

## ANTIGENIC VARIATIONS AND PATHOGENICITY BETWEEN MYCOPLASMA SPP ISOLATED FROM FISHES AND SHELLFISHES

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### Abstract

Samples from different species of native and imported fishes and shellfishes were collected for the isolation of *Mycoplasma* at different incubation temperature 25°C and 37°C. Whole cell membrane antigens of the obtained isolates were compared with each other and with other animal mycoplasma antigens by electrophoresis (SDS-PAGE). One isolate at both temperature were used for pathogenicity study in apparently healthy *Oreochromis niloticus* fish through gill scarification and intraperitoneally injection. Histopathological examination of experimentally inoculated fish revealed extensive and pronounced inflammatory lesions in gills, liver, spleen and gas bladder. Mycoplasmas in fish needs more attention and concern to evaluate its effect in combination with other fish pathogens.

### INTRODUCTION

Some bacteria are well-recognized as pathogens of fish and have received a substantial amount of attention by scientists. For other bacterial pathogens of fish, there is less information available, either because the disease is rarely encountered or because it is a new and emerging problem that is only beginning to be studied. In other cases, it is not clear if the organism should be recognized as a pathogen of fish or only an unusual concatenation of organism, host and environmental stresses (Woo and Burno, 1999).

Representatives of many bacterial taxa have been associated with fish diseases. However, not all of these bacteria constitute primary pathogens. Many should be categorized as opportunistic pathogens, which colonize and cause disease in already damaged hosts. The initial weakening process may involve pollution or a natural physiological state in the life cycle of the fish. There remains doubt about whether some bacteria should be considered as fish pathogens. As the supportive evidence is weak or nonexistent. Possibly, such organisms constitute contaminants or even innocent saprophytes (Austin and Austin, 2007).

Mycoplasmas probably evolved from clostridial ancestors by genome reduction leading to obligate parasitism of host cells. The vertebrate hosts present at the time of the origin of mycoplasmas about 400 million years ago were fish (Brown, 2002). The genus *Mycoplasma* belongs to a class of bacteria called the Mollicutes, other members of which are the ureaplasmas, spiroplasmas, mesoplasmas, entoplasmas,

asteroleplasmas, acholeplasmas and anaeroplasmas. The term mycoplasma has frequently been used to denote any species included in the class Mollicutes (Sussman, 2002). Mycoplasmas are widespread in nature as parasites in humans, mammals, reptiles, fish, arthropods and plants (Razin, 1995 and Sasaki, 2006). They are wall-less parasitic prokaryotes, phylogenetically related to low G-C Gram-positive bacteria (Weisburg *et al.*, 1989). Successful colonization of those poikilothermous ("cold-blooded") hosts must have involved adaptation to those defenses, shaping mycoplasma-host interactions for more than 125 million years before the earliest emergence of mammals. That history illuminates one aspect of the potential significance of mycoplasmosis of poikilothermous vertebrates to health and disease of other hosts including humans (Brown, 2002).

Kirchhoff and Rosengarten (1984) had isolated a mycoplasma for the first time from fish (the gills of a tench (*Tinca tinca* L.). The organism, designated as strain 163K (known later as *Mycoplasma mobile*). The most interesting property of the organism was its ability to show fast gliding motion. Several studies had made science that timedirected toward its mobility, cell morphology and cell adhesion (Rosengarten and Kirchhoff 1987 and Miyata *et al.* 2000). A full genome and proteome studies also had been done by Jaffe *et al.* (2004).

Austin and McIntosh (1991) had described mycoplasma as one of the organisms causing pathological conditions of fish. Also, Woo and Burno (1999) recorded that Mycoplasmatales (class Mollicutes) infect a wide range of bivalves and crustaceans. Austin and Austin (2007) had considered *Mycoplasma mobile* as one of the fish diseases causing gill damage.

In Egypt, Mycoplasma was first isolated by Elshabiny *et al.* (1989) from brackish water fish in El-fayoum governorate from gills, intestine and swim bladder. In 1996, they succeeded in isolation of Mycoplasma from *Clarias lazera* from different parts and finally at 1998, Husien *et al.*, it was isolated from carp.

