

**INVESTIGATIONS ON THE RESISTANCE OF THE  
CONTRACAECUM LARVAE FROM ANCHOVY, *Engraulis encrasicolus*, TO THE HOME COOKING  
AND SALTING METHODS AND THEIR PATHOGENICITY FOR THE LABORATORY ANIMALS**

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**I n t r o d u c t i o n**

In 1963 the annual fish catch of Turkey amounted to 130 000 tons. Anchovy "*Engraulis encrasicolus*" constituted nearly half of this production, that is, 60 000 tons. This indicates that the anchovy production as being one of the animal protein sources available in this country contributes substantially to the nutrition of the people of poor-income, especially of those living along the Black Sea coast. Anchovy catch in the Black Sea and to a limited extent in the Marmara Sea begins in September, reaches its peak in December, continues until March and ends in April.

During the years of 1962 and 1963 it was reported by the Chief of the Veterinary Services of the İstanbul Municipality that nearly all anchovies landed at the fishery market were infested with nematode larvae of an unknown species which were localized in the abdominal cavity and in the muscles of the fishes. As it was stated in this special report<sup>1</sup>, specimens were shipped by the Department of Parasitology and Tropical Diseases of the Faculty of the Medicine in İstanbul to the British Museum in London for identification. In reply to this request the concerned authorities of the British Museum informed that the identification could not be made accurately owing to the fact that the parasites were immature and the morphological characteristics at this stage were not fully developed.

Hereafter, upon the request of the fish inspection officials we planned to investigate the problem on the infested anchovy speci-

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mens collected from the fish markets during the catch seasons of late 1963 and early 1964 months. Works have been done for I- the identification of the larvae, its localization and distribution in the fish body, II- resistance to the common cooking and salting methods and III- pathogenicity for the laboratory animals. As the results of our studies were obtained, information was forwarded to the related Russian research institutes as being one of the main anchovy catching countries along the Black Sea coasts and in return we requested their scientific and experimental research works in this field to which we are expecting to receive very soon.

The first part of our investigations related with the identification on the nematode larvae, their localization and distribution in the fish body was partly published elsewhere <sup>8, 9</sup>.

The nematode larvae recovered from the infested anchovies were identified as *Contracaecum aduncum* from the genus of *Contracaecum* belonging to the family of *Anisakidae* of which taxonomical position is given below:

Family: Anisakidae

Genera : 1 - *Contracaecum*

2 - *Porrocaecum*

3 - *Anisakis*

4 - *Cloeascaris*

The larvae were found to be moving freely in the intestinal canal and abdominal cavity, on the surface of the visceral organs, attached or bored into the adjacent abdominal musculature and even localized in the musculature all over the body. In some fish hundreds of the living larvae, viz., 300 - 400 were counted. It was noticed that the rate of infestation was light during the months from September to November and was heavy from December to April, that is to the end of the catching season. During the transportation of the fishes to the inland it was seen that the living larvae leaving the intestinal canal through the anus were contaminating the skin of all fishes and giving a disgusting and repulsive appearance to the consumer. We failed in noticing any marked pathological lesion or change in the infested anchovies. It worth recording here that from reviewing the literature which were available before <sup>5, 7</sup> and after <sup>2, 10</sup> these works had been done, we noted that the life cycle of these genera, viz., *Contracaecum* and *Anisakis*, involve two intermediate hosts. Predatory fishes, such as cods, pollacks, mackerels, horse-mackerels, sprats, sturgeons and marine mammals and possibly some sea-birds are the final hosts of *Contracae-*

*cum aduncum*. The eggs are deposited by the adult *Contracaecum aduncum* into water. The infestive larvae developed within the eggs are eaten by the pelagic copepods and crustacea, such as, *Ascartia bifilosa* and *Eurythemora affinis* which serve as the first intermediate hosts. The fishes, such as clupides, flat fishes, cods and about 40 species, among them anchovies, feeding on the above mentioned marine planktons serve as the second intermediate hosts. According to the several reports, *Contracaecum* larvae are widespread all over the seas in the Northern Hemisphere including the White Sea and Barents Sea.

In this paper we will summarize our findings with the experiments on the resistance of the living *Contracaecum* larvae to the home-cooking and salting methods and their pathogenicity which constituted the second and third parts of our investigations.

Although some literature notes that the *Contracaecum* larvae are harmless to human or there is no report about their pathogenicity<sup>2, 10, 7</sup> we are not sure whether these statements are based on rigidly established and carefully followed medical and experimental studies and observations. The same statements had also been made for the genus of *Anisakis* which is a very close genus to that of *Contracaecum*. The literature which state that the *Anisakis* larvae do not harm humans have proved to be unsubstantiated by the medical reports<sup>11</sup> and animal experiments<sup>4</sup>. Also it has been reported in the literature<sup>3</sup> that the *Porrocaecum* larvae were recovered from humans. In view of these reports and observations and especially of those made by Van Thiel and Kuipers<sup>11, 4</sup> we have been forced to go deep into the problem and conduct experiments before making any definite conclusion. Although the habit of eating raw fish does not exist in this country, it is likely that during the insufficient cooking or salting processes, the heat or salt might not destroy the living larvae localized deep in the fish flesh. In case of probable pathogenicity, the larvae surviving the action of heat or salt could create a hazard for human health.

