Pathological and Molecular Characterization of Lymphocystis Disease Virus (LCDV) In Sea Bream Fish in Egypt

Aly S. M.[(1)], Shimaa M. Mansour [(2)], Randa Y. Thabet [(3)]

(1) Department of Pathology, Faculty of veterinary Medicine, Suez Canal University. (2) Department of Virology, Faculty of Veterinary Medicine, Zagazig University. (3) Department of Aquaculture Diseases Control, Fish Farming and Technology Institute, Suez Canal University.

Abstract

Hypertrophied nodules or papilloma like lesions were detected in the skin and fin of Sea bream farms (at Mothalath El Deba and Bardaweil Lake) in Egypt with morbidity rate reached to 70% and mortality rate up to 40%. A total of 20 affected cultured sea bream samples were collected for laboratory examinations. Histopathological examination revealed intracytoplasmic basophilic inclusion bodies in the skin and fins lesions. In addition, a thick hyaline capsule surrounding the hypertrophied fibroblast and contain heavily enlarged cells (lymphocystis) with necrosis and inflammatory response. PCR results with primers specific for the gene encoding major capsid protein (MCP) gave a predicted amplified product at 405 bp fragment by agarose gel electrophoresis. The current results encourage further investigations for this virus in Egyptian farms and recommend the development of prevention strategies against LCDV in Egypt.

Key words: Sea bream, Egypt, Iridovirus, Lymphocystis disease virus, Histopathology, Molecular detection, PCR, phylogenetic analysis.

Introduction

Aquaculture has been practiced in Egypt for millennia, but modern approaches have recently been adopted to maximize its output. Today, aquaculture production in Egypt is the largest in Africa with about one million tonnes per annum ***(Shaalan 2018)***. Fish has become an important resource in Egypt to meet the food and nutrition security needs for the rapidly expanding human population ***(Aly, 2013)***. Fish is a food of excellent nutritional value, providing high quality protein, a wide variety of vitamins and minerals, a range of micronutrients, and fatty acids particularly the omega-3 fatty acids essential for human brain

development and reproduction ***(Tacon and Metian, 2013)*** because they are required for fetal and infant growth, maturation and cognitive development ***(Michaelsen et al., 2011).*** They are also often the cheapest and most frequently consumed animal-source food in low income food deficit countries ***(World Bank, 2006),*** making an important contribution to diversity in otherwise monotonous diets dominated by starchy staples ***(Thilsted, 2013).*** In developing countries, fish contribute about 30% of the total consumption of animal protein per capita ***(Wang et al., 2015).*** Marine fish production in Egypt contributes to about 70% of the total production of marine fish from North Africa as most of the North African countries depend on fishery catches rather than aquaculture ***(Rodger and Davies, 2000; Mustapha et al., 2014).*** Many challenges face the development of mariculture in Egypt and it is important to determine the constraints and limitations facing the development of this industry and plan to alleviate these issues wherever possible. The most important obstacles hindering sustainable aquaculture development in Egypt are viral diseases which are incriminated for high economic losses and mortalities in fish farms. Besides mortalities, fish viral pathogens have a negative impact on feed conversion rates and total body weights of recovered fish post infection ***(Shaalan et al., 2018).*** Viral infections are more difficult to control due to the high susceptibility of aquatic animals to them at an early age, limited availability of therapeutics, insufficient knowledge about pathogenesis of virus infections and limited knowledge about natural resistance mechanisms in aquatic animals. In Egypt, there is not enough information about the real map of viral infections and distribution in fish due to lack of surveillance program for monitoring of viral infections in fish ***(Shaalan et al., 2018)***. Major disease problems affecting gilthead seabream farms is lymphocystis disease, the most frequently reported viral infection in farms in the South Atlantic and Mediterranean areas ***(Borrego et al., 2001; Colorni & Padros, 2011).*** Lymphocystis disease (LCD) is a self-limiting condition characterized by the appearance of hypertrophied fibroblasts in the connective tissue of fish, primarily in the skin and fins. The causative agent of the disease is the Lymphocystis disease virus (LCDV), a member of the *Iridoviridae* family, belonging to Lymphocystivirus genus. LCDV genome is a single linear double-stranded DNA molecule ***(Jancovich et al., 2012).*** LCDV is a large icosahedral non-enveloped viral particle may vary in size from 120 to 340 nm in diameter ***(Tidona & Darai 1999; Paperna et al., 2001).*** Development of the

disease is usually associated with several environmental factors and, more frequently, with stress conditions. In gilthead seabream, LCD-associated lesions have been described only in the fish skin and fins, and usually disappear after 20-45 days depending on water temperature ***(Paperna et al., 1982; Gonzalez de Canales et al., 1996; Kvitt et al., 2008).*** In Egypt, few published studies addressed the marine fish diseases. Accordingly, it is important to investigate the current situation of the emerging viral diseases among cultured marine fish in Egypt. The present study aimed to diagnose LCDV among cultured gilthead seabream (*Sparus aurata*) through clinical signs, postmortem lesions and histopathologically as well as molecular biology investigation.