This study was done to investigate the existence of Mycoplasma spp. in different types of fishes either local cultured or frozen imported (both fish and shellfish). Also, it concerned about the water temperature of isolation (25°C suitable for cold blooded and 37°C). Comparison of the antigen obtained from different isolates was compared with other animal Mycoplasma antigens to determine the common antigens between different mycoplasmas. Finally, a pathogenecity study was made on apparently healthy *Oreochromis niloticus* fish to monitor the pathological changes made by one of the obtained isolates at both tested temperatures by different routes of infection.

## MATERIALS AND METHODS

### Fish

#### Naturally infected fishes:

A total number of 45 cultured native *Oreochromis niloticus*, 45 *Hypophthalmichthys molitrix* and 5 *Clarias gariepinus* fishes were collected from Damitta and Rashid cages, Manial Sheha fish farm and desert fish farm (Table, 1). As well as a total number of 75 imported fish samples of different species were collected for the isolation of *Mycoplasma* (Table, 2). The collected fishes were examined clinically as described by Amlacher (1970).

Table 1. Native cultured Fishes

Fish species	Location	No
<i>Oreochromis niloticus</i>	Damitta cages	7
<i>Oreochromis niloticus</i>	Manial sheha Giza	1
<i>Oreochromis niloticus</i>	Desert fish farm	1
<i>Clarias gariepinus</i>	Desert fish farm	1
<i>Hypophthalmichthys molitrix</i>	Rashid cages	9

1= (pooled sample of 5 gills)

Table 2. Imported Fishes and shellfishes

Fish species	Location	No
<i>Sardine pilchardus</i>	Morocco	3
<i>Mugil cephalus</i>	Australia	1
<i>Scomber scombrus</i>	Biro	1
<i>Rastrelliger kanagurta</i>	Yamane	1
<i>Sardina pilchardus</i>	Yamane	1
<i>Solea solea</i>	Mauritania	1
<i>Cephalopholis miniata</i>	Mauritania	1
<i>Chrysophrys aurata</i>	Italy	1
<i>Penaeus Indicus</i>	Thailand	1
<i>Scomber scombrus</i>	Japan	1
<i>Sepia spp</i>	Italy	1
<i>Onchrhynchus spp</i>	Norway	1
<i>Euastacus spp</i>	UAE	1

1= (pooled sample of 5 gills)

#### Experimental fish

A total number of 100 apparently healthy *Oreochromis niloticus* were purchased from Kanater fish farm (Qualubia Governorate) with an average body weight  $80.0 \pm 20.0$  g, and transferred alive to Fish Diseases Department, Animal Health Research Institute, Dokki, Giza. Fish were divided into five equal groups and kept in chlorine-free tap water in five full glass aquaria (120×60×40). Fish were acclimated to experimental conditions for 2 weeks with continuous aeration and the water temperature was thermostatically controlled and kept at  $22 \pm 2$  °C.

**Isolation and propagation of mycoplasma** using liquid and solid media was prepared as described by Buller (2004). Incubation was done on 25 and 37 °C.

**Digitonin sensitivity and biochemical characterization** (glucose fermentation, hydrolysis of arginine, phosphatase activity, tetrazolium reduction and film and spot production) were done for the obtained isolates according to Buller (2004).

**Mycoplasma antigen** of both field isolates and other Mycoplasma reference strains (*M. bovis* and *M. gallisepticum* S6 and R strains) was prepared according to Duffy *et al.* (1998).

#### **SDS Electrophoresis**

Using Low range Molecular Weight standards, (Pharmacia) as marker, electrophoresis was done using 10% separating gel and 4% stacking gel in denatured dissociating buffer system (SDS-PAGE) following the method modified from Laemmli (1970) described by Duffy *et al.* (1998) using Hoefer Scientific Instrument vertical apparatus.

#### **Experimental design**

Fishes were randomly screened for the presence of *Mycoplasma* and were found to be free from the risk of natural infection and also free from any other pathogens.

