



The family *Iridoviridae* includes six genera; three of which are responsible for the infection of ectothermic vertebrates (*Lymphocystivirus*, *Megalocytivirus*, and *Ranavirus*), while the other three (*Iridovirus*, *Chloriridovirus* and *Decapod iridovirus*), are known to infect invertebrates such as insects and crustaceans [8]. Iridoviruses, are icosahedral large DNA viruses, enclosing a linear, double-stranded DNA genome, with approximately 120~200 nm in diameter, encoding 92-211 proteins. The primary component of the viral capsid is the major capsid protein (MCP) with molecular weight of 48 kDa, encodes between 93-135 open reading frames [9]. It has been shown that the gene encoding the MCP includes conserved sequences that can be used for identification and characterization of iridovirus isolates [10].

The clinical signs of iridovirus infected fish were severe anemia, red spots, petechiae in the gills, swelling of the spleen and kidneys [11]. Histopathological examination revealed characteristic inclusion bodies in body forming bearing cells (IBC) were found in spleen, kidneys, liver, heart, gill and the digestive tract [12]. The IBCs are hypertrophied cells encompassing large foamy or granular inclusions that extend the cytoplasm and dislodge the nucleus. There are several detection methods available for the appropriate diagnosis and identification for viruses [5, 13-15]. Formerly, several researchers identified distinguished peculiar histopathological lesions accompanied with localized or systemic iridoviral infections of fish [16-18]. Though, the existence of iridoviruses in fish is difficult to detect as it can persist for a very long time in host cells without displaying any detectable effects. As such, the host can become the asymptomatic carrier of the virus [19]. Hence, the ability to detect the carrier fish could help preventing future disease outbreak from occurring in aquaculture.

Considering the impact of these diseases on fish farming, this study aims to identify iridovirus in clinically infected and apparently healthy fish species (Sea bream, Sea bass and Mullet) and define the pathology observed in affected tissues within Damietta and Sharkia Governorates, Egypt. Four approaches were taken; i) Exploring the clinical pictures in affected fish, ii) Investigation of the histopathological changes in different tissues of fish, iii) Detection of the Iridovirus in fish using PCR, and iv) Isolation of the Iridovirus in mammalian cell line (Vero cells).

## Materials and methods

### *Clinical Samples*

Sampling was carried out from fish farms (n=7) and markets (n=3) in Damietta and Sharkia Governorates, Egypt during May 2019-September 2020. Fishes were of different ages ranged from fry to 2 years. Specimens were collected from farm fishes exhibited deaths and mortalities up to 40%. Tissue specimens (spleen, kidneys, liver, skin, gills, heart, brain, and intestine) were collected for histopathological examination, PCR, and isolation of virus. Specimens were collected from 62 infected marine fish (Sea bream n=50, Sea bass n=3 and Mullet n=9). The clinical signs of the examined fish included abnormal swimming, distended body cavity and hemorrhages on skin.

### *Histopathological examination*

The collected tissue specimens were placed in 10% neutral buffered formalin for 48 h, 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 (5  $\mu$ m) thick paraffin sections were prepared and stained with hematoxylin and eosin (H&E). The sectioned were mounted in Canada balsam and covered with cover slips, then examined microscopically [20].

### **DNA extraction and PCR**

The viral DNA was extracted directly from fish tissues (spleen and kidneys) or whole fry fish using QIAamp Viral DNA Mini Kit (QIAGEN, USA) according to manufacturer's instructions. The extracted DNA was screened using specific primer targeting MCP gene (Metabion, Germany); Irido-F: 5'-ATCAGGATCCATGTCTGCAATCTCAGGTG-3' and Irido-R: 5'-CGTCGAATTCGTCGACAGATGTGAAGTAG-3' [21]. The PCR was performed in a total volume of 25 µl in a sterile 0.2 mL nuclease free PCR tube using 2X DreamTaq™ PCR Master Mix (Thermo scientific, UK). The optimized PCR cyclic reaction conditions were performed in Creacon, Thermo cycler, Holand according to Murwantoko *et al.* [21] with minor modifications as described as followings: initial denaturation at 95°C for 2 min, followed by 35 amplification cycles of denaturation for 30 s at 94°C, annealing for 30 s at 55°C, and extension for 75 s at 72°C then final extension at 72°C for 10 min. The amplified products were visualized on 1.5% agarose gels. Electrophoresis was conducted at 100 volts for 40 min then the gel was viewed and photographed on the UV transilluminator.