Materials and methods

A total of 20 clinically infected sea bream fish samples were collected from Egyptian farms at Mothalath El-Deba, Damietta Province and Bardaweil Lake, North Sinai Province (10 fish each) during the period from January to April 2017. The collected fish showed multifocal to diffuse white, round, firm, papilloma or tumor like nodules on the skin of the body, fins, eyes and mouth easily detected by naked eye with mortalities up to 40%. The collected tissue specimens of fish (skin, muscle, fin) were used for histopathological examination, molecular detection and sequencing.

Histopathological examination

All collected sea bream fish were cleaned, washed three times in sterile distilled water and dried thoroughly with sterile towels. The collected tissue specimens (skin, fins, hepatopancrease) were fixed in 10% neutral buffered formalin for 48 hours, then washed under running water and dehydrated by using increased graded concentrations of ethyl alcohol. The specimens were cleared by xylene then blocked in paraffin. Five micron thick paraffin sections were prepared and stained with hematoxylin and eosin. The sectioned were mounted in Canada balsam and covered with cover slips, then examined microscopically ***(Suvarna et al., 2013).***

Fish samples for PCR

DNA extraction kits

A part of skin, muscle and fin are kept in separate containers at -20^{o}C for DNA extraction and diagnosis of Lymphocystis disease virus with PCR. Total DNA were extracted by following the manufacturer instructions of the extraction Kits GeneJET Genomic DNA Purification Kit (Thermo scientific, UK).

Gene amplification reaction conditions for LCDV MCP gene

Oligonucleotide Primers according to ***Kvitt et al., (2008):***

F 5’ TTTGAATGGGAGGATCAC 3’; R 5’ TCCGTAAATGCTGTTAGC 3’. that were targeting (405 bp) the

complete ORFs of structural major capsid protein of LCDV. The PCR was performed in a total volume of 50 µl in a sterile 0.2 ml RNase free PCR tube using 2X DreamTaq™ PCR Master Mix (Thermo scientific, UK). The solution phase PCRs contain the following contents: Master mix (including DNA polymerase + 10mM dNTP mix) 25 µl, Forward primer (50 pmole) 1 µl , Reverse primer (50 pmole) 1 µl , DNase free water 20 µl , Template (cDNA) 3 µl with a total 50 µl.

The optimized PCR cyclic reaction conditions were performed according to ***Poulos and Lightner (2006)*** in Creacon, Thermo cycler, Holand and described as follow: 1 cycle of an initial denaturation at 95°C for 2 minutes and followed by 39 cycles of denaturation at 95°C for 30 seconds, annealing at 65°C for 30 seconds and elongation at 72°C for 30 seconds. The PCR amplification was completed using 1 cycle of final elongation step at 72°C for 10 minutes then cool incubation at 4°C.

Gel electrophoresis

Fifty ml from 1.5% agarose was prepared in 1x TAE buffer by heating in microwave. The melted agarose was left to cool to about 45^{o}C. 50µl from ethidium bromide (stock=0.5 mg/ml) was added to give a 0.5 µg/ml as final concentration. The gel was poured. After complete solidification of agarose, the comb was removed then 1X TAE buffer was added. Five µl of the PCR products and 5 µl molecular weight marker were added into the marked wells formed in gel. Electrophoresis was done at 100 volts for 40 min then the gel was viewed and photographed on the UV transilluminator. Gel documentation system (Geldoc-it, UVP, England), was applied for data analysis using Totallab analysis software Ver.1.0.1 (www.totallab.com).

Partial sequencing:

Purification of specific PCR amplicons from agarose gel:

The PCR amplicons (405 bp) was considered specific bands for LCDV. The amplified PCR products were purified using QIAEX II Gel Extraction Kit (QIAGEN, USA) following the manufacturer's instruction.

