**Streptococcus, Centrocestus formosanus and Myxobolus tilapiae** concurrent infections in farmed Nile tilapia (*Oreochromis niloticus*)

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Stress triggered concurrent microbial/parasitic infections are prevalent in earthen pond based farmed Nile tilapia (*Oreochromis niloticus*). In the current study, a total of thirty five *O. niloticus* were collected from a commercial fish farm with a history of severe mortalities at Port Said, Egypt. Nile tilapia samples were subjected to bacteriological, parasitological and pathological examinations. Twenty one *Enterococcus fecalis* and 15 *Streptococcus agalactiae* isolates were presumptively identified utilizing the semi-automated API 20 Strept test kit. The identities of the retrieved bacteria were confirmed by the sequencing of 16 S rRNA gene. Mortibund *O. niloticus* were found to be heavily infected by one or both of *Centrocestus formosanus* encysted metacercariae (EMC) and/or *Myxobolus tilapiae* spores presenting a unique form of synergistic and/or symbiotic relationship. The identities of both parasites were confirmed through morphological and molecular characterization. Variable circulatory, degenerative, necrotic and proliferative changes were also noticed in hematopoietic organs. Interestingly, multiple myxobolus spores and EMC were noticed in some histological sections. It was obvious that the current concurrent bacterial and parasitic infections are triggered by the deleterious effects of some stressing environmental conditions. The unfavorable climatic conditions (high temperature and high relative humidity) recorded at the surge of mortalities are probable predisposing stress factors.

1. Introduction

Tilapia is the world’s second most farmed fish. The global tilapia industry has been steadily growing in recent years, reaching 4.5 million tons in 2018 and expected to reach 7.3 million tons by 2030, providing a low-cost protein source for most developing countries [1,2]. Globally, Egypt is the third major producer of Nile tilapia (*Oreochromis niloticus*) after China and Indonesia [3]. Several tilapia species are commercially farmed, but the Nile tilapia, *Oreochromis niloticus*, is the most preferred by fish farmers due to its unique characteristics including rapid growth rate, high protein content, high marketability, disease resistance and tolerance to different aquatic environmental stressors [4]. Nowadays, the majority of tilapia farmers adopt intensive culture structures with exponentially higher stocking densities to maximize economic returns [5].

Despite the remarkable tilapia’s robustness, disease outbreaks have become inevitable particularly with intensification and associated overcrowding. Remarkably, numerous parasitic and bacterial pathogenic microorganisms were identified among farmed Egyptian fishes [6,7]. A substantial amount of empirical proofs suggest symbiotic
relationships between some parasitic and bacterial infections affecting fish [6]; Elgendy et al., 2015). Numerous opportunistic bacterial infections were documented to increase concomitantly in the presence of a large variety of parasitic infestations [6,8].

The synergism between some bacterial pathogens and parasites affecting fish could be attributed to the ability of fish parasites to act as vectors/reservoir for some bacterial pathogens [Elgendy et al., 2015; 9]. Additionally, the parasitic attachment is recognized to cause harm to the fish’s skin, where attachment sites could serve as portals of entry for bacterial pathogens [10,11]. It is also possible that infection-induced stress lowers the immune function of fish, making them more susceptible to infection [12].

Streptococcus infection is one of the most significant disease challenges with deleterious impacts on tilapia industry worldwide [3,13]. Several Streptococcus sp. have been identified as potential pathogens to farmed O. niloticus including, S. agalactiae, S. dysgalactiae, S. iniae and E. faecalis [3,14,15]. Pathologically, Streptococcal infections are common causes of septicaemia and meningoencephalitis with consequent mass mortalities in farmed O. niloticus [15]. Dermal hemorrhages, panophthalmitis, ascites, and erratic swimming behavior (circling) are all symptoms of streptococcal infection [16-18].

Centrocestus formosanus is a digenean trematodes belonging to the family heterophyidae that requires three different kinds of hosts to complete their life cycle. To complete its life cycle it utilizes a snail and a fish as first and second intermediate hosts, respectively, in addition to fish-eating birds or mammals as definitive hosts [19]. Centrocestus formosanus metacercariae encyst in the gills of several farmed fish species, causing significant pathological alterations in the architecture of the gills, which may contribute to respiratory problems, reduced productivity, and sometimes mortalities, among juveniles [20]. Zoonotic cases relevant to C. formosanus have been recorded worldwide including, Egyptian Nile Delta, European and Asian countries as a result of eating raw or undercooked fish containing metacercariae [19].