### **Isolation of virus on Vero cells**

The positive PCR tested spleen samples and whole fry were subjected to virus isolation on Vero cell line. A suspension of tissue homogenates (10%) was prepared with sterile phosphate buffer saline (PBS) then centrifuged at 3000 rpm for 20 min at 4°C. The antibiotics were added to the supernatant fluids with a final concentration of 1000 IU penicillin and 1000 µg streptomycin/ml. The supernatant was filtered through syringe filter (0.45 µm). The cells were inoculated with supernatants prepared from tissue samples by 200µl/well into triplicates for each sample. Cell control was included. The plates were incubated at

28°C and at 37°C in 5% CO<sub>2</sub> incubator for 7 d with daily observation for the development of specific cytopathic effect (CPE). The inoculated cells were exposed to repeated freezing and thawing for 3 times and clarified by centrifuged at 3000 rpm for 10 min to remove cell debris [22]. The DNA was extracted from cell supernatant harvests using QIAamp Viral DNA Mini Kit (QIAGEN, USA) according to manufacturer's instructions, and then subjected to PCR.

### **Statistical analysis**

The detection rate of Iridovirus in fish was calculated as the proportion of the positive samples in which virus was detected by PCR. Both detection rate and 95% confidence interval (CI) were computed using WinPepi software, Version 11.65 [23].