Specific bands were excised using a clean scalpel. The gel slices were weighed. Three volumes of Buffer QX1 were added to 1 volume of gel for DNA fragments. (For example, 300 µl of Buffer QX1 was added to each 100 mg of gel). A 30 µl of QIAEX II was added to the sample. The tubes were incubated at 50°C for 10 min to solubilize the agarose and bind the DNA. The samples were centrifuged for 30 seconds at 13000 rpm. The supernatants were carefully removed with a pipette. A 500 µl of Buffer QX1 were added to the pellets. The pellets were resuspended by vortexing. The tubes were centrifuged for 30 seconds at 13000 rpm. The supernatants were carefully

removed with a pipette. A 500 µl of Buffer PE were added to the pellets. The pellets were resuspended by vortexing. The tubes were centrifuged for 30 seconds at 13000 rpm. The supernatants were carefully removed with a pipette. The washing process with Buffer PE was repeated twice to remove residual salt contaminants. The pellets were kept for 30 min for air-dryness.

The DNA was eluted by addition of 20 µl of Tris buffer. The pellets were resuspended by vortexing. The tubes were incubated at room temp for 5 min, centrifuged for 30 seconds at 13000 rpm. The supernatants containing the purified DNA were carefully transferred to a clean tube.

Sanger sequencing:

The purified PCR products were sequenced using the same forward and reverse primers used in PCR (Delta Scientific Consultancy Center, Alexandria, Egypt). Forward and reverse sequences were aligned together to generate a consensus sequence using DNA Baser Sequence Assembler version 4.36 (http://www.dnabaser.com/). The obtained sequences were further identified by nucleotide BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

Sequence and Phylogenetic analysis:

The nucleotide sequences obtained from the PCR products were aligned with other viral sequences available in GenBank (http://www.ncbi.nlm.nih.gov/) of the National Centre for Biotechnology Information (NCBI). Comparative alignment of nucleotide sequences was performed using ClustalW using MegAlign module of DNAStar software (Lasergene version 7.2, USA). The phylogenetic tree was generated using neighbour-joining method employing the Kimura 2-parameter correction in MEGA version 5 (www.megasoftware.net) by aligning the nucleotide sequence of virus genes with other sequences available in the GenBank with 1,000 bootstrap analyses.

Results

Gross Pathology

Diseased fish showed multifocal to diffuse white, round, firm, papilloma or tumor like nodules on the skin of the body, fins, eyes and mouth **(Figure 1)**. Hypertrophied cells and abnormal growth (white colour nodules) in the outer skin of the fish as can easily be detected by necked eye. Infected sea bream showed pale colour and some petechial hemorrhage in the internal organs

Histopathological findings:

Many clusters of Lymphocystis cells (hypertrophied fibroblasts cells) were seen in the connective tissues of the epidermis at fins and skin. Numerous hypertrophied cells with basophilic intracytoplasmic inclusion bodies were observed in the dermal connective tissues where

they were surrounded by an abundance of inflammatory epithelioid cells. The lymphocystis hypertrophied cell was surrounded by a thick smooth hyaline capsule. The nucleus of lymphocystis cell was enlarged, irregular and containing basophilic marginated chromatin. LCDV infected cells were irregular, round and connected with each other and some lack their nuclei. A summary of histopathological findings in sea bream fish lesions was shown in **Figure (3) and Table (1)** and were characteristic for LCDV. Regarding, No characteristic lymphocystis lesions were detected in the internal organs, but variable histopathological alterations were observed. Hepatocytes showed vacuolization and increased cytoplasmic basophilia with some areas of necrosis.

III- PCR results of WSSV in shrimp samples

Single-step PCR results with primers specific for the gene encoding MCP gave a predicted amplified product at 405 bp fragment by agarose gel electrophoresis as observed in **Figure (4) and Table (2)**. This PCR product was obtained from 4 LCDV samples that were previously suspected by gross lesions and histopathology as being infected with LCDV. These samples represent isolates from two localities in Egypt including; Bardaweil Lake and Mothalath El Deba.

The amplified PCR products in LCDV of infected sea bream fish samples based on the gross lesions and histopathology revealed 2 out of 4 fish samples were PCR positive.

Sequences and phylogenetic analysis of LCDV:

To assess genetic characteristics of LCDV samples and identify specific viruses for further characterization, sequencing and comparative analysis was performed. A primer pair that amplifies a 405 base pair fragment of the encoding region was used to sequence the variable regions of LCDV samples. Two fish samples were chosen for genomic sequencing and nucleotide BLASTn analysis from both localities. The percent of identity of nucleotide sequences of our samples was 96%. Comparative alignment of nucleotide sequences showed that, the two samples shared similarity 80-89.7% with LCDV sequence from Korea, Canada, USA, Tunisia and Israel.

