www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 779-788 (2016) DOI: 10.4194/1303-2712-v16_4_04

RESEARCH PAPER

Pathological Alterations in Nile Tilapia Experimentally Infected with *Streptococcus iniae* and *Candida albicans*

Samah S. Oda[1,*], Hossam G. Tohamy[1], Rawda G. Massoud[2]

[1] Alexandria University, Department of Pathology, Faculty of Veterinary Medicine, Egypt.
[2] Department of Fish Diseases, National Institute of Oceanography and Fisheries, Alexandria, Egypt.

* Corresponding Author: Tel.: +20.106503624; Fax: +20.452960450;
E-mail: samahoda@yahoo.com

Received 24 May 2016
Accepted 07 June 2016

Abstract

This study was performed to investigate the alterations that associated with experimental infection of Nile tilapia with *Streptococcus iniae (S. iniae)*, *Candida albicans (C. albicans)* or their combination. The experimentally infected fish were carefully observed for four weeks for any clinical abnormalities, mortality rates, postmortem changes, biochemical and histopathological changes. The highest mortality rate, severe clinical signs and postmortem changes occurred in Nile tilapia fish in the *S. iniae+ C. albicans* infected group. There were significant changes in the evaluated biochemical parameters in all infected groups that were marked in the *S. iniae*-and the *S. iniae+ C. albicans*-infected groups. Specimens were collected from gills, liver, kidney, spleen, brain and intestine for histopathological examination. The lesions were variable among the infected groups, however; lesions intensity was more pronounced in fish infected with both pathogens. The present study established the synergistic interaction between *S. iniae* and *C. albicans* in Nile tilapia on the bases of clinical signs, mortality rates, postmortem changes, biochemical and histopathological changes.

Keywords: Nile tilapia, *Streptococcus iniae*, *Candida albicans*, histopathology.

Introduction

Global fish production has grown steadily in the last five decades with food fish supply increasing annual rate of 3.2% and world population growth at 1.6 %, although the gap is narrowing (FAO, 2014). Undoubtedly, aquaculture is the fastest-growing food-producing sector. Intensive aquaculture fish are exposed to various stressors that affect their immune response and elicit higher susceptibility to infectious diseases and consequently important economic losses. Streptococcosis is a major aquatic problem for global fish production. *Streptococcus iniae (S. iniae)* is a hemolytic, gram-positive coccus firstly isolated in 1976 from a subcutaneous abscess of a captive freshwater dolphin (*Inia geoffrensis*) (Pier and Madin, 1976). Also, it has been reported in a number of species of fish, such as barramundi (*Lates calcarifer*) (Creeper and Buller, 2006), Nile tilapia (*Oreochromis niloticus*) (Shoemaker *et al*. 2001) and rainbow trout (*Oncorynchus mykiss*) (Lahav *et al*., 2004) as well as in mammals (Pier and Madin, 1976 and Facklam *et al*., 2005). In rainbow trout, streptococcosis is characterized by a subacute to acute course, with specific lesions of panophthalmitis and meningitis beside minor pathological changes in other organs (Eldar and Ghittino, 1999). Moreover, *S. iniae* has recently emerged as a threat to public health due to its zoonotic importance, being isolated from humans infected due to accidental injuries during handling of fresh infected fish (Weinstein *et al*., 1997; Lau *et al*., 2003; Koh *et al*., 2004; Facklam *et al*., 2005; Lau *et al*., 2006 and Sun *et al*., 2007). *Candida albicans* (*C. albicans*) is an opportunistic fungal pathogen that normally inhabits the digestive tract and mucosal regions of mammals and birds. It is isolated occasionally from aquatic habitats receiving city sewages (Cook and Schlitzer, 1981). *C. albicans* should be considered as an indicator of recent fecal pollution. Additionally, it is identified in the intestine of farmed rainbow trout (*Salmo gairdneri*), turbot (*Scophtalmus maximus*) and free-living flat-fish (*Pleuronectes platessa and Pleuronectes flesus*) (Andlid *et al*., 1995). Recently, *C. albicans* isolated from multiple skin ulcers in both Nile tilapia juveniles and Sharp toothed catfish (Eissa *et al.*, 2013). Studies on the possible interaction between bacteria and fungi in aquaculture fish are limited. Therefore, the current study investigates the possible alterations that associated with experimental infection of Nile tilapia with *S. iniae*, *C. albicans* or their combination on the bases of the clinical signs, mortality rates,

biochemical parameters, postmortem and histopathological alterations.

Material and Methods

Fish and Aquaria

A total number of 84 of apparently healthy Nile tilapia (*Oreochromis niloticus*) (50 ± 10 g. B.W) were obtained from a private fish farm at Behera province. Fish were transported alive in metal tank containing water enriched by oxygen sources. All fish were acclimatized for two weeks before beginning of the experiment. Fish were kept in prepared glass aquaria (90 ×50 ×35 cm). These aquaria were supplied with dechlorinated tap water. Continuous aeration was maintained in each aquarium using an electric air pump. Water temperature was kept at 25 ± 1 ^{0}C throughout the experiment. Fish were fed on a commercial fish diet containing 25 % crude protein. The diet was daily provided at 3% of body weight.

The Microbial Isolates

Local isolates of *Streptococcus iniae* (*S. iniae*) and *Candida albicans* (*C. albicans*) Strains were obtained from Department of Microbiology, Animal Reproduction Research Institute, Alexandria, Egypt.

Experimental Design

Fish were divided into four equal groups, 21 fish each. The first group served as control group: fish inoculated with 0.2 ml phosphate buffered saline/fish intraperitoneally, the second group (*S. iniae*-infected) fish were infected with 0.2 ml of *S. iniae* suspended in phosphate buffered saline at concentration $1.5x10^4$ colony forming units (CFU)/ml/ fish intraperitoneally. The third group (*C. albicans*-infected) fish were infected with 0.2 ml of *C. albicans* suspended in phosphate buffered saline at concentration $2x10^3$ CFU/ml/fish intraperitoneally. The fourth group (*S. iniae*+ *C. albicans*-infected) fish were inoculated with 0.2 ml of *S. iniae* suspension at concentration $1.5x10^4$ CFU/ml/fish and 0.2 ml *C. albicans* suspension at concentration $2x10^3$ CFU/ml/fish. The experiment was performed for four weeks.

Mortalities and Clinical Signs

Fish of all groups were carefully observed throughout the experiment. Abnormalities in fish behavior, clinical alterations and mortalities were recorded.