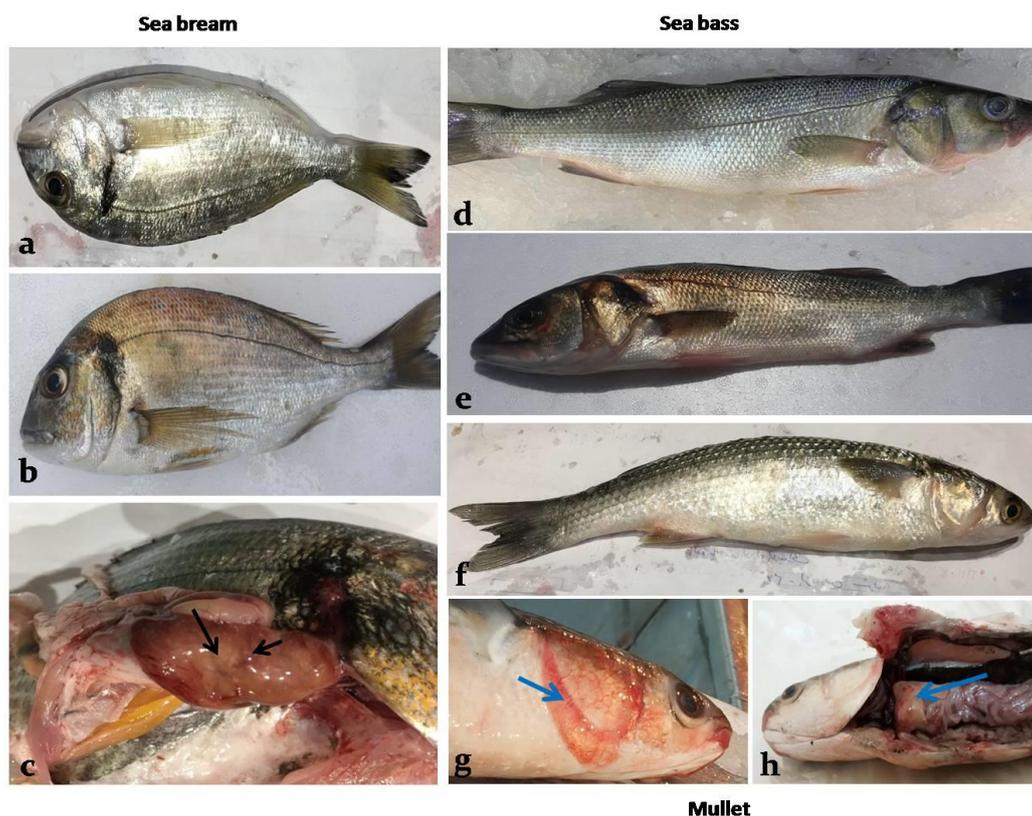
## **Results**

### **Clinical examination of different fish species**

The affected Sea bream fish become lethargic, exhibit severe anemia, petechiae in the skin and gills, enlargement of the spleen, necrotic foci on liver were observed in 20% (10/50) of examined fishes. Unilateral hemorrhages in eye were also observed in 6% (3/50) of investigated fishes besides with cloudy eye in one Sea bream with a percentage of 2%. In Sea bass fish, they were lethargy and stay in the bottom of tank. The observed pathological changes of Sea bass were hemorrhages on external surface of skin, congested liver and spleen in 100% (3/3) of examined fish. The affected Mullet fish exhibited hemorrhages on skin. On necropsy, abdominal cavities were found to be congested with hemorrhagic liquid. Petechial hemorrhages on liver, spleen and kidneys were recorded in 66.7% (6/9) of examined fishes (Figure 1). Comparative clinical pictures of investigated fish species were summarized in Table 1.

**Table 1: Comparative clinical findings in different fish species suspected to be affected with virus infections in Damietta and Sharkia Provinces, Egypt, during 2019-2020**

Clinical pictures	Fish species			Total
	Sea bream	Sea bass	Mullet	
Emaciation	4/50; 8%	0/3; 0%	0/9; 0%	4/62; 4.5%
Hg's on skin	24/50; 48%	3/3; 100%	6/9; 66.7%	33/62; 53.2%
Hg's in eye	3/50; 6%	1/3; 33.3%	0/9; 0%	4/62; 6.5%
Cloudy eyes	1/50; 2%	0/3; 0%	0/9; 0%	1/62; 1.6%
Hg's on brain	12/50; 24%	3/3; 100%	2/9; 22.2%	17/62; 27.4%
Congestion and Hg's in abdominal cavity (Liver, spleen, heart and kidneys)	16/50; 32%	3/3; 100%	6/9; 66.7%	25/62; 40.3%
Necrotic foci on liver	10/50; 20%	0/3; 0%	0/9; 0%	10/62; 16.1%

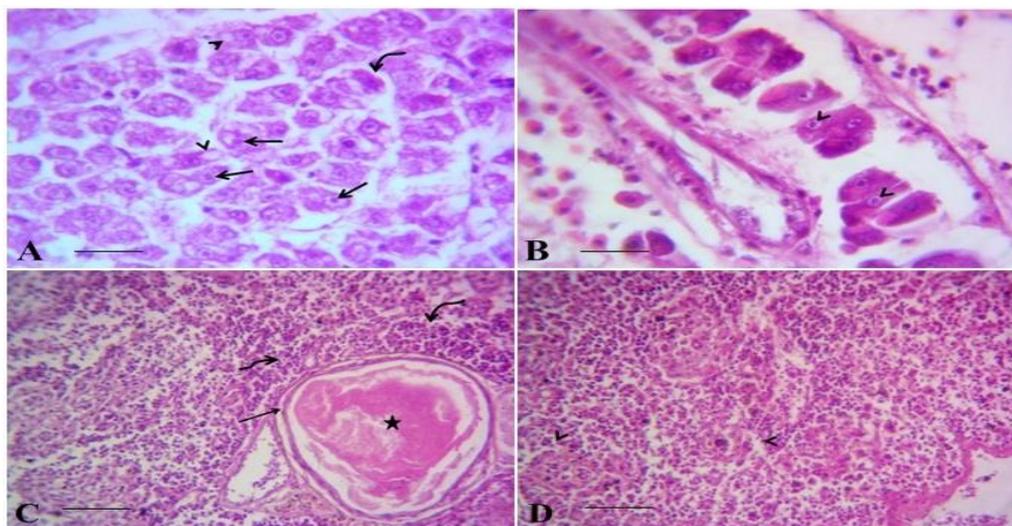


**Figure 1: Clinical picture of fish suspected to be infected with Iridovirus. (a) Normal Sea bream fish shows usual appearance of fish shape and colour. (b) The Sea bream fish shows hemorrhages on the external surface of skin, (c) necrotic foci on liver. (d) Normal Sea bass fish. (e) The Sea bass fish shows hemorrhages on skin. (f) Normal Mullet fish. (g) The Mullet fish show hemorrhages on skin, (h) petechial hemorrhages on liver.**