Myxosporeans are economically significant microscopic parasites that infect a wide range of commercially important fish, including tilapias [6,7,21]. Several Myxosporean species were detected in wild as well as farmed cichlids including; Myxobolus brachysporus, Myxobolus israelensis [22] and Myxobolus tilapiae [6]. These parasites have been linked to postmortem myoliquefaction of the host [6,23], ovarian disruption [24], and reduced respiratory ability (Molnar and Szekely, 1999). M. tilapia induces a series of external lesions in cichlids, including corneal opacity, frontal skin ulcers, and head cysts, which may develop to form holes in the head like lesions [25].

The present study aimed to investigate a concurrent infection of bacterial pathogens (S. agalactiae and E. faecalis) and parasites (M. tilapiae and C. formosanus) in an earthen pond reared Nile tilapia during the early summer season using both morphological and molecular techniques. Further, the histopathological alterations relevant to these pathogens in moribund fish were investigated.

2. Materials and methods

2.1. Fish samples

During June 2020, mass mortalities were reported among Nile tilapias reared in an earthen pond within a private fish farm at Shader Azzam locality, Port Said Egypt. The fish farm receives water from Lake Manzalla and Bahr El-Baqar drain. The latter is considered one of the most polluted drains in Egypt. This drain receives different kinds of agricultural, sewage and untreated industrial effluents from Greater Cairo as well as Sharkiya provinces. The stocking density was remarkably high with an estimate of 35,000 fish/acre. The farmer erratically reduced the water column from 1.75 m to 90 cm for a relatively long period that exceeds 2 weeks. Mortalities were concomitant with a wave of unstable weather and extreme high temperatures and excessive humidity, 41 °C and 65%, respectively, that Egypt has witnessed through the midsummer. The average recorded values for water temperatures, dissolved oxygen and pH were 32 °C, 4 mg/L and 8.9 respectively. A total of 35 moribund tilapias with an average weight of 300 ± 45 g were randomly collected and transported in an isothermal boxes filled with crushed ice to the Aquatic Animal Medicine & Management Laboratory (AAMML), Faculty of Veterinary Medicine, Cairo University. Clinical examination of moribund tilapias was performed according to Eissa et al. [6]. Parasitological and bacteriological examinations were performed immediately upon arrival (2 h s). Local optimal values versus recorded values for the management practices, water quality measures, and climatic conditions recorded during the sampling time is detailed in Table 1.

2.2. Bacteriological examination

2.2.1. Phenotypic identification

Loopfuls from, liver, kidneys, spleen, and brain of moribund tilapias were aseptically inoculated into Tryptic Soy Broth-TSB (Difco), incubated overnight at 30 °C. Loopfuls from the overnight broth cultures were then streaked onto tryptic soy agar -TSA (Oxoid) and incubated at 35 °C for 24 h. Representative random picked up colonies were further purified and then re-streaked onto tryptic soy agar. Conventional microbiological procedures were applied on the retrieved pure colonies. Such presumptive conventional morpho-chemical tests have included Gram staining, haemolytic activity and catalase reaction. The final presumptive identification of the retrieved isolates was achieved through API 20 Strept (Supplementary table).

2.2.2. Molecular examination of retrieved bacterial isolates

Genomic DNA of each bacterial isolates was extracted according to the PrepMan® Ultra Sample Preparation Reagent protocol (Applied Biosystems, USA). The complete 16 S rRNA gene from each isolate was amplified by PCR using the universal primer (FW: 5’-AGAGTTT-GATCCTGCTCAG-3’) and (RV: 5’-AAGAGGTGATCCAGCC-3’); designed by Gross et al. [26]. PCR assay was performed using Taq DNA polymerase enzyme in a 1 × PCR buffer of Maxima® Hot Start PCR Master Mix (Thermo Scientific, Waltham, Massachusetts, USA) in a final volume of 50 μL. The PCR protocol was performed according to the method described by Eissa et al. [7]. Amplicons of 16 S rRNA were then purified using GeneJET® Gel extraction kit (Thermo Scientific, Waltham, USA). The 16 S rRNA genes of two isolates were sequenced in both directions using ABI 3730XL DNA sequencer at Macrogen sequencing company (Macrogen, Seol, South Korea). Bio Edit version 7.0 was used to check and assemble the obtained sequences (Hall 1999). The identification of isolated was determined by comparing the sequences with those of 16 S rRNA prototypes deposited in GenBank. The phylogenetic tree was constructed using the neighbor-joining method, with 1000 replicates of bootstrap value and evolutionary distance calibrated by Kimura2-parameter method using MEGA version X (Kumar et al., 2018).
2.3. Parasitological examination