A total number of 100 clinically normal *Oreochromis niloticus* were divided into five groups each of 20 fish. The fish in the first two groups were experimentally inoculated with 1ml of 48 hour old *Mycoplasma* broth culture without thalium acetate ( $3 \times 10^8$  C.F.U./fish) through gill scarification (Eggbrecht, 1986). The fish in group 3 and 4 were experimentally inoculated intraperitoneally with the same dose and the 5<sup>th</sup> group were kept as control. Samples were taken every week for two weeks for reisolation and detection of cytopathic effect by histopathological examination.

#### **Histopathological studies**

Tissue specimens from gills, liver, spleen and gas bladder were taken from *Oreochromis niloticus* that were experimentally infected with Mycoplasma after 1 and 2 week post-inoculation. The samples were fixed in 10% formal saline, processed by conventional method, sectioned at 4 µm and stained with Haematoxylin and Eosin (Bancroft *et al.*, 1996 and Roberts, 2001).

## RESULTS AND DISCUSSION

Bacteria of genus *Mycoplasma* are the smallest organisms known to be capable of self-replication. They only occur in association with animal host cells on which they are dependant for many pre-formed nutrients since they lack many of the metabolic pathways associated with energy production and the synthesis of cell components found in other species of bacteria. It is generally thought that most species of *Mycoplasma* are very host specific (Pitcher and Nicholas, 2005).

Table 3. the recovery rate of mycoplasma spp from the examined samples of native cultured fishes

Fish spp	Recovery Rate				
	No. Examined	25°C		37°C	
		No.+	%+	No.+	%+
<i>Oreochromis niloticus</i>	7	6	85.7	4	57.1
<i>Oreochromis niloticus</i>	1	0	0	0	0
<i>Oreochromis niloticus</i>	1	0	0	0	0
<i>Clarias gariepinus</i>	1	0	0	0	0
<i>Hypophthalmichthys molitrix</i>	9	0	0	1	11.1

In this study mycoplasma spp. was recovered in native cultured fishes at 25 °C as 85.7% from *Oreochromis niloticus* caught from Damitta cages and 57.1% at 37°C and 11.1% from *Hypophthalmichthys molitrix* caught from Rashid cages (table 3). The recovery rate was 100% in the pooled samples at both examined temperatures taken from *Sardine pilchardus* (Morocco), *Mugil cephalus* (Australia), *Scomber scombrus* (Biro), *Solea solea* (Mauritania) and *Scomber scombrus* (Japan). While, the recovery rate was 100% in the pooled samples at 25°C from *Cephalopholis miniata* (Mauritania) and at 37°C from *Penaeus indicus* (Thailand) as explained in table (4).

By biochemical identification, a Digitonin sensitive, glucose fermenting, tetrazolium reducing, having phosphatase activity (some can't), forming film and spots isolates that did not having the ability to hydrolyse arginine were recovered from all the obtained isolates except one isolate from *Oreochromis niloticus* (Digitonin negative, *Acholeplasma*) and other isolate of *Solea solea* that produced late phosphatase activity. The isolate of *Penaeus indicus* have different biochemical characters that were (Digitonin sensitive, arginine hydrolysing and negative in glucose fermentation, phosphatase activity, tetrazolium reduction and didn't form film and spots.

Serological identification couldn't be done due to shortage of fish mycoplasmas antisera.

*Mycoplasma* had been associated with fish diseases since their first isolated by Kirchhoff and Rosengarten (1984) from gills of a tench (*Tinca tinca* L.). Kirchhoff *et al.* (1984) studied the biochemical and culture character of *M. mobile* and found that

by culturing it produces "fried-egg" colonies on Hayflick medium, which contains horse serum, after incubation for 2-6 days. Growth occurs at 4-30°C, but not at 37°C and that was on contrary to this study. While, Elshabiny *et al.* (1989 and 1996) and Husien *et al.* (1998) recorded a similar results at 25°C. Holben *et al.* (2003) surveyed the bacterial populations present in the distal intestine of salmon and found Mycoplasma to comprised approximately 96% of the total microbes in the distal intestine of wild salmon.