To recognize the association of LCDV samples with further circulating LCDV, a phylogenetic tree based on the variable region nucleotide sequences of 12 LCDV that are available in GenBank, was constructed using the neighbour-joining method. The tree **(Figure 5)** showed that our samples were closely related to LCDV isolate from Korea (AY823414), LCDV isolate from Canada (GU939626), LCDV isolate strain Leetown NFH

from USA (GU290550), isolate LCDV strain SA1.ETun.2011 from Tunisia (HE650105) and isolate LCDV-SA-Eilat from Israel (EF184306).

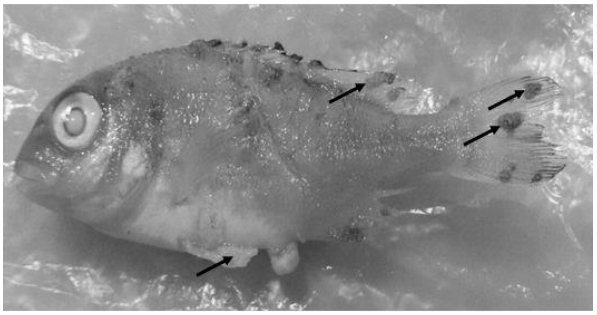

Figure 1: Sea bream fish showing multifocal to diffuse white, round, firm, papilloma or tumor like nodules on the skin of the body and fins (Arrows).

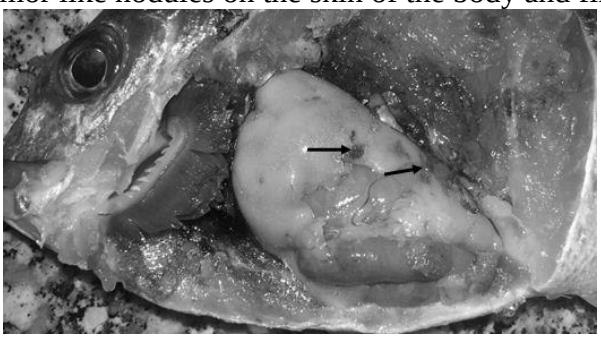

Figure 2: Sea bream fish showing pale liver with petechial hemorrhage (Arrows).

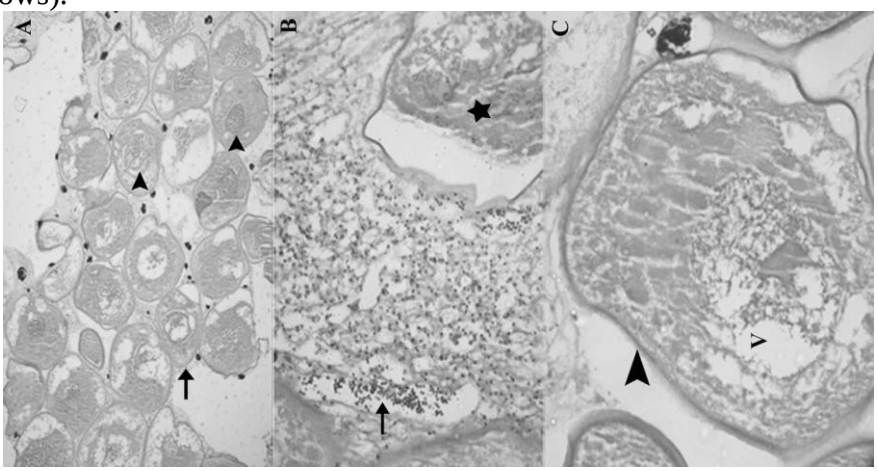

Figure 3: Histopathological examination of sea bream suspected to be infected with LCDV (H&E). (A) Fish dermis tissue showing lymphocystis in several developmental stages with numerous hypertrophied cells

containing basophilic intracytoplasmic inclusion bodies (Arrow head) surrounded by a thick smooth hyaline capsule (Arrow) in the connective tissues of the dermis X400. **(B)** Lymphocystis hypertrophied cell showing hyaline necrosis and vacuolization (Star) with inflammatory cells (Arrow) X400. **(C)** Lymphocystis hypertrophied cell was surrounded by a thick smooth hyaline capsule (Arrow head), showing vacuolization (V) and the nucleus was enlarged, round, irregular and containing basophilic clumped chromatin X400.