2.3.1. Morphometric assessment

The sampled moribund tilapias were dissected & clinically examined for the possible presence of any parasitic cysts or worms. The compression technique was used to scan different organs of the sampled tilapias for the possible presence of heterohyd EMC [27]. Gills and skin scrapings were carefully examined under microscope for the same purpose. Encysted metacercariae were counted and the average number/ microscopic field were determined according to Sohn [28]. The morphometric characteristics of the retrieved encysted metacercariae were assessed according to Woon-Mok, (2009 and Mehrdana et al. [29]. Fresh smears of gills and internal organs (liver, spleen, intestine, gonads, and gas bladder) were also primed for possible detection of myxosporean spores. Primed smears were stained with Giemsa [30] and examined microscopically using regular light microscope as well as dissecting microscope [31,32].

2.3.2. Molecular examinations of retrieved parasites

The genomic DNA from frozen infected gill tissues was extracted using QIAamp DNA Mini kit (QIAGEN, Hilden, Germany). The quality and concentration of extracted DNA were measured with a NanoDrop™ ND-1000 Spectrophotometer (Thermo Scientific, Germany). The extracted DNA was stored at –80 °C freezer for further molecular analysis. A fragment of small subunit ribosomal DNA (SSU rDNA) or 18 S rDNA gene from the retrieved Myxosporean spores was amplified using the universal primer pair 18F (5′-CTG GAT TCT GCC AGT-3′), and 18 g: (5′-CGG CAG TAG CGG GCG GTG TG-3′) as described by Andree et al. [33]. Amplification assay was set as described by Eszterbauer [34] and Abdel-Gaber et al. [35]. After that, a second nested PCR amplification was performed using MX5: (5′-CTG GGG ACG CCT CAG TAA ATC AGT-3′), and MX3: (5′-CCA GGA CAT CTT AGG GCA CAG A-3′) specific primer pair for the family Myxobolidae as described by Andree et al. [33]. PCR conditions were begun with initial denaturation for 4 min at 94 °C, followed by 40 cycles of 94 °C for 55 s, 56 °C for 50 s, and 72 °C for 90 s, with a final extension at 72 °C for 7 min. PCR products were electrophorized in 1% agarose gel (Sigma) stained with SYBR Safe DNA Gel Stain in 0.5 × TBE (Molecular Probes-Invitrogen). Three primer pairs were used for sequencing the 18 S rDNA of the present Myxosporean parasite; including (MX3-MX5) [33], (MC3-MC5) and (MB3-MB5) Eszterbauer [34]. The oligonucleotide sequences of MX3 and MX5 were mentioned previously. The oligonucleotide sequences of the rest of primers were: MC5: (5′-CCG TGG AAA ACG GGA CTA CCA CAT CCA-3′), MC3: (5′-GAT TAG CCT GAC AGA TCA CTC CAC GA-3′), and MB3: (5′-GAT GAT TAA CAG GAG CCT GTG GTG G-3′), MB5: (5′-ACC GCT CCT GTT AAT CAT CAC C-3′), as described by Eszterbauer [34] and Abdel-Gaber et al. [35]. PCR products were purified according to the QIAGEN Extraction Kit protocol (Hilden, Germany). The amplicons were sequenced in both directions using ABI 3730XL DNA sequencer at Macrogen sequencing company (Macrogen, Seoul, South Korea). The assembled sequence by Bio Edit (Hall, 1999), was aligned with other 18 S rDNA genes of myxosporean parasites available in GenBank. Then, the assembled sequence was deposited into the GenBank database. Phylogenetic tree was constructed using the neighbor joining model with maximum likelihood-parameter and 1000 bootstrap replications using MEGA X (Kumar et al., 2018).