Table 4. Recovery rate of mycoplasma spp from the examined samples of imported fishes

Fish spp	Recovery Rate				
	No. Examined	25°C		37°C	
		No. +	% +	No. +	% +
<i>Sardine pilchardu</i>	3	3	100	3	100
<i>Mugil cephalus</i>	1	1	100	1	100
<i>Scomber scombrus</i>	1	1	100	1	100
<i>Rastrelliger kanagurta</i>	1	0	0	0	0
<i>Sardina pilchardus</i>	1	0	0	0	0
<i>Solea solea</i>	1	1	100	1	100
<i>Cephalopholis miniata</i>	1	1	100	0	0
<i>Chrysophrys aurata</i>	1	1	0	0	0
<i>Penaeus indicus</i>	1	0	0	1	100
<i>Scomber scombrus</i>	1	1	100	1	100
<i>Sepia spp</i>	1	0	0	0	0
<i>Onchrhynchus spp</i>	1	0	0	0	0
<i>Euastacus spp</i>	1	0	0	0	0

Table 5. Biochemical identification of the obtained isolates from both native cultured and imported fishes

Fish spp	Biochemical Tests						
	D	G	A	P	T	F&S	
<i>Oreochromis niloticus</i>	+	+	-	±	+	+	5 (may be <i>M. mobile</i> )
	-	+	-	-	-	+	1(Acholeplasma)
<i>Hypophthalmichthys molitrix</i>	+	+	-	+	+	+	1 (may be <i>M. mobile</i> )
<i>Sardine pilchardus</i>	+	+	-	+	+	+	3 (may be <i>M. mobile</i> )
<i>Mugle cephalus</i>	+	+	-	+	+	+	1 (may be <i>M. mobile</i> )
<i>Scomber scombrus</i>	+	+	-	+	+	+	2 (may be <i>M. mobile</i> )
<i>Solea solea</i>	+	+	-	+	+	late+	1 (may be <i>M. mobile</i> )
<i>Cephalopholis miniata</i>	+	+	-	+	+	+	1 (may be <i>M. mobile</i> )
<i>Penaeus indicus</i>	+	-	+	-	-	-	unidentified

D= Digitonin sensitivity  
P= phosphatase activity

G= glucose fermentation  
T= tetrazolium reduction

A= arginine hydrolysis  
F&S= film and spot formation

Table 6. Showing The Antigenic Difference Between Some Isolated Mycoplasma From Native Cultured And Imported Fishes With Other Poultry And Bovine Mycoplasmas

	<i>M. gallisepticum</i>		<i>M. Dovis</i> Ref. strain	25°C				37°C			
	R	S6		1	2	3	4	1	2	3	4
1			349.8	349.8	349.8	349.8	349.8		349.8	349.8	
2	338.8	338.8				338.8		338.8		338.8	338.8
3	315.9	315.9									
4				303.3			303.3		303.3	303.3	
5			282.5			282.5		282.5			
6		269.8									
7	250.6				250.6	250.6		250.6		250.6	
8	234.3	234.3	234.3	234.3	234.3	234.3	234.3		234.3	234.3	
9							224.2				224.2
10	210.2	210.2	210.2	210.2	210.2				210.2	210.2	
11	172.9	172.9	172.9	172.9	172.9	172.9	172.9	172.9		172.9	172.9
12						153.4				153.4	
13	147.9	147.9			147.9	147.9	147.9	147.9	147.9		
14			131.4	131.4		131.4	131.4		131.4		
15	122.1			122.1	122.1	122.1					122.1
16	110.9	110.9	110.9		110.9		110.9	110.9	110.9	110.9	110.9
17				101.1			101.1	101.1	101.1	101.1	101.1
18	93.2	93.2			93.2	93.2		93.2	93.2		
19				88.2			88.2	88.2	88.2	88.2	88.2
20			84.9							84.9	
21				79.4	79.4	79.4					
22	76.1										
23								74.8	74.8		
24				71.0		71.0	71.0			71.0	71.0
25	69.6	69.6									
26	62.5	62.5					62.5	62.5	62.5	62.5	62.5
27	57.1	57.1	57.1	57.1	57.1	57.1				57.1	57.1
28							50.7		50.7		50.7
29			48.3		48.3	48.3	48.3			48.3	
30	46.4	46.4		46.4	46.4	46.4		46.4	46.4		
31		43.5	43.8								
32				41.1	41.1	41.1	41.1		41.1	41.1	41.1
33						37.6	37.6	37.6	37.6	37.6	37.6
34					32.7	32.7	32.7				
35	35.9	35.9									
36							31.3				31.3
37				29.6	29.6	29.6	29.6	29.6	29.6	29.6	29.6
38	27.3	27.3	27.3	27.3	27.3		27.3	27.3	27.3	27.3	27.3
39	24.3	24.3				24.3	24.3		24.3	24.3	
40				22.7	22.7			22.7		22.7	22.7
41	21.5	21.5	21.5		21.5	21.5	21.5	21.5	21.5	21.5	21.5
42	19.7	19.7		19.7	19.7		19.7				
43	18.1	18.1								18.1	18.1
44			16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5
45	15.7	15.7	15.7								
46	14.3	14.3				14.3	14.3	14.3	14.3	14.3	14.3
47	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2		13.2	13.2
48				11.8	11.8	11.8	11.8			11.8	
49					10.8	10.8		10.8	10.8		
50	9.8	9.8	9.8	9.8		9.8	9.8	9.8	9.8	9.8	9.8
51			9.0	9.0	9.0	9.0	9.0				9.0
52	8.4	8.4		8.4	8.4	8.4	8.4		8.4	8.4	
53	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7
54			7.3	7.3		7.3	7.3	7.3	7.3	7.3	7.3