Biochemical Studies

Blood samples were collected from the caudal vein of Nile tilapia of all experimental groups during 1st, 2nd and 4th weeks of the experiment and were left to coagulate then centrifuged at 3000 rpm for 10 minutes. The resultant serum was stored at -20 ^{0}C for biochemical analysis. Serum activities of aspartate amino transaminase (AST) and alanine amino transaminase (ALT) beside alkaline phosphatase (ALP) were estimated colorimetrically according to Reitman and Frankel, 1957 and Kid and King, 1954 respectively by using commercial kits produced by Pasteur Lab. Moreover, serum total protein was determined according to Domuas *et al*., 1981. Serum creatinine level was determined colorimetrically according to Henry, 1974 and serum urea concentration was determined by colorimetric kinetic method according to Patton and Crouch, 1977.

Pathological Studies

Necropsy was performed for moribund, freshly dead and sacrificed fish from each group up to four weeks. Following necropsy, tissue specimens were collected from gills, liver, spleen, kidney, brain and intestine. These specimens were collected during 1st, 2nd and 4th week from each group. Tissue specimens were rapidly fixed in 10% neutral buffered formalin solution for at least 24 hrs. The fixed specimens were processed through the routine paraffin embedding technique. Five µm thick sections were obtained and were stained with hematoxyline and eosin (HE) according to the method described by Culling, 1983.

Statistical Analysis

The analysis of variance for the obtained data was performed using Statistical Analysis System (SAS, 2004) software to assess significant differences.

Results

Mortality Rates, Clinical Signs and Postmortem Findings

Mortalities in all groups were recorded throughout the experiment and summarized in Table 1 and Figure 1. No mortalities were recorded in the control group throughout the experimental period. Table 1 shows that the highest mortality rate occurred in Nile tilapia fish in the *S. iniae*+ *C. albicans* infected group (76.19%). The first mortality was recorded in the *S. iniae*+ *C. albicans* infected group on day 1 and reached to the maximum during the 1st week (38.10%), followed by (23.81%) and (14.29%) during the 4th week and the 2nd week of the experiment, respectively. However, the group infected with *S. iniae* alone showed mortality rate (61.9%) that started on the 2nd day post infection and the highest mortality rate (28.57%) was recorded during the 1st week, then (19.05%) and (14.29%) during the 4th and 2nd week post infection, respectively. Regarding to the *C. albicans* infected group, mortality rate was the

Table 1. Mortality % and biochemical changes of *Streptococcus iniae* (*S. iniae*), *Candida albicans* (*C. albicans*) and *S. iniae+C. albicans*-infected Nile tilapia fish

Parameters	Weeks	Groups			
		Control	*S. iniae*	*C. albicans*	*S. iniae+C. albicans*
Mortality %		0 %	61.9%	57.14%	76.19%
ALP (U/l)	1	13.50±0.29^{b}	18.00±1.15^{a}	14.20±0.58^{b}	17.80±0.06^{a}
	2	13.33±0.57^{b}	16.10±0.06^{a}	13.30±0.36^{b}	16.33±0.47^{a}
	4	11.00±0.58^{c}	15.40±0.38^{b}	12.25±0.14^{c}	16.90±0.06^{a}
ALT (U/l)	1	58.20±1.27^{b}	82.65±2.51^{a}	59.85±1.07^{b}	61.50±0.87^{b}
	2	63.00±1.00^{b}	78.50±0.12^{a}	64.67±1.86^{b}	64.67±1.45^{b}
	4	66.00±1.00^{d}	81.00±1.5^{a}	73.00±0.58^{b}	69.67±0.33^{c}
AST (U/l)	1	83.50±0.87^{c}	116.00±9.24^{a}	93.20±3.00^{b}	124.50±1.44^{a}
	2	88.67±2.40^{d}	135.00±1.73^{a}	104.10±4.05^{c}	121.57±1.57^{b}
	4	80.67±0.67^{b}	137.33±5.04^{a}	69.20±16.86^{b}	121.67±0.88^{a}
Total protein (g/dl)	1	11.05±0.55^{a}	9.55±0.43^{b}	10.30±0.12^{a}	8.80±0.46^{b}
	2	11.70±0.25^{a}	10.30±0.20^{b}	9.60±0.23^{c}	9.07±0.07^{c}
	4	8.63±0.13^{a}	8.80±0.55^{a}	8.30±0.17^{a}	8.57±0.07^{a}
Urea (mg/dl)	1	16.25±0.32^{c}	25.50±3.18^{ab}	21.80±1.50^{b}	26.20±2.02^{a}
	2	17.53±0.62^{c}	32.50±0.87^{a}	23.90±2.46^{b}	31.93±0.07^{a}
	4	20.47±0.18^{b}	35.20±0.57^{a}	34.90±0.06^{a}	35.20±0.20^{a}
Creatinine (mg/dl)	1	0.55±0.09^{c}	1.29±0.29^{b}	1.20±0.17^{b}	1.70±0.03^{a}
	2	0.73±0.13^{c}	0.84±0.03^{c}	1.10±0.15^{b}	1.71±0.01^{a}
	4	0.63±0.07^{d}	0.87±0.04^{c}	1.95±0.03^{a}	1.73±0.03^{b}

Values are means ± standard errors
Means with different superscripts within the same row differ significantly (P<0.05)
ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase

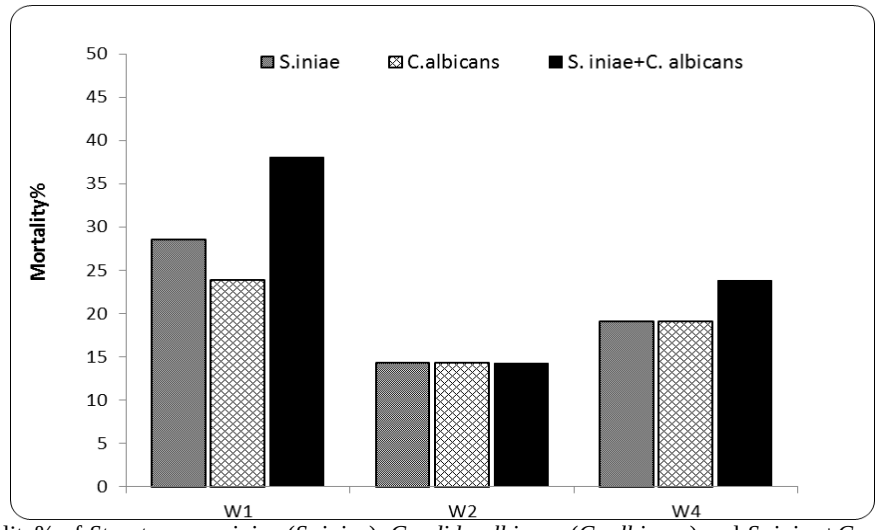

Figure 1 Mortality% of *Streptococcus iniae* (*S. iniae*), *Candida albicans* (*C. albicans*) and *S. iniae+C. albicans*-infected Nile tilapia fish. No mortalities were recorded in the control group.