On the other hand, PCR amplification of The ITS2 region of the retrieved Centrocestus formosanus was amplified utilizing the specific primers, BD2 (5′-TAT CCT GCT TAA ATT CAG CGG GTG-3′), and 3S (5′-GGT ACC GGT GGA TCA CTC GCC TCC TG-3′). The PCR reactions were conducted according to Wongsawad et al. [36], using MyTaq™ Red Mix kit (Bioline, Taunton, MA, USA). PCR assay started with initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 57 °C for 1 min, and extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were sequenced using the same primer pair in both directions as mentioned above.

2.4. Histopathology

Tissue specimens of liver, spleen, kidneys, intestine gills and eyes from freshly dead & moribund fish were fixed in 10% buffered formalin, washed in tap water and processed for further sectioning and staining by H&E stain according to [37].

3. Results

3.1. Clinical examination

Moribund tilapias were presented with signs of emaciation, darkening and pale gills. Some fish showed petechial hemorrhages on the skin. Fin erosions, skin ulcerative lesions, corneal opacity, subcutaneous abscess in heads of diseased fish were commonly noticed. Internally, liver was pale and friable while spleen was congested.

3.2. Bacteriological examination

A total of 36 bacterial isolates that were grouped into 21 E. faecalis and 15 S. agalactiae were retrieved from moribund O. niloticus. The patterns of streptococcal infections in O. niloticus are demonstrated in Table 2. Isolates were retrieved from brain, kidney, liver and spleen of diseased fish. Isolates were Gram-positive cocci, catalase and oxidase negative. The final identity of the retrieved isolates was achieved by the API 20 Strept. The morphochemical characteristics of retrieved isolates are illustrated in (Supplementary table).

3.3. Molecular examination of retrieved isolates

Streptococcus agalactiae and E. faecalis isolates were identified by conventional methods and the sequencing of their 16 S rRNA genes were assigned the GenBank accession numbers (MZ076660 and MZ076661), respectively. The accession number (MZ076660) yielded 1306-bp, and showed 99.92% similarity with S. agalactiae (MW17560), and 99.69% similarity with M. tilapiae (MW807752-LC586806-MW093154), 99.84% similarity with E. faecalis (MT626756, MT527545, MT012246, MT626756, CP020387 and LS483342). Therefore, the accession number (MZ076660) was confirmed to be S. agalactiae. On the other hand, the accession number (MZ076661) was 1270-bp and exhibited 99.92% similarity with E. faecalis (MT611694-CP046108-MW087752-LC586806-MW093154), 99.84% similarity with E. faecalis (MW17560), and 99.69% similarity with E. faecalis (MT484109). Therefore, the accession number (MZ076661) was confirmed to be E. faecalis. The phylogenetic analysis revealed that both bacterial isolates (S. agalactiae and E. faecalis) were grouped with their relevant accession numbers and separated from each other (Fig. 1).

3.4. Parasitological examination

3.4.1. Concomitant parasitic infection

Centrocestus formosanus metacercaria and M. tilapiae were identified in some investigated O. niloticus specimens. Centrocestus formosanus was noticed in the gills of 9 tilapias. The metacercarial intensities were ranged between 3 and 8/infected tilapias. On the other hand, M. tilapiae was identified in 13 Nile tilapia samples. The M. tilapiae spores were detected in the skin, gills, muscles and kidneys of infected tilapias. Four tilapia samples were found to be concomitantly infected with the C. formosanus and M. tilapiae. All tilapias infected with pathogenic S. agalactiae and E. faecalis were found to be concomitantly infected with the previously mentioned myxosporean spores and EMC (Table 2).

3.4.2. Morphological and molecular identification of C. formosanus

Centrocestus formosanus EMC were found encysted within the primary filaments of gills. The metacercariae are oval in shape, averaged 200 - 149 μm. The oral sucker is prominent at the body end, measuring
about 54–26 μm and armed with two rows of spines, which is prominently located at the most anterior part of the metacercarial body. The excratory bladder is characteristic X shape. The esophagus is short leading to a wide cæcum which reaches posteriorly to the level of excretory vesicle. Two small ocular dark spots appear at a level of pharynx (Fig. 2).