1-3 =isolates of native cultured fish (*Oreochromis niloticus*)

4= isolate of imported fish (*Sardine pilchardus*)

Polyacrylamide gel electrophoresis (PAGE) of whole-cell proteins was first used for differentiation of *Mycoplasma* species by Razin and Rottem (1967). The principal reasons for examining whole-cell proteins are to achieve sufficient separation on the basis of molecular mass to allow individual proteins to be identified. This technique is suitable for most applications and allows direct comparisons to be made between different samples run on the same gel (Duffy *et al.*, 1998).

The antigen of some isolates obtained from *Oreochromis niloticus* from native cultured fishes and *Sardine pilchardus* from imported fishes at 25 °C and 37°C were electrophoresed and compared with antigen of *Mycoplasma gallisepticum* (R and S6) and *Mycoplasma bovis* as shown in table (6) and fig. (1-4). The *Mycoplasma* isolated on 25 °C gave 24-28 bands ranged from 7.3- 359.8 kDa while isolates at 37°C gave 26-32 bands with the same molecular weights. At the same time, *M. gallisepticum* R and S6 strains produced 27 and 20 bands respectively ranged from 7.7-338.8kDa and *M. bovis* ref. strain revealed 26 bands varied from 7.3- 359.8 kDa. All used *Mycoplasmas* shared in some bands that were almost present at 7.7, 9.8, 13.2, 21.5, 27.3, 110.9, 172.9 and 234.3 kDa that may considered structural antigen.

Fish isolates characterized by the presence of specific bands at 10.8, 11.8, 22.7, 29.6, 37.6, 41.1, 50.7, 71.0, 88.2, 101.1 and 303.3 kDa that didn't occur at other *Mycoplasmas*, also imported fish characterized by a specific bands at 31.3 and 224.2 kDa and only one band in one of the native isolates at 153.4 kDa. Some antigens appeared only at 25 °C as 19.7 kDa common with MG ref. strains, 32.7 and 79.4 kDa that were unique bands to this temperature of isolation. At 37 °C, there was a band at 84.9kDa common with *M. bovis*, 18.1 kDa with both MG ref. strains and a single band at 74.8 kDa. The other shared antigens are detailed in table 6.

*Mycoplasmas* lacking a cell wall and intracytoplasmic membranes, the mollicutes have only one type of membrane, the plasma membrane. Proteins constitute over two-thirds of the mycoplasma membrane mass, with the rest being membrane lipids (Razin *et al.*, 1998). Membrane proteins in prokaryotes function as transporters, enzymes, energy metabolism, sensors, and motility devices (Åke and Rosén 2002).