least (57.14%) and started on the 2nd day post infection. The highest mortality rate in the latter group was (23.81%) during the 1st week, then (19.05%) and (14.29%) during the 4th and 2nd week post infection, respectively. Clinically, the *S. iniae*-infected fish exhibited loss of appetite, loss of equilibrium with erratic movement, swam near to the surface of water, detachment of scales, hemorrhages on the different parts of body surface, slight abdominal dropsy, slight exophthalmia (unilateral or bilateral) with presence of skin erosions and ulcerations (Figure 2a). There was hyperemia of the internal organs. Bloody ascetic fluid was found in the abdominal cavity (Figure 2b) beside distended gall bladder (Figure 2c). Moreover, gills were covered with mucus and were congested during the first week then became pale towards the end of the experiment. Fish infected with *C. albicans* showed clinical signs similar to those infected with *S. iniae* beside darkening of the skin and loss of all reflexes just prior to death. Additionally, there was a characteristic cotton wool-like growth on various parts of the body (Figure 2d) as well as button-like ulcers on the area of the caudal peduncle (Figure 2e). Grossly, there were enlargement and congestion of all

internal organs with distended gallbladder (Figure 2f). Fish in *S. iniae*+ *C. albicans*-infected group showed severe clinical signs of loss of appetite, loss of equilibrium and respiratory distress in the form of gasping, rapid operculum movement and gathering at the source of oxygen. Moreover, there were marked exophthalmia and cloudiness of eye (Figure 2g). Loss of scales and deep diffuse ulcerations with necrotic myocytes were noticed (Figure 2h). Also, gills were severely congested during the 1st week then became pale at the end of the experiment and enlarged pale liver was observed (Figure 2i).

Biochemical Findings

Table 1 illustrates that serum ALP activity was significantly ($P<0.05$) increased in the *S. iniae* and the *S. iniae*+ *C. albicans*-infected groups all over the experimental period and no significant changes ($P>0.05$) occurred in the *C. albicans*-infected group compared to the control group. Also, the serum ALT activity was significantly increased in the *S. iniae* and the *S. iniae*+ *C. albicans*-infected groups along the experimental period and in the *C. albicans*-infected group only at the end of the experiment in comparison to the control group. Regarding to serum AST activity, there was a significant increase in the *S. iniae* and the *S. iniae*+ *C. albicans*-infected groups till the end of the experiment and in the *C. albicans*-infected group only during the 1st and 2nd weeks of the experiment. Serum level of total protein showed a significant decrease in the *S. iniae* and the *S. iniae*+ *C. albicans*-infected groups during the 1st and 2nd weeks of the experiment and in the *C. albicans*-infected group during the 2nd week of the experiment. As shown in Table 1 there was a significant increase ($P<0.05$) in the serum urea and creatinine levels in all infected groups all over the experiment compared to the control group.

Histopathological Findings

The histopathological findings were observed in gills, liver, kidney, spleen, brain and intestine of infected groups. Lesion scoring was illustrated in Table 2.

Gills

Fish of the control group had normal structures without any significant microscopic lesions (Figure 3a). Fish of the *S. iniae*-infected group during the 1st week post infection showed focal and multifocal fusion of the secondary gill lamellae due to hyperplasia of the basal epithelium of the primary lamellae with mononuclear infiltrates (Figure 3b). During the 2nd week, there was filamentous clubbing of the tip of the primary gill lamellae. Additionally, lamellar telangiectasis was evident at the end of the experiment. The *C. albicans*-infected fish showed congestion of branchial blood vessels with multifocal fusion of secondary lamellae during the 1st and 2nd weeks of the experiment. At the end of the experiment, there were severe leukocytic aggregations at the base of the primary gill lamellae (Figure 3c). Fish in the *S. iniae*+ *C. albicans*-infected group showed similar lesions but more in the intensity, particularly at the end of the experiment. These lesions were filamentous clubbing, hyperplasia of mucous cells at the tip of the primary gill lamellae,

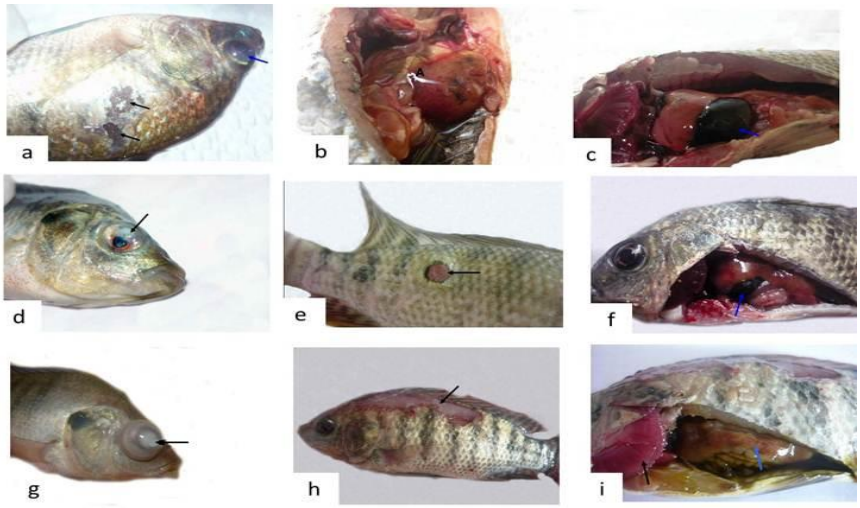

Figure 2 Photograph of Nile tilapia fish. *Streptococcus iniae*-infected fish showing (a) Skin ulceration (black arrow) and Slight exophthalmia with cloudy eyes (blue arrow). (b) Bloody ascetic fluid in the abdominal cavity (A). (c) Congestion of the internal organs and distended gall bladder with bile (blue arrow). *Candida albicans*-infected fish showing (d) Cotton wool-like growth on the eye (arrow) (e) Button-like ulcer on the area of caudal peduncle (f) Congestion of internal organs and distended gall bladder with greenish bile (blue arrow). *Streptococcus iniae*+*Candida albicans*-infected fish showing (g) Severe exophthalmia and cloudiness of the eye (arrow) (h) Severe skin ulceration with necrotic muscles (arrow) (i) Paleness of the gills (black arrow) and the liver (blue arrow).

