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Research Article

PREVALENCE INVESTIGATION OF CESTOIDEA CONTAMINATION IN MARINE SHRIMP IN THE COASTS OF THE CITY OF BUSHEHR

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ABSTRACT

Introduction: In Cestodiasis disease in shrimp, shrimp is intermediate hosts of the parasites in three genera Prochristianla, Parachristianla and Polyposefalus and their adult kinds can be seen in fish. Usually the adult parasites are seen in the place of the digestive glands, while they are sunken in to them. In shrimp, the larvae of parasite can be seen in the hepatopancreas tissues and its surrounding. The larvae of this parasites which exists in the surrounding tissues of hepatopancreas and the other tissues of shrimp, can be seen with the naked eye. There are also likely to see the eggs of parasites in shrimp muscles. This type of contamination due to strain to the abdominal area of shrimp, and partially destruction of tissues, especially the hepatopancreas tissue, and also using of food reserves of shrimp from the other parts, causes reduce of growth and weaken the body against the other illnesses, so with passing the time, can even cause the death of shrimps. Given the above, the main aim of the present study is investigation of the infection rate of cestodia pollution in marine shrimps in the coasts of the city of Bushehr.

Methods: For this study, 100 pieces of marine shrimps were hunted from the coasts of Bushehr, in summer. Since the marine shrimp fishing is just allowed in summer season, so this season was selected for the shrimp fishing. The hunted shrimps had various ages, since hunting was done randomly. Immediately after hunting, the shrimps were fixed by using the fixative solution (Davidson's fixative), then they were transferred to the Kazeroon laboratory and histopathological sections, were prepared from them.

Results: By investigation of prepared samples, no cestodia parasitic pollution was observed in hunted shrimp. Histological studies showed that 4% of observations relate to artifacts, 10% relate to lymphocytes, 10% to the accumulation of mononuclear, 33% to the melanization, 41% to the gill hyperplasia and 2% relates to the other observations.

Conclusion: The global reporting of parasitic pollution is low; the main cause of this low level of research is the low amount of damages caused by worms in shrimp. It means; these organisms are not able to create large damages and losses in shrimp population in the city of Bushehr.

Keywords: Prevalence, Cestodiasis Disease, Marine Shrimp, Bushehr.

INTRODUCTION

Crustaceans are accounted as the largest sub-branch in arthropods category that they fit more than 42000 species into their category. Crustaceans are mostly marine organisms and some of them also live in freshwater¹⁻³.

The brine shrimp are approximately 318 species which relate to the Penaeidae family, and their genus is Penaeus and Meta Penaeus. The brine shrimp are economically, 52 important species. These species live at the both northern and southern hemispheres and have been developed where the sea surface temperature is above 20 °C, in the summer. By the impact of

two steams of cold water, along the eastern coasts of the Pacific Ocean, the Penaeidae shrimp are just found in limited areas in California Peninsula, Mexico, Central America to Colombia and Ecuador. Penaeidae shrimps, in the terms of the number of species and quantity are more abundant along the West Coasts than the East Coasts. Of the 52 economically important species of this family, 33 species of them exist in the Indian Ocean and West of Athletic Ocean, which nowadays, 28 species of them can be reproduce artificially.

Zoology Classification of shrimps^{2,4}:

Phylum: Arthropoda

Class: Crustacea

Series: Eumalacostraca
 Super order: Eucarida
 Order: Decapoda
 Sub order: Natantia
 Infraorder: Penaeidea
 Super family: Penaeoidea
 Family: Penaeidae
 Genus: Penaeus
 Species: Indicus

Shrimps are formed a large group of crustaceans, which their sizes are variable from microscopic dimensions up to 35 cm. Although, nearly 25000 species of them are identified, but economically less than 300 species are considered. A large amount of fishing in the world, relates to 100 species of shrimp. Shrimp with having three pieces in front of their chest, which are attached to the head and these pieces have become to the "jaw- feet", are account as the swimmer crustaceans of sea.