Kusumoto *et al.* (2004) , Uenoyama *et al.* (2004) , Seto *et al.* (2005), Uenoyama and Miyata (2005) localized the Gli521, Gli349 and Gli123 which were known to be involved in glass binding, haemadsorption gliding and adhesion. Also, Ohtani and Miyata (2007) identified a protein with a molecular mass of 42 kDa (P42) from *Mycoplasma mobile*, one of several mycoplasmas that exhibit gliding motility, was shown to be a novel NTPase (nucleoside triphosphatase) which is the key ATPase in the gliding motility of *M. mobile*.

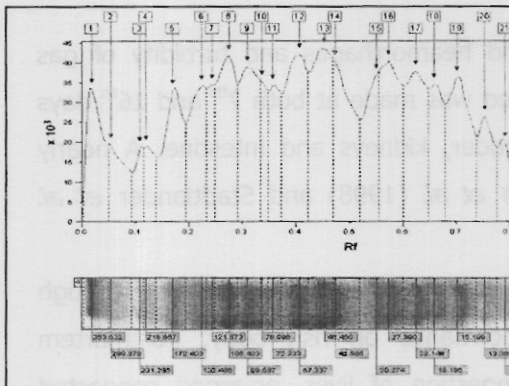


Fig. 1. the electrophoretic pattern of Mycoplasma isolated from native cultured fish at 25°C

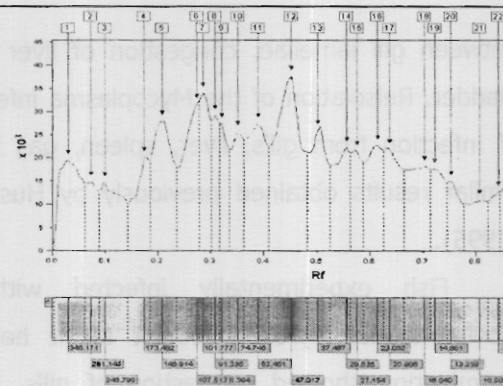


Fig. 2. the electrophoretic pattern of Mycoplasma isolated from native cultured fish at 37°C

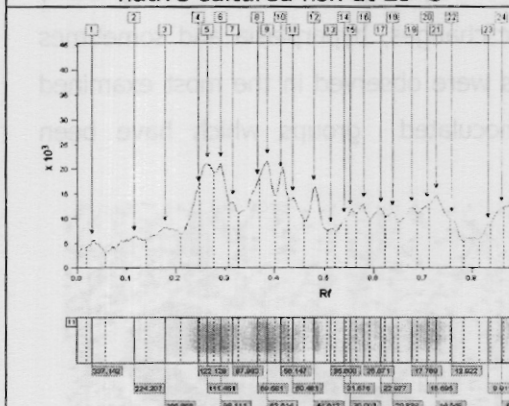


Fig. 3. the electrophoretic pattern of Mycoplasma isolated from imported fish at 25°C

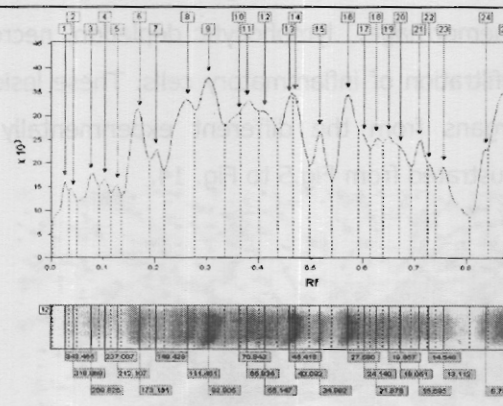


Fig. 4. the electrophoretic pattern of Mycoplasma isolated from imported fish at 37°C

*Mycoplasma mobile* isolated from fish, belongs to the *Mycoplasma hominis* group of the mycoplasma phylogeny, which also includes *Mycoplasma pulmonis* and *Mycoplasma hyopneumoniae* is believed to be pathogenic (Jaffe *et al.*, 2004). Kirchoff *et al.* (1984) made their first research on the pathogenicity of flask-shaped Mycoplasmas with a more or less defined terminal structure together with gliding motility and adherence properties and found that all flask-shape organisms ferment glucose. Miyata *et al.* (2000) indicating that mycoplasma gliding motility depends on cytodherence-associated components, suggesting a certain role of gliding motility in the parasitic life-cycle of mycoplasmas.