Table 2. The severity of histopathological lesions in gills, liver, kidney, spleen, brain and intestine of the infected Nile tilapia

Organ / Lesions	*S. iniae*			*C. albicans*			*S. iniae+C. albicans*		
	1st wk	2nd wk	4th wk	1st wk	2nd wk	4th wk	1st wk	2nd wk	4th wk
Gills									
Lamellar telengiectasis	+	++	+++	+	++	+	++	++	+++
Fusion of secondary lamellae	++	++	+	++	+	+	++	++	++
Filamentous clubbing	++	++	+	++	+	+	++	++	++
Liver									
Circulatory disturbances*	+++	++	++	++	++	+	+++	+++	++
Hepatocytic vacuolation	++	+++	+++	-	++	++	++	+++	+++
Hepatic necrosis	-	-	++	-	-	++	+	+	++
Activation of MMCs	-	-	++	-	+	+	+	+	+
Kidney									
Circulatory disturbances	++	++	+	+	+	+	++	++	++
Tubulonecrosis	-	-	+	+	++	+	-	++	-
Interstitial nephritis	-	-	-	-	-	+	-	-	-
Activation of MMCs	+	+	-	-	-	-	++	+	-
Spleen									
Activation of MMCs	+++	++	++	+++	++	+	+++	+++	++
Depletion of white pulp	-	++	++	-	+	+	-	++	+++
Brain									
Circulatory disturbances	+	++	+	+	++	++	++	++	++
Neuronal necrosis	+	+	+	+	+	-	+	+	++
Purkinje cells depletion	-	+	+	-	-	-	-	-	+
Intestine									
Submucosal EGCs infiltration	+	+	+	-	-	-	+	+	+
Hyperplasia of the goblet cells	-	-	-	-	+	+	-	+	+
Epithelial necrosis	-	-	-	-	-	-	-	++	+

*Circulatory disturbances: congestion and /or hemorrhage. MMCs: melanomacrophage centers; EGCs: eosinophilic granular cells.
Lesion scoring: (-) none, (+) Mild, (++) Moderate, (+++) Severe.

lamellar telangiectasis and complete fusion of secondary lamellae (Figure 3d).

Liver

Liver of the control fish revealed a normal histoarchitecture and there were no significant pathologic abnormalities (Figure 4a). Liver of fish of the *S. iniae*-infected group exhibited eosinophilic granular cells (EGCs) aggrergations around the blood vessels with hepatocytic vacuolation during the 1st week of the experiment. During the 2nd week of the experiment, fatty degeneration of hepatocytes was evident which characterized by large empty vacuoles of sharp borders replaced almost the cytoplasm (Figure 4b). At the end of the experiment, there were coagulative necrosis of the hepatocytes accompanied by mononuclear cell infiltrates and hemorrhage. Liver of the *C. albicans*-infected fish showed congestion during the 1st week of the experiment, fatty degeneration and mild activation of melanomacrophage centers (MMCs) during the 2nd week of the experiment (Figure 4c). Later hepatocytic necrosis was evident. Fish in *S. iniae+C. albicans*-infected group showed more severe lesions that were time dependent. Congestion of the hepatic sinusoids with consequent atrophy of the hepatocytes and also fatty changes were observed during the 1st and 2nd weeks of the experiment (Figure 4d). At the end of the experiment, there was activation of MMCs with nuclear pyknosis of the hepatocytes.

Kidney

The kidneys of control fish revealed no histopathologic alterations (Figure 5a). Kidney of fish in the *S. iniae*-infected group showed congestion of blood vessels, hemorrhage and depletion of hemopoietic elements (Figure 5b). Kidneys of the *C. albicans*-infected fish were congested and had focal tubular necrosis (Figure 5c) during the 1st and the 2nd week of the experiment, respectively. At the end of the experiment, there was interstitial nephritis which characterized by mononuclears infiltration in the interstitial tissue. Fish in the *S. iniae+C. albicans*-infected group showed congestion of the blood vessels, hemorrhage and activation of MMCs all over the experimental period (Figure 5d).

Spleen

Sections from control tilapia revealed no histopathological alterations. Spleen of fish in all-infected groups showed activation of MMCs all over

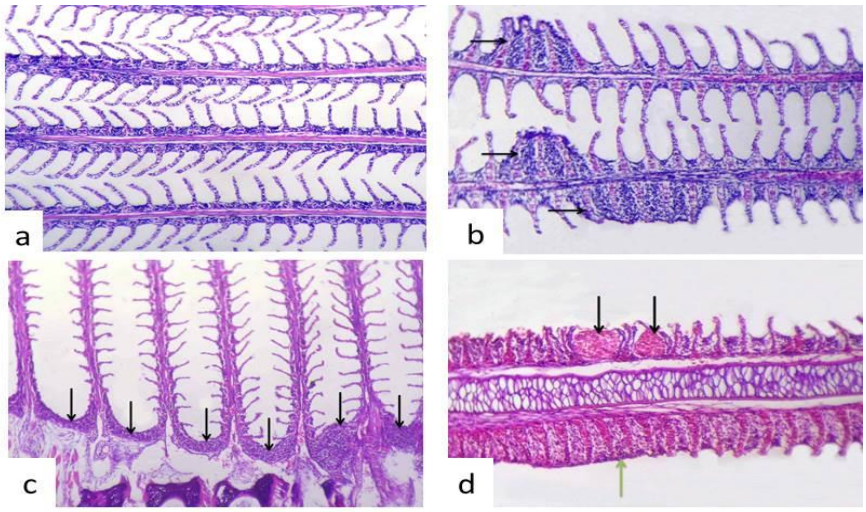

Figure 3 Photomicrograph of Nile tilapia gills stained with HE. (X 160). (a) Control fish (b) *Streptococcus iniae*-infected fish showing multifocal fusion of the secondary gill lamellae with mononuclears infiltration. (c) *Candida albicans*-infected fish showing severe leukocytic aggregations at the base of the primary gill lamellae. (d) *Streptococcus iniae*+ *Candida albicans*-infected fish showing lamellar telangiectasis (black arrows) and complete fusion of secondary gill lamellae (green arrows).