Polluting with cestodea, is a disease which can cause economic losses to the shrimp farming industry. In fact shrimp are the intermediate hosts of the parasite and their adult forms can be seen in fish and can be seen more in the hepatopancreas and its surrounding tissues. This type of contamination due to the pressure to the abdominal enclosure of shrimp and partially destruction of the tissues, especially the hepatopancreas tissue and on the other hand, using the host's food supply can cause slowdown and weakening of the body in the fight against other diseases and so, by passing the time, can even cause mortality in shrimp. Rodelphi in 1808 for the first time, proposed the name of Cestoidea (derived from a Greek word Cestos that means belt), for this group of parasitic worms. These organisms were previously had local and native names. They were called in Persian as articulated, embryonic or tape worms, and idiomatically it is called as gourd worms. Human is known these worms from thousands of years ago. So far, 5- 6 thousands species of them have been described, however, determining the actual number of species is not possible except by speculation. Because some of the reported names were synonymous with each other and many others still have not been reported. Cestodes are all parasites. Mature Cestodes live in intestine or bile ducts of vertebrate hosts. Their body length is changed from several millimeters to several meters. Clearly or in undetermined, their body is articulated. The number of articulations may be from 3 to several thousands. The dorsal- ventral surfaces in worms are flattened for complete coordination to parasitic life. The majority of them are hermaphrodite and each articulation in the stage of maturity is perfect sexually. Their immature forms or their infants that traditionally have been known as bladder worms are living in the form of bags in muscles and other tissues of vertebrate or invertebrate animals. In general, the damaged caused be worms in shrimp, is not impressive. These organisms cannot cause massive casualties, and in this sense are not important for pathogenesis. Therefore, their pathogenesis discussion especially Cestodes have been reviewed very briefly⁵.

Body organs of the host that are invaded by this parasite are different. For example, the strip- shaped flat worms usually are found in sefalotoraks and also in the intestine. And leaf

worms, usually can be seen as in the form of cysts in adjacent tissues to the limbs and sefalotoraks's organs as well as in abdominal muscles and the external skeleton. Natural locations of worms in shrimp's body are shown in the Figure⁵. Cestodes usually are located in vicinity of digestive glands, nematodes in the limbs and in the sefalotoraks's organs also in the intestine and trematodes, they are also can be seen in the organs and adjacent organs of sefalotoraks and muscles of the abdominal cavity and under the external skeletons. About the lack of contamination of farmed shrimp, it should be noted to a number of parasitic factors particularly worms. Since most of worms in a stage of their life cycles requires to one of the oysters, crab or fish as their hosts. And because breeding farms are being controlled, so the cycle of life in worms is not complete and because of this reason, these contaminations are less occurred to be reported. In some cestodes, fish is raised as the intermediate host and in the others is a final host⁶. In this study, we want to review the existence of these contaminants in marine shrimp in the coasts of Bushehr city, and in the case of the presence of these contaminations, to review the level of pollutions and their interpretation and the relationship between them and the other population reducing agents of marine shrimp in Bushehr.

METHODS

In this study, marine shrimp were caught from several fish catching stations of Bushehr's beaches, by using boats which were equipped with the trawl net insoles. Then, immediately by using the insulin syringe, the Davidson fixative solution, was injected into the shrimp's abdominal cavity. And after several transects in the whole body of shrimp, they were transferred into the Davidson's solution which its amount was at least twice the body volume of the shrimp and then were transferred to the histopathology laboratory for the preparation of microscopic section.

The best fixative solutions for shrimp histology works is Davidson's solution.