### Histopathological examination

Histopathological examination revealed changes in different tissue specimens. Microscopically, various degrees of necrosis, vacuolar degenerative changes, apoptotic changes and leucocytic infiltration were predominantly observed in the most investigated tissues. Histologically, the liver showed characteristic intranuclear inclusion bodies within degenerated hepatocytes and epithelial lining pancreatic acini in both Mullet (Figure 2A) and Sea bream (Figure 2B) liver tissues. Cytoplasmic vacuolations in hepatocytes, and apoptotic hepatocytes with pyknotic nucleus and more eosinophilic cytoplasm were also observed. Moreover, multifocal hepatocellular necrosis which replaced by inflammatory cells mainly lymphocytes and eosinophils were recorded. Spleen showed granuloma represented by eosinophilic central necrotic area which enclosed in a thick fibrous capsule and surrounded by inflammatory cells (Figure 2C). Hypo cellular white pulp spaces around the ellipsoid's arterioles which represented by empty areas (Figure 2D) or marked depleted were observed.

The intestine showed extensive necrosis of the surface of entire mucosa with an accumulation of necrotic enterocytes within intestinal lumen. The submucosa exhibited congested blood vessels and perivascular inflammatory cells infiltration and edema. Moreover, the gills showed focal necrotic areas of almost secondary lamellar epithelium which represented by eosinophilic necrotic debris infiltrated by inflammatory cells, thickening of the basal membrane of the secondary lamellae. Congestion and interstitial hemorrhages were also observed in the gills. In brain, the microscopic examination revealed coagulative necrosis of numerous Purkinje cells. The examined skin and muscles of fish showed thickening of dermal layer, hyaline, vacuolar degeneration, and multifocal area of necrosis in muscle fibers replaced by reactive inflammatory cells including lymphocytes and eosinophils. Furthermore, congestion and hemorrhage of dermal blood vessels and interstitial edema between muscle bundles was also seen.



**Figure 2: Photomicrograph of liver and spleen sections from different investigated fishes (Stain: H&E). (A) Liver of Mullet showing cytoplasmic vacuolations (arrow heads), and intranuclear inclusion bodies within degenerated hepatocytes (arrows) Bar: 20  $\mu$ m. (B) Liver of Sea bream showing intranuclear eosinophilic inclusions within the epithelial lining of the pancreatic acini (arrowheads) Bar: 20  $\mu$ m. (C) Spleen of Mullet showing granuloma represented by eosinophilic central necrotic area (stars), encapsulated by fibrous connective tissue (arrow) and inflammatory cells (curved arrow) Bar: 50  $\mu$ m. (D) Spleen of Mullet, showing mild depletion the white pulp around the ellipsoids arterioles (arrowheads) Bar: 50  $\mu$ m.**

**Molecular detection of Iridovirus in fish tissues using PCR**

Viral DNA of the MCP gene of iridoviruses yielded amplified product of 1000 bp (Data not shown). The virus was detected in tissues collected from both farm and markets. The iridovirus was molecularly detected in 4 out of 33 pools with a percentage of 12.1%. It was noticed that the freshly obtained and tested samples (n=3) from markets yielded positive results with a percentage of 66.7% (2/3) compared to stored tissues which yielded positive results in 2 out of 30 pools with a percentage of 6.7%. The detection rate of iridovirus was explored according to 5 factors; fish species, Governorate, origin of sampling, age of fish and disease condition (Table 2). The detection rates ranged from 6.7% in Damietta to 66.7% in Sharkia Governorate. The highest Iridovirus detection (60%) was recorded in Mullet.