All experimentally infected fish failed to exhibit clinical signs with low mortality (one of each groups (1 & 2) 5% infected by gill scarification at the 1<sup>st</sup> and 2<sup>nd</sup> days post-infection). Fish experimentally infected with the isolated Mycoplasma through gills revealed external haemorrhages especially on fins and operculum, fin and tail rot and ulceration. Postmortem examination showed congestion of gills with adhesion

between gill lamellae, congestion of liver and haemorrhages and turbidity of gas bladder. Reisolation of the Mycoplasma infected was made at both 7<sup>th</sup> and 16<sup>th</sup> days of infection from gills, liver, spleen, gas bladder, kidneys and intestine. A nearly similar results obtained previously by Husien *et al.* (1998) and Stadlander *et al.* (1995).

Fish experimentally infected with the isolated Mycoplasma through intraperitoneal injection exhibit slight haemorrhages on fish body. Postmortem examination showed congestion of gills, congestion of liver, enlarged congested spleen and haemorrhages and turbidity of gas bladder. Temperature of incubation had no prominent effect on the observed lesions.

The histopathological findings were mainly represented as congestion, haemorrhage, lymphocytic depletion, necrotic changes, hyperplasia and sometimes infiltration of inflammatory cells. These lesions were observed in the most examined organs from the different experimentally inoculated groups which have been illustrated from Fig.5 to Fig. 14.



Fig. 5. liver of *O. niloticus* group I 7 day showing hydropic Degeneration ( H & E X 400).



Fig. 6. pancreas of *O. niloticus* group I 7 day showing haemorrhage of the pancreatic lumen (H & E X 400)

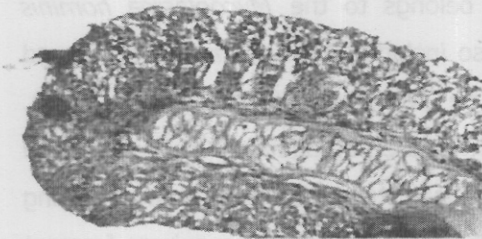


Fig. 7. gills of *O. niloticus* group I 7 day showing epithelial hyperplasia, fusion of secondary lamellae and focal haemorrhages in the primary lamellae (H & E X 100)

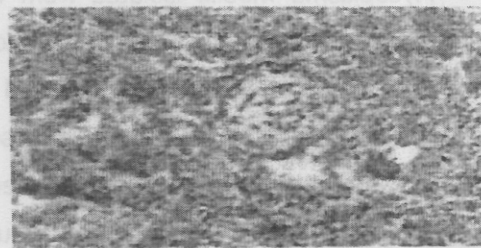


Fig. 8. spleen of *O. niloticus* group I 7 day necrotic changes replacing germinal centers accompanied with focal haemorrhages (H & E X 400)

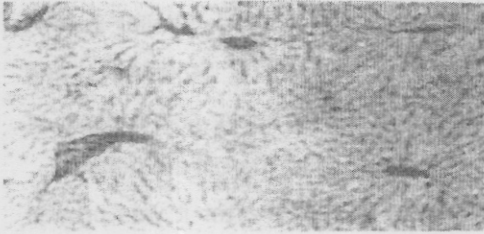


Fig. 9. liver of *O. niloticus* group I 16 day showing congestion of hepatic sinusoids (H & E X 100)

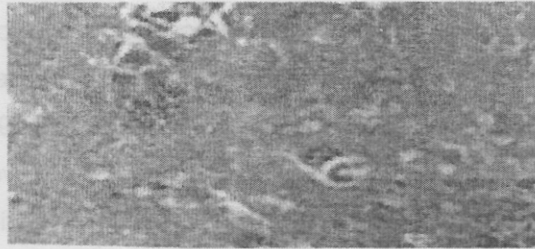


Fig. 10. spleen of *O. niloticus* group I 16 day showing necrotic changes in (H & E X 100).