Table 1: *Scores of histopathological lesions in 20 collected sea bream fish suspected to be infected with LCDV:*

Histopathological lesions	**LCDV**
Necrosis in dermis, epidermis	++++
I/N inclusion bodies	-
I/C inclusion bodies	++++
Vacuolar degeneration	+
Lymphoid organ spheroids (LOS)	++
Zenker's necrosis	+++
Cellular degeneration	+++
Nuclear hypertrophy	+++
Pyknosis and karyorrhexis	+++
Lymphocytic infiltration	+++
Accumulation of fluid (edema)	+++
Degeneration & hypertrophy	+
Lymphocystis cells (hypertrophied fibroblasts cells)	++++
Inflammatory cells	++++
Thick smooth hyaline capsule	++++
Enlarged, irregular cells and nucleus and containing basophilic marginated chromatin	++++
Altered shape of Hepatocytes	++++
Hyaline degeneration	++++
Signs of vacuolization	++++

The criteria of scoring were done according to Shackelford et al., (2002):

++++ Severely affected (100% of examined samples have lesions)
+++ Highly affected (75% of examined samples have lesions)
++ Moderately affected (50% of examined samples have lesions)
+ Mild affection (25% of examined samples have lesions)
- No affection (no lesion in all examined samples)

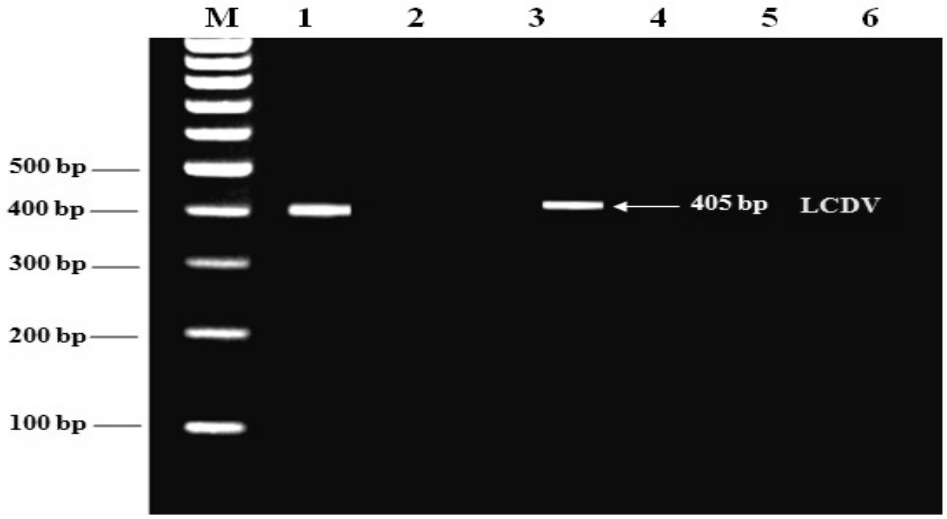

Figure 4: Molecular detection of LCDV in sea bream fish using PCR. The amplified products from fish tissues following 1.5% agarose gel electrophoresis and ethidium bromide staining. **M:** DNA marker (100 bp), **lane 1 and 3:** Positive LCDV samples, **lane 2 and 4:** Negative samples, **lane 5 and 6:** Negative control.

Table 2*: Molecular detection of sea bream fish virus (LCDV) using PCR in samples collected from Mothalath El Deba and Bardaweil Lake, during 2017*

Locality	Fish (LCDV)		
	Total No. of samples	No. of Pooled samples	No. of Positive samples
Mothalath El Deba	10	2	2/2
Bardaweil lake	10	2	0/2