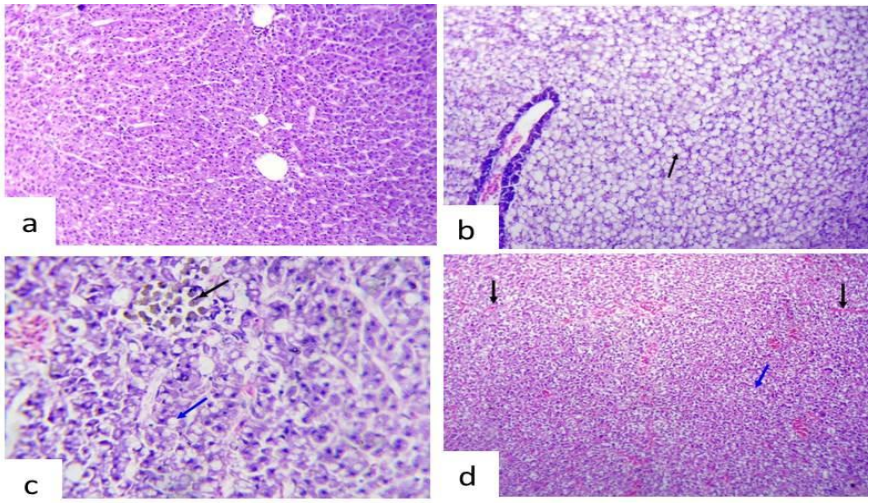

Figure 4 Photomicrograph of Nile tilapia liver tissue stained with HE. (a) Control fish. (X 160) (b) *Streptococcus iniae*-infected fish showing fatty degeneration of hepatocytes which characterized by large empty vacuoles of sharp borders replaced almost the cytoplasm. (X 250)**.** (c) *Candida albicans*-infected fish showing fatty degeneration (blue arrow) and mild activation of melanomacrophage (black arrow). (X 250). (d) *Streptococcus iniae*+ *Candida albicans*-infected fish showing congestion of the hepatic sinusoids (black arrows) with fatty changes of the hepatocytes (blue arrow). (X 160).

the experimental period wherein, the melanophores appeared heavily loaded with dark brown melanin pigment. As well there was white pulp depletion in all in infected groups, particularly in the *S. iniae*+*C. albicans*-infected group (Figure 6).

Brain

Brain of control fish was within normal histologic limits. Brain of fish in the *S. iniae*-infected group showed congestion of the blood vessels and small shrunken pyknotic neurons during the 1st week of the experiment. At the end of the experiment, there were pyknosis and depletion in the number of purkinje cells besides pericellular edema. Brain of the *C. albicans*-infected fish had congested blood vessels with mild neuronal pyknosis and the *S. iniae*+*C. albicans*-infected fish exhibited similar lesions to those of the *S. iniae*-infected group.

Intestine

Intestine of the control group exhibited a normal tunica mucosa, submucosa, muscularis and serosa and

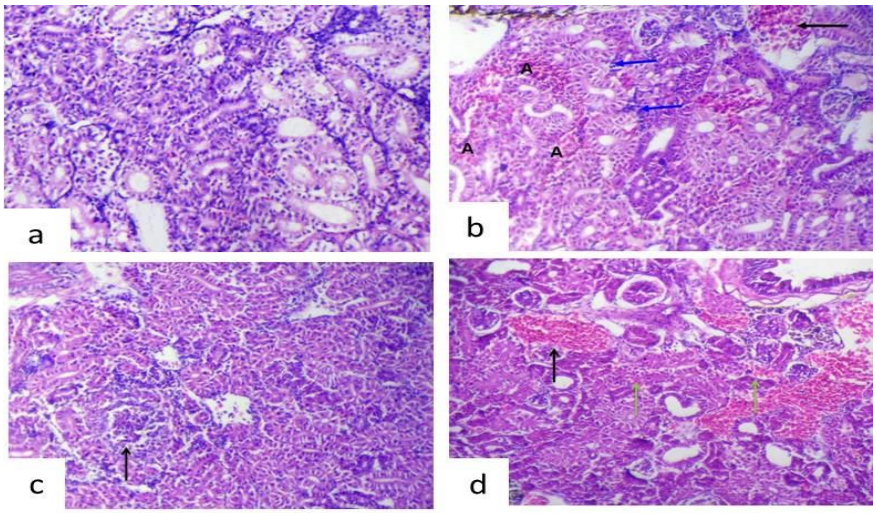

Figure 5 Photomicrograph of Nile tilapia kidney stained with HE. (X 250). (a) Control fish. (b) *Streptococcus iniae*-infected fish showing congestion of blood vessels (black arrow), hemorrhages (A) and depletion of hemopoietic elements (blue arrows). (c) *Candida albicans*-infected fish showing focal tubular necrosis (arrow). (d) *Streptococcus iniae*+ *Candida albicans*-infected fish showing congestion of the blood vessels (black arrow) and hemorrhages (green arrows). (X 160).

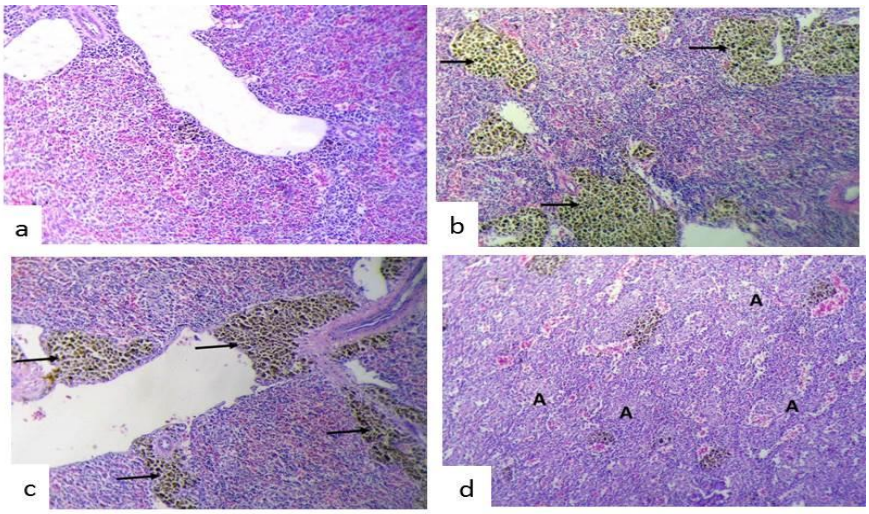

Figure 6 Photomicrograph of Nile tilapia spleen stained with HE. (X 160). (a) Control fish. (b) *Streptococcus iniae*-infected fish showing hyperactivation of melanomacrophage centers with overloaded melanophores (arrows). (c) *Candida albicans*-infected fish showing hyperactivation of melanomacrophage centers (arrows). (d) *Streptococcus iniae*+ *Candida albicans*-infected fish showing hyperactivation of melanomacrophage centers and depletion of white pulp (A).

there were no pathologic changes. Intestine of fish in the *S. iniae*-infected group showed submucosal eosinophilic granular cells (EGCs) infiltration during the 1st week of the experiment. There was hyperplasia of the goblet cells in the *C. albicans*-infected group during the 2nd week of the experiment. Regarding to the *S. iniae*+*C. albicans*-infected fish, there were focal necrosis of the intestinal epithelium with mononuclear inflammatory cell infiltrations, particularly during the 2nd week of the experiment.