Ingredients to prepare 1 liter of this solution, include:

- 330 milliliter of 95% ethanol
- 220 milliliter of 37% formalin
- 115 milliliter of glacial acetic acid
- 335 milliliter of distilled water

A fixative solution should be kept in a sealed container. For fixing the larvae and post- larvae of shrimp, they must be directly kept in the 10 times amount of sample size with Davidson's solution. For the post- larvae larger than 20 mm, making several small incisions in the dorsal midline of sefalotoraks's organ and the first abdominal articulation, cause a rapid penetration of the fixative solution into the tissue. The injection for larger shrimp should be performed in the hepatopancreas areas and into the muscles in abdominal areas. The volume of injection depends on the size of shrimp. For large- sized shrimp, the injection in hepatopancreas should be done in several different areas. Immediately after the injection, the color of the shrimp's tissue becomes white. Shrimp in this work, have received amount 2 ml of Davidson's solution. Injection areas were in lateral areas in both sides of the carapace and into the hepatopancreas, that approximately 60%

of the volume of the solution was injected in these areas. The remaining solutions were injected into the 6 regions of the lateral areas of muscles in the first to sixth abdominal articulations. Due to the sensitive nature of hepatopancreas against autolysis, the pectoral area of shrimp was cut as far as the hepatopancreas gland became appear and then the whole of shrimp were fixed in a fixative solution in a volume equal to 10 times of the sample volume. Because the Davidson's solution is acidic, so duration time for curing of the samples depending on its size varies between 24 to 72 hours. Given to the sizes of the samples in this study, after passing of 24 hours, the container of the samples became empty from Davidson's solution. Then the samples were transferred into the container which contained 70% ethanol solution and was kept in this solution till the histological works were performed (Figure 1)⁷. Then the prepared slides were examined microscopically in the terms of the amount of artifacts, accumulation of lymphocytes, nuclear accumulation, melanization, hyperplasia and the other items. The obtained results were analyzed by SPSS statistical software.



Figure 1: The fixative injection into some parts of the shrimp's body

RESULTS

During the study that was performed to determine the level of Cestoidea contamination, on 100 pieces of marine shrimp which were brought from coasts of the city of Bushehr, no contaminations were found (Chart 1).

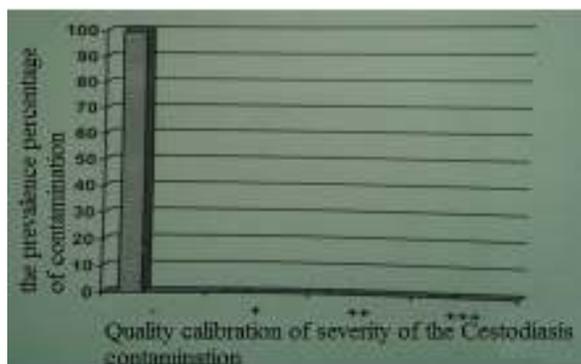


Chart 1: A column of quality intensity of Cestoidea contaminants in hunted marine shrimp from the coasts of the city of Bushehr

Any Cestoidea was not observed, during the study and the other percentage of the observations were included as following, artifacts 4% of the observations, lymphocytes 10%, accumulation of mononuclear 10%, melanization 33%,

hyperplasia of the gills 41% and the other items were included 2% of the observations (Table 1). (Figures 2- 6)

Table 1: Microscopic observations of slides

Observations	Percentage
Cestodiasis	0
Artifacts	4
Lymphocytes	10
Accumulation of Nuclear	10
Melanization	33
Hyperplasia	41
other items	2



Figure 2: A healthy gill tissue, Original magnification 40X, hematoxylin and eosin staining



Figure 3: A healthy muscle tissue, Original magnification 40X, hematoxylin and eosin staining



Figure 4: A gill tissue affected by Hyperplasia, Original magnification 40X, hematoxylin and eosin staining



Figure 5: A healthy muscle tissue of lymphocyte sample, Original magnification 40X, hematoxylin and eosin staining



Figure 6: A healthy muscle tissue of Artifacts sample, Original magnification 40X, hematoxylin and eosin staining

DISCUSSION

During the study, no parasitic contaminant of Cestoidae was observed in marine shrimp of the Bushehr's coasts. During a research that was performed about the parasitic contaminant of Cestoidae on shrimp and squid that lived in the state of Georgia in November 1997, 83 percent of the shrimp were polluted by Cestoidae's contaminant in their hepatopancreas tissues and nervous systems. This is the only study that among the examinations of different articles, reports the high percentage of parasitic contaminations; and the other studies have noted the low percentages⁸.