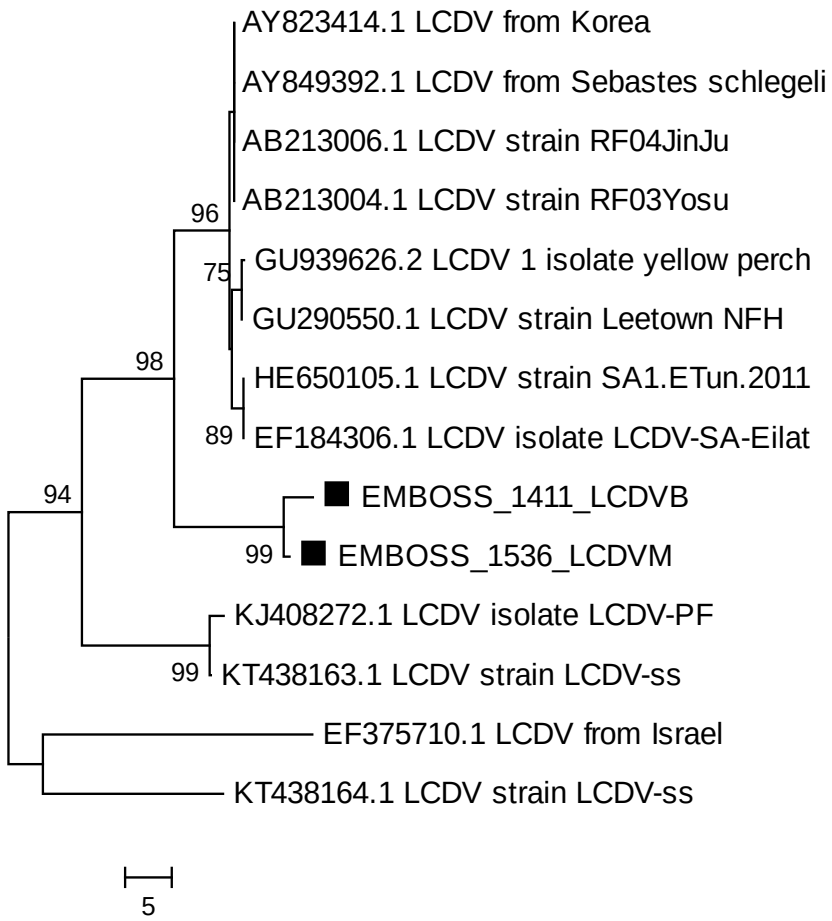

Figure 5: Phylogenetic tree of LCDV based on a partial nucleotide sequence. The tree was constructed using the Neighbor-joining method in MEGA5. The robustness of individual nodes of the tree was assessed using 1000 replications of bootstrap re-sampling of the originally aligned nucleotide sequences. Bootstrap values ≥70% is shown above the branches. The virus isolated in this study is marked with solid quadrilateral.

Discussion

Emerging disease epizootics frequently cause substantial, often explosive, losses among populations of shrimp and fish, resulting in large economic losses in commercial aquaculture and threats to valuable stocks of wild aquatic animals ***(Walker & Winton, 2010)***. A large number of pathogens threaten the fish aquaculture industry with a majority of these being viral in etiology, so viral illnesses constitute the main problem faced by penaeid shrimp and seabream fish farms worldwide

(Verônyca Coelho-Melo et al., 2014). In Egypt, few published studies addressed the marine fish diseases. Accordingly, it is important to investigate the current situation of the emerging viral diseases among cultured marine fish in Egypt.

To better understand the circumstances of some emerging viral diseases among fish farms in Egypt, twenty fish were collected from Mothalath El Deba, Damietta Province as well as Bardaweil Lake, North Sinai Province that were examined for clinical signs of disease and then investigated for the presence of viral infection. Under field conditions, it was possible to suspect clinically affected seabream fish. The gross lesion and histopathological pictures were recorded in some cases and later confirmed by molecular detection and sequencing of the viruses.

LCD outbreaks are frequently observed in the Mediterranean gilthead seabream aquaculture. Generally, viral diseased fish show low growth rates, which may be caused by the anemia generally associated with this disease as mentioned by ***Iwamoto et al. (2002),*** although it is usually described as a self-limiting disease, there are several reports on mortalities up to 45 % in juvenile fish, which may be related to secondary bacterial infections or with particularly large growth of lymphocystis, which severely impaired fish osmoregulation, breathing or feeding, cannibalism and/or parasitic infestations ***(Colorni & Padrós, 2011; Haddad-Boubaker et al., 2013; Dezfuli et al., 2012).*** In heavily affected fish, lymphocystis may cover the entire body, spreading from the gills to the fins and less frequently, they have also been described on eyes, causing exophthalmia, and internally over the mesenteries, peritoneum and several internal organs as mentioned by ***Xing et al. (2006).*** LCDV is considered a dermotropic virus so diagnosis of LCD is generally based on typical skin lesion observation. In the current study, abundant and extensive nodules were seen all over the skin especially in the pectoral and dorsal regionas well as caudal fins of cultured sea bream fish. The pathognomonic signs of LCD include the appearance of small pearl-like nodules on the skin and fins that are usually grouped in clusters, papillomatous in appearance, and can cover the entire body surface of the fish as similarly reported by ***Wolf (1988).*** These nodules consist of LCDV-infected hypertrophied dermal fibroblasts (up to 1 mm in diameter), named lymphocysts or lymphocystis cells ***(Bowden et al., 1995).***