Discussion

S. iniae is considered as one of the most important bacteria infecting aquaculture fish causing great economic losses. Clinically, this study revealed that the *S. iniae*-infected fish exhibited loss of appetite, loss of equilibrium with erratic movement, swam near to the surface of water, detachment of scales and hemorrhages on the different parts of body surface. These encountered clinical signs in the infected Nile tilapia were indicative of respiratory distress which may be attributed to decrease of the gills surface epithelium, hyperplasia of the epithelium at the base of secondary lamellae and consequently complete lamellar fusion of the majority of the lamellae. Additionally, there were nervous signs that characterized by erratic swimming and loss of

equilibrium. These signs were similar to those found by Chen *et al*., 2011 who observed that channel catfish infected with *S. iniae* exhibited abnormal behavior in the form of erratic swimming, lethargy and cutaneous hemorrhages and also were consistent with clinical signs observed in the *S. iniae*-infected red-tail black shark *Epalzeorhynchos bicolor* and rainbow shark *E. erythrurus* Russo *et al.*, 2006. Histopathologically, multiple lesions were developed in different organs such as gills, liver, kidney, spleen, brain and intestine. These lesions indicate that the infection with *S. iniae* induces acute systemic inflammation. Chen *et al*., 2011 reported that acute septicemia in the *S. iniae*-infected channel catfish resulting in sudden death. Gills have important functions as they are responsible for respiration, osmoregulation, acid -base balance and nitrogenous waste excretion Au, 2004. There was inflammatory cells infiltration in the base of primary lamellae, mainly lymphocytes; these lymphocytes may release cytokines which in sequence can activate macrophages. Chen *et al*., 2011 observed many macrophage infiltrates in different affected organs of the *S. iniae*-infected channel catfish, suggesting that macrophages are the predominant inflammatory cells in the *S. iniae*-infected channel catfish (Chang and Plumb, 1996). The damage effects induced by *S. iniae* could be attributed to bacterial invasion and/or bacterial toxins. It is noteworthy that *S. iniae* infection induces rapid elevation of reactive oxygen species (ROS) and nitrite oxide radical (NO), resulting in loss of mitochondrial membrane potential and induction of apoptosis (Zhu *et al*., 2015). Regarding biochemical results, there was a significant increment in the serum activities of ALT and AST in all infected groups and this may be attributed to hepatocellular damage and enzymatic leakage. Similar results were recorded by Chen *et al*., 2004. Also, the serum level of alkaline phosphatase was significantly increased in all infected groups and this may be due to bile pathway obstruction caused by pressure of degenerated hepatocytes (Duncan, 1995). Moreover, damage of liver and kidney causes a significant reduction in serum proteins synthesis by liver and increase protein loss from damaged kidney leading to decrease in serum protein levels. This was parallel to the results recorded by Chen *et al.*, 2004. The encountered liver lesions could be explained as the liver is the site of replication of many bacterial pathogens (Neely *et al.*, 2002). The serum creatinine and urea levels were significantly increased in all infected groups reflecting the histopathologic lesions found in kidneys of all infected groups. These observations could be attributed to renal dysfunction either by bacterial invasion and/or by bacterial and mycotic toxins. Similar results were achieved by Rehulka and Minarik, 2007.

Fungal diseases cause severe losses among different species especially Nile tilapia. It also acts as a source of human infection (Calderone, 2002). Chao *et al*., 2010 assessed the use of zebrafish as a host model to study the pathogenicity of invasive *C. albicans*. They found that the *C. albicans* induced mortalities among adult zebrafish in a dose-dependent manner. Moreover, histopathological findings revealed that *C. albicans* colonized multiple anatomical sites in the adult zebrafish (Chao *et al.*, 2010). Epithelial cells play an important role in signaling specific cells to support an immune response to *C. albicans* (Gratacap *et al*., 2013). Wherein, *C. albicans* may colonize the host's epithelial surface and persist on growing resulting in deep penetration into tissues and organs, causing severe damage and probable host mortality (Chen *et al*., 2013). This explanation was confirmed in our study by appearance of button-like ulcers on the area of the caudal peduncle and cotton wool-like growth on various parts of the body. Many fungal pathogens affect fishes are considered opportunists attacking the fishes when they are stressed or immunocompromised due to unsuitable environmental conditions, or secondary to bacterial or viral infections, or when they have lost their mucus protection because of trauma or unwarranted management (Roberts, 1989 and Quiniou *et al*., 1998). These opportunistic pathogens can produce both non-lethal localized mucosal and life-threatening systemic infections. Cavalcanti *et al*., 2016 reported that biofilm growth and hyphal filament production by *C. albicans* is enhanced by *S. oralis* and there was growth synergy between oral bacteria *Actinomyces oris* and *C. albicans*, and between *S. oralis* and *C. albicans*, resulting in augmentation of total biofilm biomass in each case. Since *S. oralis* facilitates invasion of oral mucosal epithelium by *C. albicans* (Diaz *et al*., 2012), concurrently *S. oralis* growth is enhanced by the fungus. In addition, the pathogenic properties and invasive potential of streptococci bacteria could be augmented by *C. albicans* (Falsetta *et al*., 2014 and Xu *et al*., 2014).Our results revealed that the highest mortality rate occurred in Nile tilapia fish in the *S. iniae+ C. albicans* infected group (76.19%) which was maximum during the 1st week post infection (38.10%), followed by those infected with *S. iniae* alone (61.9%) and then the *C. albicans* infected group (57.14%). The combined infection *(S. iniae+C. albicans*) exerted severe exophthalmia and cloudiness of the eye, severe skin ulceration with necrotic muscles and paleness of the gills and the liver. Lesions were more severe in the combined infected group that consisted of congestion in branchial blood vessel, excessive lamellar fusion and lamellar clubbing, while in gill arch there were mononuclear cell infiltrations and severe hemorrhages. Telangiectasis of secondary lamellae was produced as a response to branchial injury in which there is break down of vascular integrity due to rupture of pillar cells and pooling of blood (Ferguson, 1989, De Oliveira Ribeiro *et al*., 2002 and Giari *et al*., 2008). The hyperactivation of melanomacophage centers in

the spleen may be caused by stimulation of this organ by the action of bacterial toxin and/or mycotoxins. In this study cerebral lesions were mild to moderate in the intensity in the *S. iniae*- and *S. iniae +C.albicans*-infected groups and were consisted of cerebral congestion and edema as well as neuronal degeneration and necrosis. Eldar *et al.*, 1995 and Chen *et al*., 2007 observed more severe lesions in tilapia infected with *S. iniae* such as meningoencephalitis and panophthalmitis. This may be explained by the difference in *S. iniae* serotypes or the variance in the infective dose of the *S.iniae*. Therefore, further studies are needed to categorize the *S. iniae* serotypes and use the different infective doses of *S. iniae*.