In another study was reported that Peneus Sesti Frous shrimp, was became contaminated to Cestodiasis pollution. The mentioned shrimp that was became contaminated to the parasitic pollution in its hepatopancreas, had been infected by slow growth of dense cyst including hemocytes, fibroblasts and collagen fibers around the parasite³. Since the prevalence of Cestoidae parasitic contaminant in shrimp is low in the country, and the articles from sources of other parts of the world is also low, and to learn more about this contamination, we have been expressed some reports from this disease in Iran's fish.

Bothriocephalus which is caused by a parasite species of Bothriocephalus that are pathogenic Cestodes for fish are widely prevalent in the aquaculture breeding farms of the country and have had major losses in juveniles of carp and grass fish or have led to reduce of growth in 1- or 2- year- old fish and therefore have caused economic losses for breeding fish companies. Legionnaires' disease that may also be called Legionellosis is another common disease that there is in some of important and economical fish species such as bream fish with a scientific name "Abramis Brama orientalis" and Rutilus rutilus caspicus fish that all exist in fresh water resources of the country. According to the specific lesions on fish's reproductive system, Legionellosis disease has lead to infertility and so has reduced the fish stocks. Hence the importance of Cestodes in the development of aquaculture in the country, been tried, to be investigated the common disease caused by them and methods of control and treatment of this parasites group, more fully. In the past, Bothriocephalus Gowkongensis has been reported in several species of carp fish in Sefidrood River, and also Caryophyllaes Fimbriceps has been reported in the category of carp fish. In this report,

the species "Polroserkuid Diphylo bothrium Latum" has been introduced in the river dodder fish⁹.

The species Caryophyllaes Fimbriceps of the Caryophyllidea family has been reported in 15.8 percent of carp fish and 7.1 in mullet fish (Mugil Auratus) of the Caspian Sea. It seems that this is the first report in the world about the presence of this parasite in the mullet fish¹⁰.

Different species of Cestodes from litocestoidea family have been reported from the intestine of the carp fish from Far East, Europe, Azerbaijan Republic and Iran; the Cestodes in Iran from the species Khavia Arminakea which lives in Zayandeh Rood River, is isolated from the Capoeta Capoeta fish species and Capoeta Bush fish (egg yolk fish or subterranean fish)¹¹.

According to the report of Yamaguti in 1959, from Diplokotilide family and the species of Bothrimonos Falaks, this worm is one parasite of the Asiptos Estalatus and Aspiners Golden ashtadet sturgeon of the Caspian Sea¹².

Other investigation on Iran's sturgeon was reported by Mokhayer in 1352¹³.

CONCLUSION

According to the reports of parasitic infections in the world, just small amount of reports are devoted to these infections. The main reason for this low level of research in this field is shortage of damage caused by worms in shrimp. Since these organisms cannot cause broad damage and casualties in shrimp; and in losses basically are not important in pathogenesis, therefore the studies has been done in this case are low. And given to the obtained results that does not show any contamination in marine shrimp of the coasts of Bushehr, can be partially realized the little importance of this studies. And that observed lack of pollution in the shrimp of the coasts of Bushehr, is not indication for the absence of contaminants; because some factors can be involved in lack of accuracy of these results, including:

- 1) The low number of samples
- 2) Sampling form a limited area of coast
- 3) Experimental errors
- 4) Sampling in a particular season
- 5) The selectivity of the nature in reduction of infected shrimp
- 6) Marine contaminations and therefore the reduction of parasites and the main hosts

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