Histopathological examination of LCDV-infected fish showed the presence of LCDV in the skeletal muscle and gill lamella. The cytoplasm of lymphocystis cells were changed, developing basophilic, intracytoplasmic

inclusion bodies that appeared as dense vacuolated bodies with enlarged nucleus and cellular hypertrophy. In addition, a thick hyaline capsule surrounding the hypertrophied fibroblast was observed in the cytoplasm, especially in the mature lymphocystis cells as previously confirmed by ***Hossain and Oh (2011).*** Histopathological studies carried out in LC-diseased fish have been focused on the description of lymphocystis cells, with few reports dealing with histological observations of the internal organs, except when lymphocysts were also present ***(Sheng & Zhan, 2004). I***n this study, LC-diseased gilthead seabream specimens showed lymphocystis cells only in the dermis of the caudal fin, with histological characteristics resembling those previously described in this fish species as recorded by ***Gonzalez de Canales et al. (1996).*** Histopathological alterations of varied severities were also observed in other organs, including necrotic changes in the liver and kidney, inflammatory response in the intestine and intraventricular hemorrhage. On the other hand, necrotic changes in the epithelium were the only histological alterations described so far in gilthead seabream fishes affected by LCD ***(Cano et al., 2009).*** In the present study, hepatocytes showed vacuolization and increased cytoplasmic basophilia with some areas of necrosis.

The second approach of this work was to molecularly detect the viral infection in diseased fish. Fish tissues were analyzed by PCR using primer set to LCDV. Although these methods are quite accurate, they are too expensive to be used viably as mentioned by ***Mello et al. (2011).*** The availability of sensitive and specific tests for detection of pathogens in sea bream fish is essential for accurate diagnosis of diseases affecting cultured population. PCR is a rapid, sensitive and highly specific detection method for fish viruses, which can be a powerful tool to detect iridovirus infections; LCDV in sea bream fish as reported by ***Mao et al. (1997).*** A primers set was used to detect 405 bp amplicons in LCDV using PCR. The primers was shown to be specific for Major capsid protein (MCP) gene of LCDV and no amplicons were detected using DNA extracted from sea bream fish infected with other fish viruses as indicated by ***Kvitt et al. (2008).*** The PCR has been used to amplify a portion of LCDV genome using specific primer as stated by ***Kvitt et al. (2008).*** It was used for definite identification of LCDV and the results showed higher sensitivity to detect the LCDV like those described before. In this study, 20 samples were collected, pooled together in 4 tubes and submitted to DNA extraction using specific primer to detect viral DNA in the

tissue. 2 out of 4 samples were positive in LCDV from locality Mothalath El Deba, which showing that affection is high in this area than another one.

The PCR has been used to amplify a portion of LCDV genome using specific primer of MCP gene. It was used for definite identification of LCDV, the results showed higher sensitivity to detect the LCDV like those described before as recorded by ***Kvitt et al. (2008).*** In this study, 4 samples were collected, pooled together and submitted to DNA extraction and PCR using MCP gene specific primer to detect viral DNA in the tissue. 2 out of 4 samples were positive.

The third approach of this work was to assess genetic characteristics of viruses via nucleotide sequencing and phylogenetic analysis. Thus, nt sequence analysis is needed to completely identify the viruses of collected samples as stated by ***Mello et al. (2011).*** The phylogenetic tree based on sequence of specific gene for Lymphocystis disease virus showed that samples are clustered and compared with other related nucleotide sequences on Genbank. In Egypt, there are no records for LCDV until now.

In sea bream fish, LCDV of our samples was related to Korea, Canada, USA, Tunisia and Israel with homology 80-89.7%.

Although the reported LCDV samples showed similar macroscopic and microscopic picture with those detected by ***Hossain and Oh (2011).*** However, low identity was reported between our nucleotide sequences and those published sequences derived from Korea, Canada, USA, Tunisia and Israel isolates, as the published sequences recorded that the isolates from those countries collected from different species of fishes not from sea bream fish.