Conflict of Interest

The authors declare that there is no conflict of interests

References

Andlid, T., Va´zquez-Jua´rez, R.V. and Gustafsson, L. 1995. Yeast colonizing the intestine of rainbow trout (*Salmo gairdner*i) and turbot (*Scophthalmus maximus*). Microbial Ecology**,** 30: 321–334. doi: 10.1007/BF00171938.

Au, D.W.T. 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. Marine Pollution Bulletin**,** 48: 817–834. doi:10.1016/j.marpolbul.2004.02.032

Calderone, R.A. 2002. Candida and candidiasis. Washington DC: ASM Press p 451.

Cavalcanti, I.M.G., Del Bel Cury, A.A. , Jenkinson H.F. and Nobbs A.H. 2016. Interactions between *Streptococcus oralis*, *Actinomyces oris*, and *Candida albicans* in the development of multispecies oral microbial biofilms on salivary pellicle. Molecular Oral Microbiology, doi: 10.1111/omi.12154.

Chang, P.H. and Plumb J.A. 1996. Histopathology of experimental *Streptococcus* Sp. Infection in Tilapia *Oreochromis niloticus* (L.) and Channel Catfish, *Ictalurus punctatus*. Journal of Fish Diseases**,** 19: 235-241. doi: 10.1111/j.1365-2761.1996.tb00130.x

Chao, C.C., Hsu, P.C., Jen, C.F., Chen, I.H., Wang, C.H., *et al*. 2010. Zebrafish as a model host for *Candida albicans* infection. Infection and Immunity, 78: 2512–2521. doi: 10.1128/IAI.01293-09.

Chen, C.Y., Chao, C.B. and Bowser, P.R. 2007. Comparative histo-pathology of *Streptococcus iniae* and *Streptococcus agalactiae*-infected tilapia. Bulletin- European Association of Fish Pathologists Journal**,** 27: 2–9.

Chen, C.Y., Wooster, G.A. and Bowser P.R. 2004. Comparative blood chemistry and histopathology of tilapia infected with *Vibrio vulnificus* or *Streptococcus iniae* or exposed to carbon tetrachloride, gentamicin, or copper sulphate. Aquaculture, 239: 421-443. doi:10.1016/j.aquaculture.2004.05.033.

Chen, D.F., Wang, K.Y., Geng, Y., Wang, J., Huang, L.Y. and Li, J.M. 2011. *Streptococcus iniae*, isolated from Channel Catfish (*Ictalurus punctatus*) in China. Israeli Journal of Aquaculture-Bamidgeh**,** 63: 593–599.

Chen, Y.Y., Chao, C.C., Liu, F.C., Hsu, P.C., Chen, H.F., Peng, S.C., Chuang, Y.J., Lan, C.Y., Hsieh, W.P. and Wong, D.S.H. 2013. Dynamic Transcript Profiling of *Candida albicans* Infection in Zebrafish: A Pathogen-Host Interaction Study. PLOS ONE**,** 8: e72483.doi.org/10.1371/journal.pone.0072483.

Cook, W.L. and Schlitzer, R.L. 1981. Isolation of *Candida albicans* from freshwater and sewage. Applied and Environmental Microbiology, 41: 840–842.

Creeper, J. and Buller N. 2006. An outbreak of *Streptococcus iniae* in barramundi (*Lates calcarifera*) in freshwater cage culture. Australian Veterinary Journal, 84: 408–411. doi: 10.1111/j.1751-0813.2006.00058.x.

Culling, C.F. 1983 *Handbook of Histopathological and Histochemical Staining Techniques*. 3rd (Ed), Butter Worth-London.

De Oliveira Ribeiro, C.A., Belger, L., Pelletier, E. and Rouleaux, C. 2002. Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). Environmental Research, 90: 217–225. doi: 10.1016/S0013-9351(02)00025-7.

Diaz, P.I., Xie, Z. and Sobue T. *et al*. 2012. Synergistic interaction between *Candida albicans* and commensal oral streptococci in a novel in vitro mucosal model. Infection and Immunity**,** 80: 620-632. doi: 10.1128/IAI.05896-11.

Doumas, B.T., Bayson, D.D., Carter, R.J., Peters, T. and Schaffer, R. 1981. Estimation of total serum protein. Clinical Chemistry, 27: 1642 –1643.

Duncan, L.L. 1995. Blood chemistry. In: Sirois, M. (Ed.) Veterinary Clinical Laboratory Procedures. MosbyYear Book, St. Louis, MO, USA, pp. 101 – 121.

Eissa, A.E., Tharwat, N.A. and Zaki, M.M. 2013. Field assessment of the mid-winter mass kills of trophic fishes at Mariotteya stream, Egypt: Chemical and biological pollution synergistic model. Chemosphere**,** 90: 1061–1068. doi.org/10.1016/j.chemosphere.2012.09.010.

Eldar, A., Benjerano, Y., Livoff, A., Horovitcz, A. and Bercovier, H. 1995. Experimental streptococcal meningoencephalitis in cultured fish. Veterinary Microbiology, 43: 33–40. doi:10.1016/0378-1135(94)00052-X.

Eldar, A. and Ghittino, C. 1999. *Lactococcus garvieae* and *Streptococcus iniae* infections in rainbow *trout Oncorhynchus mykiss*: similar, but different diseases. Diseases of Aquatic Organisms, 36: 227–231.

Facklam, R., Elliott, J., Shewmaker, L. and Reingold, A. 2005. Identification and characterization of sporadic isolates of *Streptococcus iniae* isolated from humans. Journal of Clinical Microbiology, 43: 933–937. doi:10.1128/JCM.43.2.933-937.