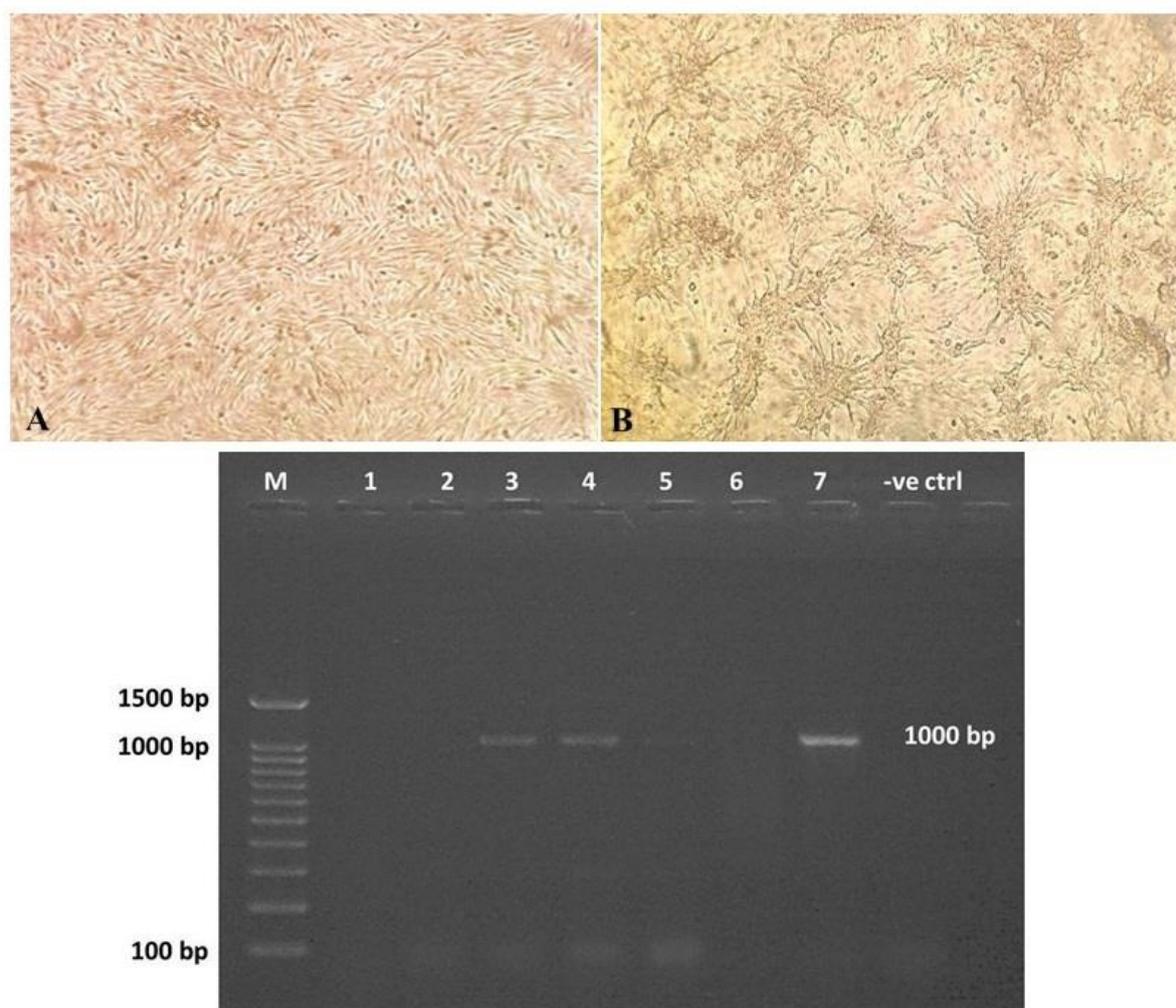
**Virus isolation on Vero cells**

The PCR positive spleen tissues (n=3) and whole fry (n=1) were subjected to virus isolation on Vero cells. Mock-infected cells showed confluent monolayer sheet (**Figure 3A**). When the inoculated Vero cells were incubated at 28°C, the samples showed CPE in the form of cell rounding and/or cell aggregation. The CPE started 4 d post inoculation and gradually become obviously to reach 70% of the cell sheet in some samples after 6 d (**Figure 3B**). Meanwhile, incubation of cells at 37°C led to no obvious virus replication. The inoculated Vero cell harvests were submitted to PCR using MCP gene specific primers with generation of 1000 bp amplicon following gel electrophoresis. No bands were noticed from the mock-infected cells and free-template control (Figure 3).

**Table 2: Molecular detection of Iridovirus in fish collected from farms and markets in Damietta and Sharkia Provinces, Egypt during 2019-2020 by PCR using sets of MCP gene specific primers**

Criteria	No. of samples Examined	No. of positive samples	Percent of positive samples	95% CI Confidence interval
<b>Governorate</b>				
Damietta	30	2	6.7%	1.1%-20.3%
Sharkia	3	2	66.7%	1.4%-99.2%
<b>Species</b>				
Sea bream	26	1	3.8%	0.1%-19.6%
Sea bass	2	0	0%	0%
Mullet	5	3	60%	14.7%-94.7%
<b>Sample Origin</b>				
Farm	30	2	6.7%	1.1%-20.3%
Market	3	2	66.7%	1.4%-99.2%
<b>Age</b>				
Fry	9	1	11.1%	0.3%-48.2%
Adult	24	3	12.5%	2.7%-32.4%
<b>Disease condition</b>				
Diseased	26	3	11.5%	2.4%-30.2%
Apparently healthy	7	1	14.3%	0.4%-57.9%
<b>Total</b>	<b>33</b>	<b>4</b>	<b>12.1%</b>	<b>3.4%-28.2%</b>

The detection rate and 95% CI were computed using WinPepi software, Version 11.65.