Fig. 11. gas bladder of *O. niloticus* group I 16 day showing hyperplasia of epithelial lining associated with subepithelial edema and presence of inflammatory cells (H & E X 100).

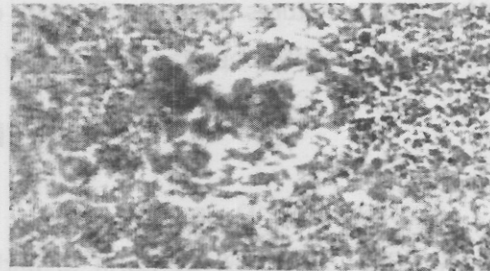


Fig. 12. spleen of *O. niloticus* group II 7day showing lymphocytic depletion with extensive haemorrhage and aggregation of melano-macrophages (H & E X 400).



Fig. 13. gas bladder of *O. niloticus* group II 16 day showing marked epithelial hyperplasia and presence of oedematous thickening (H & E X 100)

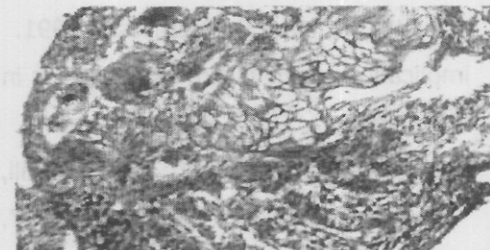


Fig. 14. gills of *O. niloticus* group II 16 day showing severe haemorrhage in the primary lamellae (H & E X 400)

In the present study, Mycoplasma generally induce multisystemic inflammatory lesions which include necrotic changes of the germinal centers of spleen with extensive haemorrhage and depletion of lymphocytes. Lesions of gills and gas bladder were also characterized by hyperplastic changes, haemorrhage and oedematous thickening of the subepithelial. Additionally, liver and pancreas exhibited degenerative changes in association with haemorrhage and congestion. These pathological alterations could possibly be attributed to the development of disseminated mycoplasmal infection of the blood and the ability of Mycoplasma to colonize and

adhere intimately to the host cell's surface ( mucosal and serosal ). Similar lesions were described by Brown (2002), Baseman and Tully (1997), Stadländer and Kirchoff (1995) and Stadlander *et al.*, (1995) whom revealed heavy colonization of mycoplasmas on gill rakers, resulting in severe tissue damage of the gill epithelium.

In conclusion, Mycoplasma in fish still need more research after it was proven by many authors to be pathogenic, its combination with other fish pathogens and other environmental stress factors. Also, other mollicutes causing infection of fish should be studied in Egypt especially spiroplasma and the economic losses occurring in shrimp aquacultures in combination with other viral diseases and Erysipelothrix that considered of zoonotic importance especially from imported fish.

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## الإختلافات الأنتيجينية والتأثير المرضى بين أنواع الميكوبلازما المعزولة من

### الأسماك و القشريات

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تم تجميع عينات من انواع مختلفة من الاسماك المحلية و المستوردة والقشريات لعزل الميكوبلازما فى درجات حرارة مختلفة وهى ٢٥ °م و ٣٧ °م. تمت المقارنة بين الانتيجينات للعترات المعزولة ببعضهم البعض وكذلك مقارنةهم بالميكوبلازما المعزولة من الحيوانات الاخرى عن طريق الفصل الكهربى (اس.دى.اس). تم استخدام عترة واحدة من الميكوبلازما عند ٢٥ °م و ٣٧ °م لاجراء عدوى تجريبية فى اسماك البلطى النىلى عن طريق الخياشيم والحقن البريتونى. اظهر الفحص الهستوباثولوجى للاسماك التى تم اجراء عدوى تجريبية لها اعراض التهابية واضحة وشديدة فى الخياشيم و الكبد والطحال والمثانة الهوائية. وبناء على ذلك تحتاج الميكوبلازما فى الاسماك الى مزيد من الاهتمام و العناية لتقييم تأثيرها بجانب امراض الاسماك الاخرى.