gastrointestinal distress such as diarrhea, vomiting and abdominal pain.

Regarding to the gross findings of the internal organs of the experimentally infested puppies, variable sized and shaped ulcers were recorded in the gastric and intestinal mucosa in addition to congestion and edematous thickening of the gastric mucosa. Our obtained results are agreement with **(3, 1)**. Liver and kidneys of the infested puppies were pale in color. These results are similar to that result recorded by **(28, 10)**. Regarding to the microscopical changes in our study the more obvious lesions were recorded in the **stomach** which showed chronic ulcerative gastritis with granuloma formation in addition to the presence of the cross sections of the parasite together with focal sloughing of the gastric mucosa. Other cases showed epithelial metaplasia of the gastric mucosa with fibrous connective tissue proliferation and inflammatory cellular infiltration mainly eosinophils and gaint cells. Our results are agreement with **(3, 29, 27 and 1)** they mentioned that, in Japan the gastric Anisakidosis is far more common than intestinal Anisakidosis(95%) of cases while in Europe the intestinal Anisakidosis is the more common. Also they recorded that ***Anisakis*** larvae causes numerous mucosal erosion and sections of the parasite present in the submucosa together with eosinophilic granuloma induced tumor formation and gastrointestinal obstruction. The **microscopical** findings of the **intestine** in our study showed congestion of the intestinal blood vessels with sloughing of the tips of the villi and eosinophilic infiltration of the mucosa. Our results were nearly in agreement with that result obtained by **(1)** who mentioned that diffuse inflammatory cells infiltration of the jeujunal wall predominantly eosinophil granulocytes

together with numerous mucosal erosions were recorded in case of anisakiasis. The **microscopical** changes of **kidneys** and **liver** in our study showed hydropic and vacuolar degeneration of both hepatocytes and renal tubular epithelium together with the presence of cross sections of the parasite are found in the liver. There was no available literature about the kidneys and liver lesions. Only few literature mentioned that ***Anisakis simplex*** larvae can penetrated the wall of the gastrointestinal tract intestinal organs such as peritoneum, lungs, lymph node, ovaries, liver pancreas, especially stomach and small intestine, nevertheless cases involving colon and extra spleen and kidneys have been recently reported by **(30, 27).** On the same line **(31)** reported that the risk of human infection with ***Anisakis simplex*** larvae is not only for viscera but also, invasion of the 3 rd stage larvae to liver, pancreas and probably the lungs. These results indicated that the Anisakiasis disease not only involve stomach and intestine but also extend and affect other organs .This was clearly noticed in our work and supported by the presence of cross sections of the parasite in the liver.

Although the hieghest infestation of ***A.simplex*** in the abdominal cavity among the infested fish, their importance as a potential source of human infection can not be excluded, as larval migration to the muscles in dead fish is possible as mentioned by **(10).**This beside serious complication, including eosinophilic gastritis, intestinal perforation , intestinal heamorrhage and migration of larvae to other tissues **(32,26)** .Finally, an allergic reaction may develop in human anisakiasis, and this condition is sometime termed a gastroallergic reaction **(33).**

From these results we concluded that the ***Anisakis simplex*** larvae are dangerous either live or dead, as it causes many pathological lesions to consumers and therefore cause public health hazards.

Control measures

Current European Community regulations on food fish and productes (Directive 91/493/EC and Decision 93/140/EC) require the visual examination of the fish, extraction of the visible parasites and the removal of those that are heavily parasitized from the market. Moreover, species

intended for marinating or salting at temperatures <60C° must be stored at -20C° for 24 hours. The US Food and Drugs Administration (FDA) agnecy demands that all fish products not intended for cooking or processing at temperatures >60C° should be deep frozen at -32C° for a minimum period of seven days which, could denaturat the thermolabil protien to nonantigenic form to avoid alleregy and should reduce infection **(34, 26)**.

A simple but effective control measure is to inform the general public about ***A.simplex*** infection and the risks of eating raw or undercooked fish. Nevertheless, such measuresmight not be adequate considring the thermostability of the allergen involved in allergic reactions. For many people, it is difficult to avoid eating fish, but the simple precaution of avoiding feeding whole small fish (***Atherina boyeri***) and the abdominal region of ***Trachurus mediterraneus*** could be effective.

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نسبة تواجد والاثار الباثولوجيه لبرقات الأنيساكس سيمبلكس فى بعض الأسماك المصرية

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الملخص العربى

اجريت هذه الدراسة لتحديد مدى تواجد يرقات الأنيساكس سيمبلكس فى أنسجة بعض الأسماك المصرية (الباساريا, الباغة, السردين) و دراسة الأثار المرضية لهذه اليرقات على الأسماك المصابة طبيعيا بها والكلاب المعدية معمليا بها. اظهرت نتائج الفحص الباراسيتولوجى اصابة كلا من اسماك الباساريا , الباغة, وعدم تواجدها فى سمك السردين. وكانت توزيع اليرقات داخل الانسجة بواقع ٨٨,٩%, ٢٢,٢%, ٣٣,٣% فى التجويف البطنى و المبايض و العضلات على التوالى فى سمكة الباساريا. و كانت التوزيع فى سمكة الباغة بواقع ٩٥,٧%, ١٩,١% فى التجويف البطنى و المبايض على التوالى ولم توجد فى العضلات فى العينات محل الفحص. واظهر الفحص الباثولوجى للاسماك المصابة طبيعيا وجود عدد كبير من اليرقات محاطة بكبسولة من الانسجة الضامة فى التجويف البطنى للسمك المصاب. كما اظهرت عدوى الكلاب الآكلة للاسماك المصابة وجود التهابات و تقرح مزمن فى جدار كلا من المعدة و الامعاء. كما اظهر الفحص الهستوباثولوجى للمعدة وجود تجمعات لعدد كبير للخلايا الالتهابية المزمنة مكونة ورم حبيبي (الجرانيولوما) . واظهر فحص الامعاء عن سقوط قمم الانسجة الطلائية لجدر الامعاء, كما ظهر فى كلا من الكلية و الكبد وجود تنكسات مختلفة ووجود بقايا لليرقات فى الكبد.وقد تم دراسة الأهمية الصحية لتناول الاسماك المصابة بهذه اليرقات وكيفية الوقاية منها .