Falsetta, M.L., Klein, M.I. and Colonne, P.M. *et al*. 2014. Symbiotic relationship between *Streptococcus mutans* and *Candida albicans* synergizes virulence of plaque biofilms in vivo. Infection and Immunity**.** 82: 1968-1981. doi: 10.1128/IAI.00087-14.

Ferguson, H.W. 1989. Systemic Pathology of Fish, Iowa State University Press, Ames pp. 263.

Food And Agriculture Organization of the United Nations (FAO). 2014. The State of World Fisheries and Aquaculture Opportunities and challenges. Rome.

Giari, L., Simoni, E., Manera, M. and Dezfuli, B.S. 2008.

Histocytological responses of *Dicentrarchus labrax* (L.) following mercury exposure. Ecotoxicology and Environmental Safety. 70: 400–410. doi: 10.1016/j.ecoenv.2007.08.013.

Gratacap, R.L., Rawls, J.F. and Wheeler, R.T. 2013. Mucosal candidiasis elicits NF-kappaB activation, proinflammatory gene expression and localized neutrophilia in zebrafish. Disease models and mechanisms**.** 6: 1260-70. doi: 10.1242/dmm.012039.

Henry, R.J. 1974. Clinical Chemistry, Principles and Techniques. 2nd (Ed), Harper and Row, Hagerstown, MD, USA, pp. 525.

Kid, P.R.N. and King, E.G. 1954. Colorimetric determination of alkaline phosphatase activity. Journal of Clinical Pathology, 6: 330–332.

Koh, T.H., Kurup, A. and Chen, J. 2004. *Streptococcus iniae* discitis in Singapore. Emerging Infectious Diseases. 10: 1694–1696. doi: 10.3201/eid1009.040029.

Lahav, D., Eyngor, M., Hurvitz, A., Ghittino, C., Lublin, A. and Eldar, A. 2004. *Streptococcus iniae* type II infections in rainbow trout *Oncorhynchus mykiss*. Diseases of Aquatic Organisms**.** 62: 177–180. doi: 10.3354/dao062177.

Lau, S.K.P., Woo, P.C.Y., Luk, W.K., Fung, A.M.Y., Hui, W.T., Fong, A.H.C., Chow, C.W., Wong, S.S.Y. and Yuen, K.Y. 2006. Clinical isolates of *Streptococcus iniae* from Asia are more mucoid and B-hemolytic than those from North America. Diagnostic Microbiology and Infectious Disease**.** 54: 177–181. doi: org/10.1016/j.diagmicrobio.2005.09.012.

Lau, S.K.P., Woo, P.C.Y., Tse, H., Leung, K.W., Wong, S.S.Y. and Yuen, K.Y. 2003. Invasive *Streptococcus iniae* infections outside North America. Journal of Clinical Microbiology, 41: 1004–1009. doi:10.1128/JCM.41.3.

Neely, M.N., Pfeifer, J.D. and Caparon, M. 2002. Streptococcus-Zebrafish Model of Bacterial Pathogenesis. Infection and Immunity, 70: 3904–3914.doi. 10.1128/IAI.70.7.3904-3914**.**

Patton, J.R. and Crouch, S.R. 1977. Annual Chemistry**,** 49: 464-469.

Pier, G.B. and Madin, S.H. 1976. *Streptococcus iniae* sp. nov., a beta hemolytic *Streptococcus* isolated from an Amazon freshwater dolphin, *Inia geoffrensis*. International journal of systematic bacteriology, 26: 545–553.

Quiniou, S.M.A., Bigler, S., Clem, L.W. and Bly, J.E. 1998. Effects of water temperature on mucous cell distribution in channel catfish epidermis: a factor in winter saprolegniasis. Fish and Shellfish Immunology**,** 8: 1-11. doi: 10.1006/fsim.1997.0115

Rehulka, J. and Minarik, B. 2007. Blood parameters in brook trout *Salvelinus fontinalis* (Mitchill, 1815) affected by columnaris disease. Aquaculture Research**,** 38: 1182-1197. doi: 10.1111/j.1365-2109.2007.01786.x**.**

Reitman, S. and Frankel, S. 1957. A colorimetric method for determination of oxaloacetic transaminase and serum glutamic pyruvic transaminase. American Journal of Clinical Pathology**,** 28: 56-63.

Roberts, T.R. 1989. The freshwater fishes of Western Borneo (*Kalimantan Barat, Indonesia*). Memoirs of the California Academy of Sciences, 14: 210.

Russo, R., Mitchell, H. and Yanong, R.P.E. 2006. Characterization of *Streptococcus iniae* isolated from *ornamental cyprinid* fishes and development of challenge models. Aquaculture, 256: 105–110. doi:10.1016/j.aquaculture.2006.02.046.

SAS Statistical Analysis System. 2004. Statistical user's Guide. International Cary, New York City USA.

Shoemaker, C.A., Klesius, P.H. and Evans, J.J. 2001. Prevalence of *Streptococcus iniae* in tilapia, hybrid striped bass, and channel catfish on commercial fish farms in the United States. American Journal of Veterinary Research**,** 62: 174–177. doi: 10.2460/ajvr.2001.62.174.

Sun, J.R., Yan, J.C., Yeh, C.Y., Lee, S.Y. and Lu, J.J. 2007. Invasive infection with *Streptococcus iniae* in Taiwan. Journal of Medical Microbiology**,** 56: 1246-1249. doi: 10.1099/jmm.0.47180-0.

Weinstein, M.R., Litt, M., Kertesz, D.A., Wyper, P., Rose, D., Coulter, M., McGeer, A., Facklam, R., Ostach, C., Willey, B.M., Borczyk, A. and Low, D.E. 1997. Invasive infections due to a fish pathogen *Streptococcus iniae*. The New England Journal of Medicine**.** 337: 589–594. doi: 10.1056/NEJM199708283370902.

Xu, H., Sobue, T. and Thompson, A. *et al*. 2014. Streptococcal co-infection augments Candida pathogenicity by amplifying the mucosal inflammatory response. Cell Microbiology, 16: 214-231. doi: 10.1111/cmi.12216.

Zhu, J., Li, C., Ao, Q., Tan, Y., Luo, Y., Guo, Y., Lan, G., Jiang, H. and Gan, X. 2015. Trancriptomic profiling revealed the signatures of acute immune response in tilapia (*Oreochromis niloticus*) following *Streptococcus iniae* challenge. Fish and Shellfish Immunology**,** 46: 346-353. doi: 10.1016/j.fsi.2015.06.027.