**Figure 3: Isolation of iridovirus in mammalian cell culture (Vero cells). The cells were inoculated with supernatants of PCR positive spleen tissue and whole fry, incubated at 28°C with daily observation for the development of specific CPE. (A) Normal Vero cells, showing confluent monolayer sheet of cell. X 100. (B) At 6 days post-inoculation, the CPE appeared in the form of cell rounding and cell aggregation. X 100. (Upper panel). Molecular confirmation of iridovirus infection in Vero cells (Lower panel). The PCR amplified products from positive isolates of Vero cells inoculation following 1.5% agarose gel electrophoresis. M: DNA marker (100 bp), lane 3-7: Positive samples, -ve Ctrl: Negative control (Mock-infected cells and free-template control).**

## Discussion

Fish is an essential and a rich source of protein and other nutrients for human consumption. Emerging disease epizootics commonly cause considerable losses among fish populations, resulting in huge economic losses in commercial aquaculture and threats to valuable stocks of wild aquatic animals [24]. 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 fish farms worldwide [2]. Outbreaks of diseases that cause noteworthy morbidity and/or mortalities in the aquaculture industry are always a major concern. This is exacerbated when a new pathogen was introduced into a region which has not previously been affected. Epizootic iridoviral infection has threatened the aquaculture industry worldwide causing diseases that range in severity from subclinical to lethal and resulting in high mortality and severe

economic losses [5]. In Egypt, few studies addressed the viral infections in fish. Accordingly, it is important to investigate the iridovirus infections in clinically infected and apparently healthy fish species (Sea bream, Sea bass, and Mullet) and define the pathology observed in affected tissues.

To better understand the circumstances of iridovirus among fish farms and markets in Egypt, sixty-two fish were collected from Damietta and Sharkia Governorates during 2019-2020. The first approach of this work was to explore the clinical pictures in affected fish. The investigated fish farms suffered from sudden deaths and high mortalities up to 40% with suspicion of viral infections. A range of 20-60% mortalities were recorded in outbreaks of iridoviral disease infection in cultured red sea bream fish in Shikoku Island, Japan in 1990 [12]. The disease was widely detected also in East and South-East Asian countries [19, 25, 26]. A systemic iridovirus infection accompanied with high mortality was recorded in cultured Mullet [26]. Experimental infection of red sea bream with iridovirus isolates results in high mortality, 60-90% [11]. Externally, the fish collected from different disease outbreak ponds and markets exhibited a range of clinical signs, including severe hemorrhages on body surfaces characteristically in abdomen region, around base of pectoral and ventral fins, operculum, mandible and around eyes as recorded by He *et al.* [27]. Unilateral hemorrhages in eye (3/50; 6%), cloudy eye (1/50; 2%), and exophthalmia were recorded only in Sea bream fish. Exophthalmia was observed in Nile tilapia in Lake Volta, Africa [28]. At necropsy, the abdominal cavities were found to be congested with hemorrhagic fluid. Petechial hemorrhages on liver, spleen and kidneys were recorded in 40.3% (25/62) of examined fishes. Similar gross lesions were recorded by Inouye *et al.* [12]. More recently, the same PM lesions in Tilapia fish including enlarged and hemorrhagic organs including the spleen, brain, heart

and gills, but most notably kidney and liver were demonstrated [28]. Additionally, necrotic foci on liver were seen in 20% of examined Sea bream fish. These findings are consistent with the study of Hazeri *et al.* [29]. Correspondingly, Zilberg *et al.* [30] recorded the evidence of necrosis in the gills, heart and gastrointestinal mucosal epithelium of experimentally infected *Micropterus salmoides* with Santee-Cooper Ranavirus.