The assembled sequence of ITS2 region of this C. formosanus was 439-bp in length and deposited in the GenBank under the accession number MZ074320. Based on its sequence alignment, the present sequence of C. formosanus is firmly embedded within the genus Centrocestus. The accession number (MZ074320) showed 99.09% similarity to that of C. formosanus (KX430147- KX430148- KX430149), 98.86% similarity to that of C. formosanus (KX430147- KX430148- KX430149), 89.18% similarity to that of Apophallus species (MF438073), and 89.12% similarity to that of Cryptocotyle lingua (MW544130). The derived phylogenetic tree of ITS2 region of C. formosanus exhibited strong nodal support for two major lineages (Fig. 3). The first major clade is comprised of the genus Centrocestus. The first major clade is a monophyletic clade and grouped the present parasite with other C. formosanus with a 100% bootstrap value.

3.4.3. Morphological and molecular identification of M. tilapiae

Myxobolus tilapiae spores were medium sized, ovoid in shape with 2 small polar capsules generally located at anterior end, in plane parallel to sutural plane. Sporoplasm contains one rounded iodinophilous vacuole and 2 small polar capsules. Each spore was ellipsoidal in shape with length of polar capsules 1/3 of spore length. The polar capsules were ovoid, their length <1/4 of spore length, and they are equal in size. The triangular inter-capsular process is absent. The length of the spore was 10–17 μm (12.5 ± 0.67) while its width ranged between 4.9 and 7 μm.

<table>
<thead>
<tr>
<th>No. Examined fish</th>
<th>No. Infected fish</th>
<th>No. bacterial isolates</th>
<th>No. EMC/fish</th>
<th>No. M. tilapiae spores/field</th>
<th>No. of fish with mixed infections</th>
</tr>
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<tbody>
<tr>
<td>35</td>
<td>14</td>
<td>21</td>
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<td>35</td>
<td>13</td>
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<td>6-10 plasmodia</td>
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*a* Refers to existence of relevant infections in fish.

– Refers to absence of infection in fish.

![Fig. 1. Phylogenetic tree constructed by neighbor joining method with 100 bootstrap replicates showed the comparative analysis of the partial 16 S rRNA gene sequence of S. agalactiae and E. faecalis infecting O. niloticus and other related bacterial isolates.](image1)

![Fig. 2. Light microscopic micrograph of O. niloticus gills showing the gill trematoda (Centrocestus formosanus); A: B: Encysted metacercaria with characteristic excratory bladder X shape. C: D: excysted metacercaria showing X shape excratory bladder and note the spine rows.](image2)
(5.7 ± 1.2) (Fig. 4).

The assembled sequence of 18 S rDNA of this M. tilapiae was 1305-bp in length and deposited in the GenBank under the accession number MZ0900095. Based on its sequence alignment, the present sequence of M. tilapiae is firmly embedded within the family myxobolidae. The accession number (MZ0900095) showed 99.46% similarity to that of M. tilapiae (KX632950), 98.85% similarity to that of M. brachysporus (KX632949), 98.77% similarity to that of Triangula egyptica (KX632951), and 97.45% similarity to that of M. cerebralis (JN134175). The derived phylogenetic tree of 18 S rDNA gene of M. tilapiae exhibited strong nodal support for two major lineages (Fig. 5). The first major clade is comprised of myxosporean parasites infecting gills and separated from the second clade that comprised of Myxobolus sp. infecting the head-cartilages. The first major clade is further subdivided into two subclades. The first and second subclades were strongly supported with a 99% bootstrap value, where the first subclade grouped the present M. tilapiae (MZ0900095) with M. tilapiae (KX632950), Triangula egyptica (KX632951), M. brachysporus (KX632949), and M. fomenai (KX632947). The second major clade is a monophyletic and comprised of 3.5. Histopathological examination

The histopathological examination revealed that nearly all internal organs were affected together with eyes and brain. In liver, vacuolar degeneration and massive area of necrosis that appeared as homogenous deep eosinophilic cells with pyknosis of some nuclei (Fig. 6A). Moreover, severe congestion of hepatoporal blood vessels was also noticed (Fig. 6B). Marked activation of melanomacrophage centers (MMcs) and thrombosis were common in splenic parenchyma (Fig. 6C). Necrosis in subcapsular area of the splenic tissue was obvious (Fig. 6D). The renal tissue exhibited similar picture to that of spleen where MMcs activation and thrombosis were common together with marked aggregation of myxobolus spores in the subrenal tissues and musculature. The spores appeared unicellular with its characteristic polar capsules (Fig. 6E). In such areas, eosinophilic granular cells infiltration and perivascular oedema were common (Fig. 6F). In the intestine, submucosal oedema and dilatation of the lymph vessels were noticed (Fig. 7A). The gills showed prominent congestion of the branchial blood vessels and parasitic cysts in the branchial cartilage (Fig. 7B). The eyes showed hemorrhage and congestion in the area of subcleral cartilage (Fig. 7C) and many eosinophilic granular cells in the optic nerve together with aggregation of melanophores (Fig. 7D and E). In the brain, the most common lesions were congestion of the blood vessels and intracellular oedema (Fig. 7F).