In the Mediterranean Sea, the major constraints in aquaculture of sea bream were LCDV as stated by ***García-Rosado et al. (2007).*** Since the 1980s, LCDV has been reported in different countries of the Northern Mediterranean coasts as recorded by ***Menezes et al. (1987); Le Deuff and Renault (1993).*** In the Southern coasts, only a partial MCP sequence was reported in Tunisia in 2005, but with no information about its epidemiological impact or its geographic origin as mentioned by ***Cano et al. (2010).*** LCDV has been reported more than 100 different marine and freshwater fish species, it seems obvious that, the existence of differences regarding the viral genome structure, gene organization and DNA sequence depending on the host fish or the geographical location as indicated by ***Kitamura et al. (2005).*** In fact, although MCP gene is highly conserved and contains sufficient variable regions to allow phylogenetic analysis, the use of different genes may be more significant to trace the epidemiological origin in such a

context. Unfortunately, this analysis requires availability of relevant sequences of entire LCDV genome but currently a few number of LCDV genome sequences are available ***(Haddad-Boubaker et al., 2013).***

Conclusion

The present study revealed detection of LCDV from sea bream fish cultured in Egypt that might constitute a negative impacts on the national economy.

Gross lesion and histopathology may aid in diagnosis of LCDV among fish however, molecular detection; PCR and sequencing as well as phylogenetic analysis confirm the diagnosis.

The active international trade with lack implementation for strict regulations regarding fish transfer between countries may be responsible for the dissemination of viral strains, especially in the absence of a certification as free stocks.

The current results recommend further studies for the diagnosis and development of preventive measures and control strategies against LCDV in sea bream fish in Egypt.

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التوصيف الباثولوجى والجزيئى لفيروس الحويصلات الليمفاوية فى أسماك الدنيس البحري في مصر

صلاح الدين مصيلحي على[1] , شيماء محمد منصور[2] , رندا يسري ثابت[3]

1- قسم الباثولوجيا بكلية الطب البيطري - جامعة قناة السويس
2- قسم الفيروسات بكلية الطب البيطري - جامعة الزقازيق
3- قسم مكافحة أمراض الاستزراع المائي بمعهد الاستزراع السمكي وتكنولوجيا الأسماك - جامعة قناة السويس

مرض الحويصلات الليمفاوية الفيروسى (LCDV) هو مرض منتشر جغرافيا يؤثر على أكثر من 150 نوعًا مختلفًا من أسماك المياه العذبة والبحرية في جميع أنحاء العالم. يتميز المرض الذي يسببه فيروس مرض الحويصلات الليمفاوية (LCDV) بمظهر الورم التي تحتوي على خلايا متضخمة بشكل كبير (الحويصلات الليمفاوية) للأسماك المصابة التي عادة ما تحل نفسها بنفسها مع مرور الوقت. تعد أسماك الدنيس من أهم أنواع الأسماك المستزرعة اقتصاديًا في منطقة البحر الأبيض المتوسط. فيروس مرض (LCDV) في أسماك الدنيس البحرية المصرية غير مفهوم بشكل جيد، ولا توجد معلومات متاحة بشأن العدوى المحتملة للـ LCDV في مصر. لذلك كان الهدف الرئيسي من هذا العمل هو تشخيص LCDV في الدنيس البحري المستزرع فى مصر عن طريق فحص الأعراض الظاهرية وفحص النسيجي المرضي ومن خلال اختبار الطرق الجزيئية والتسلسل الجزيئي واختبار التتابعات الجينية وتحليل السلالات عن طريق عمل تحليل الشجرة الجينية. وأظهرت عينات من 20 عينة من أسماك الدنيس البحري أورام في الجلد والزعانف تم جمعها من مزارع الدنيس البحري في مصر. وأظهر الفحص النسيجي بعض التغيرات في أنسجة الجلد والزعانف مع نخر واستجابة التهابية. نتائج الـ PCR مع بادئات محددة لبروتين (MCP) أعطى نتيجة عند bp405 بواسطة الفصل الكهربائي. تشجع النتائج الحالية المزيد من التحقيقات لفيروس مرض الحويصلات الليمفاوية lymphocystis في المزارع المصرية ويوصي بوضع استراتيجيات الوقاية والمكافحة ضد فيروس مرض الحويصلات الليمفاوية LCDV في مصر.

الكلمات الافتتاحية: الدنيس، مصر، فيروس مرض الحويصلات الليمفاوية، الفحص المرضي للأنسجة، الفحص الجزيئي، تفاعل البلمرة المتسلسل، الشجرة الجينية.