The second approach of this work was to demonstrate the histopathological changes in different tissues of examined fish. In this study, the pathological lesions are variable but necrosis in internal organ is common. Histologically, many authors noticed a characteristic IBCs development in infected fish tissues (spleen, kidneys, and liver) with Iridovirus (Megalocytivirus) [31-33]. Here, intranuclear inclusion bodies were seen in hepatocytes of Mullet and Sea bream fish. The existence of inclusion bodies and enlarged cells in histopathological evaluations is an imperative diagnostic indication of systemic iridoviral infections. In the present study, the occurrence of multifocal necrosis and various degree of inflammation in different fish tissues were observed. Similar microscopic lesions were previously reported [34-36]. Systemic necrotizing syndromes mainly included serious necrosis of splenic and hepatic tissues. Previous studies have recorded similar pathologies in fish infected with sheatfish iridovirus [37], catfish iridovirus [38], *Rana temporaria* iridovirus [39] and grouper iridovirus [29]. The noticeable cellular inflammatory responses observed in the spleen and liver could be either due to vascular endothelial damage from neighboring infected cells, a reaction to severe viremia, or cells containing centrarchid-type inclusion bodies as observed in iridoviral disease [40]. In previous study, basophilic and eosinophilic enlarged cells were seen in liver, kidneys and gills and it was one of the distinctive features of grouper iridovirus infection [29].

The clinical pictures as well as pathological findings recorded in this study are suggestive of iridovirus infections in fish. The appearance of inclusion bodies is pathognomonic for Megalocytivirus [41]. Unfortunately, the confirmatory inclusion bodies are usually only distinguished in early stage of infection due to the considerable necrosis. Therefore, the sole reliance on clinical and pathological changes for diagnosis is not possible and other tools should be used. Here, some samples showed clinical signs, but the inclusion bodies were not found in the tissues. However, they tested positive in PCR amplification. As well, the presence of iridoviruses in fish is difficult to detect because it can persist for a very long time in host cells without manifesting any detectable effects. As such, the host can become the asymptomatic carrier of the virus [19]. Hence, the ability to detect the carrier fish could help in preventing future disease outbreak from occurring in aquaculture.

The third approach of this work was to molecularly detect the iridovirus infection in different fish species. The PCR is a rapid, specific, and sensitive assay for detection of fish viruses, which can be a powerful tool to detect iridovirus infections. A primer set was used to detect 1000 bp amplicons in iridovirus using PCR. The primer set was shown to be specific for MCP gene of iridoviruses [21]. In the present study, the molecular detection using PCR revealed positive iridovirus in the examined fish with a percentage of 12.1%. Meanwhile, no iridovirus MCP gene was detected in negative controls, indicating that the amplified viral DNA was specific and did not originate from contamination.

In this study, the PCR tests on stored samples at -20°C from diseased fish with clinical and pathological pictures relevant to iridovirus infection revealed low detection rate of 6.7% comparable with fresher tissues during 2020 which yielded higher detection rate of 66.7%.

Consistently, Ramírez-Paredes *et al.* [28] reported negative PCR results of all archived samples of diseased tilapia. Additionally, samples from affected farms during 2016 were screened for detection of red sea bream iridovirus (RSIV) /infectious spleen and kidney necrosis virus (ISKNV), and tested negative for the virus [42].

The aforementioned results explained the variable iridovirus detection rate among fish species in this study; 60% in Mullet, 3.8% in Sea bream and no virus in Sea bass. Two thirds of Mullet samples (n = 6) was from fresh samples. Apparently healthy and asymptomatic Megalocytivirus infected fish cases were reported [43-45] leading to difficult diagnosis of the disease and allowed its spread through exporting overseas during its carrier stage. Subramaniam *et al.* [46] have recorded several positive cases of ISKNV on ornamental fishes which did not display any clinical signs. Jeong *et al.* [19] also found Megalocytivirus in marine fish species that were apparently healthy, a condition that could be called asymptomatic or persistent infection. They proved a high DNA concentration of the megalocytivirus in asymptotically infected tissues. Here, the iridovirus was detected in apparently healthy as well as clinically infected fish with a percentage of 14.3% and 11.5%, respectively. As well, the iridovirus was detected in both fry and market size fish with a percentage of 11.1% and 12.5%, respectively. Iridovirus-like agent was detected by electron microscopy in turbot fry from a turbot rearing unit in Denmark [47].