4. Discussion

Natural concomitant infections with bacterial pathogens and parasites are widespread in farmed fish. Concurrent ectoparasitic infections potentiate susceptibility to bacterial pathogens in fish and lead to substantially greater mortality, particularly when coupled with poor water quality [10,38,39]. A sum of 40% of the investigated O. niloticus fish were found infected with streptococcal infections. A total of 36 isolates of streptococcus strains, E. fecalis (21) and S. agalactiae (15) were retrieved from moribund O. niloticus. The final identity of S. agalactiae and S. fecalis were confirmed by conventional morpho-chemical as well as molecular assays. Isolates were further identified by API 20 Strept showing diverse profiles in agreement with [40]. Identity of the isolates was confirmed by sequencing 16 S rRNA and comparing the
oligonucleotide sequences with other relevant genes deposited in GenBank.

*Streptococcus agalactiae* and *E. fecalis* have been frequently isolated from many farmed fish in Egypt suffering from mortalities [41–43]. Outbreaks in cultured fish is associated with multiple undesirable environmental circumstances including, high water temperatures, low dissolved oxygen levels, high ammonia and nitrite levels since these conditions exhaust the natural defense capacity of fish [41, 44, 45].

Streptococci are excreted in the feces of infected fish into the water, ultimately infecting other cohabitant fish. Majority of pathogenic streptococci can resist many adverse environmental circumstances that destroy other microorganisms [46]. Contaminated water, diseased and carrier fish are major sources of infection [37, 47].

Parasitic diseases present one of the most potential challenges to tilapia production and sustainability worldwide [48]. Poor water quality and erratic management plays a significant role in development and pathogenesis of parasitic diseases [49]. In the current study, Nile tilapias were concurrently infected with *C. formosanus* and *M. tilapiae*. Identification of *C. formosanus* and *M. tilapiae* parasites were achieved by studying their main morphological characteristics. Parasites morphological data were similar to those reported in previous literatures [25, 29, 50–52]. Further identification was confirmed by sequencing of 18 S rDNA and ITS2 regions for *M. tilapiae* and *C. formosanus*, respectively.

**Fig. 5.** Phylogenetic tree constructed showed the comparative analysis of the partial 18 S rDNA sequence of *M. tilapiae* infecting *O. niloticus* and other related sequences retrieved from various Myxobolidae.

**Fig. 6.** Histopathological examination of *O. niloticus* fish showing A: liver showed vacuolar degeneration and area of necrosis (n). B: severe congestion (c) of hepatoporal blood vessels. C: spleen showed activation of melanomacrophage centers (m) and thrombosis in the blood vessels (th). D: Necrosis (n) in subcapsular area of the splenic tissue. E: Muscular tissue in subrenal region showed multiple myxobolus spores (mx). F: eosinophilic granular cells infiltration (egc) and perivascular oedema in the subrenal tissue. (H&E stain).
Migratory birds also have significant role in spreading infection among farmed fish. Many of the heterophyids metacercariae present in fish have definitive hosts in aquatic migratory birds. The eggs move through the feces of birds to pond’s water. In water, eggs hatch then pass with different morphological/developmental stages till the free swimming cercariae arises. The free swimming cercariae penetrate the gill tissues through the secretion of certain proteolytic enzymes (hyaluronidase enzyme) with consequent encystation [54,55]. Centrocestus formosanus has a potential of public health importance where human infection is possible through eating infected raw fish [19]. Human intestinal heterophyasis causes mild and transient symptoms such as gastric pain, nausea, vomiting, and diarrhea. Furthermore, cases of ectopic lethal heterophyasis have been identified as a result of cercarial migration or the movement of immature flukes from the intestinal tract to other organs such as the heart and brain [56,57]. Centrocestus formosanus was described in Egypt for the first time by [51]. Other Centrocestus sp. than C. formosanus have been reported in Egypt as, C. cuspidatus [58], C. unequiorchalis [59] and C. armatus [60].