In order to elucidate these points and to follow the suggestion made by Prof. Dr. Reichenbach - Klinké<sup>6</sup>, we decided to carry out experimental feedings with the laboratory animals and to investigate the resistance of the larvae to the common cooking and salting methods.

At the same time we called attention of the staffs of the hospitals located at the main cities along the Black Sea coast to the fact of the probable danger of the infection with these larvae and requ-

ested to report us of any pathological finding which would be traced upon eating the infested fishes.

### **Resistance of the Living Larvae to the Home Cooking and Salting Methods**

#### *Experimental Procedures*

The design and the results of the experiments were as follows:

A - The effect of cooking on the living larvae.

The dishes prepared according to the local methods of cooking vary greatly. However, there are some dishes that are very common. They are as follows: The anchovies are fried in oil in shallow pans, cooked in water with rice, baked with corn flour what is called "anchovy bread" and simmered in water.

For the experiments we chose frying in oil, cooking with rice in water and simmering in water. Before cooking the fishes were gutted, beheaded and sometimes deboned. To be sure, additional living larvae were placed deep into the center of the flesh before the infested fishes were cooked. After cooking in usual way, examinations made for the viability of the larvae (examining the recovered larvae in saline with naked eye or preferably using a binocular magnifier) revealed that none of the fishes had harbouring any living larvae.

B - The effect of salting on the living larvae.

The common method applied for home preservation with salt consists of placing the fishes in single layers into the big tins and salting heavily with coarse salt between the layers. Before salting the fishes are gutted and beheaded. The salted fishes are never consumed unless standing preserved for many weeks. At the experiments with heavily infested fishes none had been found to have any surviving larvae after 15- 20 hours of the preservation.

C - Resistance of the larvae to the different salt concentrations at the laboratory.

1 - Experiments with the living larvae alone: The collected living larvae were placed into 20 % and 25 % table salt solutions and into the dry salt. The larvae

placed into the salt solutions were killed within 30 minutes and those placed into dry salt were killed within 20 minutes.

- 2 - Experiments with the infested fishes: Here we used the same concentration of salt solutions. The larvae survived for 1 hour in both solutions and 5 hours in dry salt. The delay in killing with dry salt is explicable with the slow penetration of dry salt into the fish flesh.

### **Pathogenicity of the Living Larvae for the Laboratory Animals**

#### *Experimental Procedures*

The design and the results of the experiments were as follows:

For this experiment we used 2 cats, 5 mice, 5 rats and 5 guinea pigs. All the laboratory animals, with the exception of cats, were supplied from the healthy stock of the animal house of the Faculty. The two cats were physically examined prior to the experiments followed by fecal examinations for other parasitological infestations in order to be sure that they were safe and eligible for the tests.

The living larvae were fed to the laboratory animals, with the exception of the cats, through gastric tube and pipette. The cats were given 3 - 5 fishes daily for 30 succeeding days. The fishes fed were whole and heavily infested.

The mice and rats were given 100 living larvae daily for 10 succeeding days, totaly 1000 larvae for each animal.

The guinea-pigs were given 100 living larvae daily for 15 succeeding days, in total 1 500 larvae for each animal.

None of the test animals showed any clinical symptoms and in their autopsies which were carried out 10-20 days after the completion of the experimental feedings no pathological changes whatsoever were observed in macroscopical and microscopical examinations. No larvae, living or dead, were noticed in the abdominal cavities, in the organs or in their feces which were checked regularly.

### **Conclusions**

- 1 - Experimentally it has been shown that the *Contracecum* larvae occuring in anchovies have no pathogenicity or toxicity

for the laboratory animals. However, before deriving any conclusion for the pathogenicity of the larvae in humans from these experiments on the laboratory animals, it is advisable to go ahead with carefully carried out medical observations and surveys in areas where these fishes constitute the staple food of the population.

2 - Experimentally it has been shown that the *Contracaecum* larvae are not resistant to the common cooking and salting methods used for the preparation of anchovy dishes.

3 - Although the experiments on the pathogenicity for the humans have thrown a light on the problem, the decision to be taken for the consumption of the heavily infested fishes presents a complicated situation. Earlier, as it was stated, the infestation appears to be light during the early months of the catching season starting from September and continuing to December and getting heavier from January to April. All the catch during the heavy infestation period has a disgusting appearance which makes the fresh fish extremely repulsive to the average consumer. The Code of Sanitary Food Inspection which is in force in this country as well as in some other countries contain general provisions which prohibits the use of fishes infested with parasites and especially of those lots which are heavily infested and carry the encysted and living larvae or worms abundantly on or in their musculature and which cannot be eliminated by any means before marketing and which present a repulsive appearance to the consumers. In some countries, e.g., in Bulgaria, suggestions are made by authorities that during the peak infestation periods the anchovy fishing be abandoned.