Cell cultures are valuable tools for investigating cellular responses and viral pathogenicity in fish species. It was recorded that the target organ for fish iridoviruses is spleen [48, 49]. The fourth approach of this work was to demonstrate the ability of molecularly identified iridovirus from fish tissues to replicate in mammalian cells such as Vero cell cultures. The growth of virus was observed in Vero

cells incubated at 28°C. Nevertheless, incubation of infected cell cultures at 37°C led to no obvious virus replication. These findings are congruent with those reported earlier by Marschang *et al.* [22]. Moreover, Huang *et al.* [50] recorded that the CPE of ISKNV in GS-1 cells produced between 20°C and 30°C with yields of  $10^{7.7}$  TCID<sub>50</sub>/ml and the maximum titer was  $10^{8.4}$  TCID<sub>50</sub>/ml at 25°C. To be assured that the cytopathogenic agent in cell cultures is iridovirus, the infectious cell culture supernatants were submitted to DNA extraction and PCR amplification. The isolates were identified as iridovirus with generation of specific amplicon.

### Conclusion

The results of this study confirmed the presence of iridovirus infection in fishes within Damietta and Sharkia Governorates, Egypt. The virus was detected in both young/fry fish and adult fish as well as in clinically infected and asymptomatic fishes. Detection of iridovirus can be influenced by storage time and temperature as long storage period may decrease the rate of virus detection comparable with freshly collected tissues. The virus was able to replicate in mammalian cell line (Vero cells) at 28°C, but not at 37°C. The existence of the characteristic inclusion bodies in hepatocytes of Mullet and Sea bream fishes is an imperative diagnostic indication of iridoviral infections. Further studies are recommended to investigate its presence on a wider scale with genomic sequence analysis to map the circulation of Iridovirus in different localities within Egyptian aquaculture.

### Conflict of interest

No potential conflict of interest was reported by the authors.

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## الملخص العربي

الكشف عن وجود فيروس الايريديو في الاسماك في محافظتي دمياط والشرقية، مصر

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تهدد عدوى الفيروس القزحي (فيروس الايريديو) صناعة الاستزراع السمكي في جميع أنحاء العالم. وقد ارتبطت بأمراض خطيرة وخسائر اقتصادية في الأسماك. في هذه الدراسة، تم تجميع عدد 62 سمكة (الدنيس = 50، القاروص = 3، البوري = 9) من محافظتي دمياط والشرقية وذلك خلال الفترة 2019-2020. وقد تم تسجيل الأعراض السريرية والصفة التشريحية والتغيرات المرضية النسيجية المصاحبة لعدوى الفيروس القزحي وتأكيد اصابتها عن طريق الكشف الجزيئي لتواجد الفيروس باستخدام تفاعل البلمرة المتسلسل في أنسجة أنواع الأسماك المختلفة. إلى جانب ذلك، تم محاولة عزل واكتشاف الفيروس القزحي الذي تم تحديده المسبق جزيئياً في الأنسجة المصابة في مزارع خلايا الفيرو المبطن من اصل حيواني. وكشفت نتائج الفحص الاكلينيكي للمرض في المزارع عن وجود خمول، انيميا حاده، وجود انزفة دموية على منطقة الجلد والخياشيم. ووجود بعض حالات انزفة من جانب واحد في العين مع وجود عكاره في أسماك الدنيس فقط. كشفت الصفة التشريحية عن وجود احتقان بالأعضاء الداخلية وأنزفه مبعه في الطحال والكبد والكلى والقلب. كما لوحظ وجود بؤر نخرية على الكبد. وكشف الفحص النسيجي عن وجود احتقان الأوردة البابية، وشهدت أجسام متضمنة داخل النواة في خلايا الكبد. كما لوحظ وجود نخر متعددة البؤر والتهابات في الأنسجة بدرجات مختلفة. وكشفت نتائج اختبار تفاعل البلمرة المتسلسل عن تواجد جين بروتينالMCP عن 1000 bp. وكما أثبتت الدراسة قدرة الفيروس القزحي المحدد مسبقاً جزيئياً على التكاثر في خلايا فيرو عند 28 درجة مئوية، وليس عند 37 درجة مئوية. وعلى حد علمنا، هذا هو التقرير الأول لعزل فيروس الايريديو في خلايا Vero والكشف عنه في الأسماك بمحافظة دمياط والشرقية، بمصر. كما نوصى بإجراء مزيد من الدراسات للتحقق من وجود فيروس الايريديو باستخدام تحليل التسلسل الجيني على نطاق أوسع و في أنواع أسماك أكثر.