Myxobolus tilapiae was detected in 37% of collected Nile tilapias. Similarly, M. tilapiae spores were detected in the earthen pond reared Nile tilapias with high prevalence (80%) [25]. Myxobolus tilapiae spores can survive for long periods (more than a year) in the tubificid oligochaet worm intermediate host as well as in pond’s mud [61]. Spores remain endemic in affected farms and recurrent infections occur when favorable conditions for these parasites are re-established [6]. Carrier migratory aquatic birds facilitate the transmission, establishment and spread of the active spores from one farm to another through the continuous shedding of their droppings [62]. Myxobolus tilapiae secretes a variety of proteolytic enzymes that can digest the whole skin, hypodermis, and underlying musculature, leading to hole-in-the-head-like lesions especially in the skin of scaly less areas of the fish body, eventually become predisposed to opportunistic bacterial infections [25].

In this study, fish-pathogenic S. agalactiae and S. fecalis infections were concomitantly identified from moribund farmed O. niloticus. Moreover, all streptococcal infections were detected in Nile tilapias with concurrent infection of C. formosanus or M. tilapiae parasites indicating synergy and/or symbiotic relationship. Several reports of concurrent infections with diverse bacterial pathogens and invasive parasites have been frequently reported in farmed Nile tilapias [6,63].

Similar synergy has been previously identified between numerous bacterial pathogens and fish parasites. In Egypt, mass kills have been reported among farmed O. niloticus during the early summer of 2010 where mortalities were attributed to concurrent infection with both F. columnare and the M. tilapia [6]. The substantial break down of infected tilapias’ skin was possibly initiated by M. tilapia lytic enzymes followed by competent attachment sites which potentiate F. columnare colonization [6]. The M. tilapia secretes proteolytic enzymes, which can digest the whole skin, including hypodermis and muscles, leading to hole-in-the-head lesions [25]. Similarly, severity of F. columnare infections was potentiated by co-infection of fish with the crustacean parasite Argulus coregoni [64]. Additionally, channel catfish parasitized by Trichodina sp. was more susceptible to S. iniae or S. agalactiae infections [8]. Ichthyophthirius multifiliis infection predisposed the hybrid red tilapia to contract clinical infection of the facultative intracellular bacteria, Francisella noatunensis subsp. orientalis [39]. Further, parasitism by Gyrodactylus niloticus potentiates the severity of S. iniae infections in Nile tilapia [65].

The mechanisms involved in such symbiotic relationship between parasitic and bacterial infections affecting fish are diverse. Also, avenues initiated for bacterial invasions [10], some parasites may also acts as mechanical vectors e.g. Ichthyophthirius multifiliis theronts have served as a mechanical vector for the transfer of Edwardsiella ictaluri to fish [66], G. niloticus could act as a vector for S. iniae [65], Gyrodactylus anguillae for V. vulnificus [10] as well as Sea louse, Lepeophtheirus salmonis was also reported as a possible vector for A. salmonicida [67].

Elgindy et al. (2015) documented a link between enhanced infections with P. damselae subsp. piscicida and Caligus elongatus ectoparasitic copepods in Dicentrarchus labrax, suffering wide scale mortalities. The majority of fish tissues were co-infected with both P. damselae and C. elongatus. P. damselae subsp. piscicida was retrieved from C. elongatus infesting fish signifying their possible role in propagation and dissemination of photobacteriosis among cultured fish. Fish parasitic crustaceans feed on blood/tissues of their hosts with consequent spread of bacterial and viral pathogens among different fish species and individuals within the same aquatic environment [68].

Multiple concurrent infections of several bacterial pathogens (Flavobacterium columnare, Aeromonas veronii, S. agalactiae, Plesiomonas shigelloides and Vibrio cholerae) together with Iridovirus were reported in disease outbreaks affecting cultured O. niloticus [69]. A. veronii and F. columnare were the two major pathogens responsible for the noticed mortalities, with other pathogens acting as opportunistic infections during disease outbreaks. Furthermore, Basri et al. (2012) identified Tilapia Lake Virus, A. hydrophila and S. agalactiae concomitant infections in a mass mortality of adult red hybrid tilapias. Further, Eissa et al. [9]
have reported an extreme case of concurrent larval nematode (Anisakis L3) infection together with Vibrio vulnificus in kidney of wild caught scorpionfish (Scorpaena porcus).