### **S u m m a r y**

This paper deals with the occurrence of a nematode larvae in anchovies caught during the recent years, the works done for their identification, experiments made on their resistance to the common cooking and salt preservation methods and their pathogenicity for the laboratory animals.

### **Ö z e t**

#### **Hamsilerde Bulunan Contracaecum Larvalarının Pişirme ve Tuzlamaya Karşı Dayanıklılığı ve Laboratuvar Hayvanlarında Patogenitesi Üzerinde Araştırmalar**

Son yıllarda Hamsi balıklarında Nematoda sınıfından fazla miktarda larvalara tesadüf edilmiştir. Bu durumun bilhassa gıda kontrolü

bakımından da önemini ortaya koyabilmek için şu çalışmalar plânlaştırılmıştır:

- 1- İdentifikasyonu, balığın vücudunda dağılışı ve biyolojisi.
- 2 - Balığın ev usulleri ile pişirilmesi ve tuzlanması esnasında ısı derecesi ve tuza karşı larvaların dayanıklılığı.
- 3 - Larvaların laboratuvar hayvanlarında patogenitesi.

Yukarıda bildirilen plana göre, 1963/64 hamsi avlama mevsimi sonunda literatür incelemelerine ve eksperimental araştırmalara başlanmıştır.

1 - İdentifikasyonu ve balığın vücudunda dağılışı konusundaki incelemelere başladığımızda, memleketimizde larvaların idantifikasyonu yapılamamıştı. İstanbulda ilgililerce İngiltere'ye British Museum ve Ziraat Vekâletine teşhis için gönderilmiş olan larvalar hakkında alınan cevapta, gelişmemiş oldukları ve binaenaleyh teşhis imkânı olmadığı bildiriliyordu. Çalışmalarımızın ilk safhasında larvaların **Contracecum** soyuna bağlı nematodlardan oldukları ilk defa tarafımızdan tespit edilmiş ve bunlar hakkındaki bilgiler yayınlanmıştır.

II - Çalışmalarımızın ikinci safhasında, larvaların gerek serbest ve gerekse balıkların vücudunda iken, belli başlı pişirme ve tuzlama usullerine dayanma kabiliyetleri üzerinde eksperimental araştırmalar yapılmıştır. Alınan sonuçlara göre, larvaların dayanıksız oldukları ve mutad usullere göre pişirilmiş ve tuzlanmış balıklarda telef oldukları anlaşılmıştır.

III - Çalışmalarımızın üçüncü safhasında, canlı larvaları gerek serbest olarak ve gerekse enfekte balıklarla birlikte yedirmek suretiyle laboratuvar hayvanlarında eksperinental enfeksiyonlar meydana getirilmesine gayret edilmiştir. Bu konuda, literatürde larvaların zararsız olduğu bildirilmekte ise de, **Contracecum** soyuna yakın olan **Anisakis** soyuna bağlı nematod larvalarının insanlarda enfeksiyonlar yaparak ince barsaklarda şiddetli patolojik leziyonlara sebebiyet verdiklerine dair kayıtlara tesadüf edilmiştir. Aynı durumun Contracecumlar için de varit olabileceği düşünülerek bu konudaki kesin hükümlerimizi bir takım tahminlere değil, ve fakat dencysel çalışmalar sonucuna dayandırmayı uygun bulduk.

Almış olduğumuz sonuçlara göre, larvaların deney hayvanlarında herhangi bir envazyon yapmadıkları ve patolojik leziyonlara sebebiyet vermedikleri anlaşılmıştır.

Ancak , doğrudan doğruya insanlar için patogenitesi hakkında tam ve kesin bir hükme varabilmek için, ilgililerce medikal observasyonların ihmâl edilmemesi lüzumunu belirtiriz.

V - Şimdiki çalışmalarımıza göre, larvaların dayanıksız ve apathogen oldukları anlaşılmış ise de, hamsilerde envazyonun bilhassa fazla olduğu aylarda, kaslar içerisinde yaygın bir halde bulunan larvaların hiçbir tedbirle balık vücudundan bertaraf edilmesi mümkün olamadığından, arzettikleri çok iğrenç ve tiksindirici manzara dolayısıyla ve halen yürürlükteki Gıda Tüzüğü'nün bu hususlarla ilgili ve açıklıkla belirtilmiş olan hükümlerine uyularak, *bilimsel ve idarî zorunluğu kabul ile*, yedirilmesine musaade edilemeyeceği kanısında olduğumuzu teyit ederiz.

VI - Bu konularda, diğer ayrıntılı noktaların aydınlanması için Tarım Bakanlığı ilgilileri ile birlikte devam edecek çalışmalar ileride yayınlanacaktır.

Deney hayvanlarında patolojik incelemeleri yapan ve balıkların kaslarından kesitleri hazırlıyarak mikroskopik muayenelere yardım eden Fakültenin Patoloji kürsülerinden Dr. C. Köküslü ile E. Ertürk'e teşekkür ederim.

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