The concurrent bacteria and parasitic infections noticed in the studied farm are interrelated with some environmental predisposing factors [39]. The unfavorable bad climatic conditions recorded during the onset of mortalities are probable stress factors. It is well hypothesized that the pathogenicity of many opportunistic infections is enhanced with unfavorable environmental conditions such as high temperature, elevated water pH, and low dissolved oxygen which perfectly accords with the disease eruption reported in the current study [42]. Fish reared in such bad aquatic environmental conditions are commonly immunocompromised and accordingly become infected with diverse varieties of ubiquitous bacterial pathogens and parasites existing in the pond’s water. Outbreaks of Streptococcal infections commonly erupt among farmed fish during summer season associated with high water temperature [41].

The deteriorated water quality during the hot weather have drastically impacted the normal pond environment with consequent waves of mass mortalities due to the colossal increase in microbial load and their pathogenicity [70]. Similarly, Elgendy et al. [71] have linked the eruption of Vibriosis outbreaks in cultured Panus indicus to the unstable climatic circumstances that resulted in increased bacterial loads pond’s water because of sediment disturbance. Spread of ectoparasites is directly related to water quality and pond management [72]. Parasites and their hosts usually live in equilibrium; however, invasion commonly increases in polluted aquatic environments [73]. Parasites spread rapidly in overcrowded aquaculture conditions causing high mortalities [74]. Abundance of snail intermediate hosts is also affected by pond’s water quality. The water temperature has significant effect on the transmission of encysted metacercariae by impacting the parasite intra-snail development rate as well as the survival of emerged cercariae [75]. In the current study, a major portion of the water supplying the investigated Nile tilapia farm is mainly from Lake Manzalla and Bahr El-Baqr drain where the latter is considered one of the most polluted waterways in Egypt [45,76]. This type of water usually brings up unlimited number of infectious agents into the aquaculture facility. Therefore, fish reared in this poor quality water are liable to different kinds of viral, bacterial and parasitic invasions [6]. Further, water loaded with substantial amounts of organic matter which eventually accumulates in the sediment forming sludge like layer at the bottom of the pond is a rich source of biological and chemical deleterious factors [77].

In the current research, the histopathological examination of tissue sections from moribund Nile tilapias demonstrated variable circulatory, degenerative, necrotic, and proliferative changes in hematopoietic organs, like that reported in microbial infections of fish. In this regard, the deleterious effect of parasitism on the immune system of their host fish cannot be ignored. Also, such changes clinically mean remarkable immunosuppression that triggered pathogenic invasion in these immuno-compromised fishes. The aforementioned alterations were concordant with the erupting intense buccal ulcerations in Large Mouth immuno-compromised fishes. The aforementioned alterations were perfectly accorded with the disease eruption reported in the current study concordant with the erupting intense buccal ulcerations in Large Mouth immuno-compromised fishes. The aforementioned alterations were investigated Nile tilapia farm is mainly from Lake Manzalla and Bahr El-Baqr drain where the latter is considered one of the most polluted waterways in Egypt [41].

In conclusion, bad weather, poor water quality and farm erratic management are the potential triggers of concurrent parasitic/bacterial invasion which have badly jeopardized fish immune system with an ultimate result of mass mortalities. Further, the intensity of infections with both EMC and streptococci could be used as a real biological indicator of aquatic environmental pollution.

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Ethics approval

All standard national and international ethical guidelines dealing with fish sampling were fully adopted and approved by the Ethics Committee of the Faculty of Veterinary Medicine, Cairo University.

Availability of data and material

The data are available within the article.

Code availability

Not applicable.

Author’s contributions

This study was conducted in cooperation between all authors. Eissa A.E., Derwa H. M., Abdelsalam M. conceived and designed the study. Eissa A.E., Gehad A. I., Nader M. S., Ghada O. El-Demerdash, performed fish sampling and clinical examination; Eissa A.E., Mamdouh Y.E., Prince A., Abdelsalam M., Gehad A. I., Nader M. S., Ghada O. El-Demerdash, isolated the bacteria and conducted the molecular study; Marwa M.A., performed parasitological work; Mahmoud A.M., performed pathological study, all authors wrote and concluded the manuscript; all authors have drafted, revised and approved the final manuscript.

Declaration of competing interest

All Authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2021.105084.

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