Vet.Med.J.,Giza. Vol.46, No.4. A (1998): 407-416.

# PRELIMINARY STUDIES ON MYCOPLASMA INFECTION IN NILE CARP (LABEO NILOTICUS) IN EGYPT

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## SUMMARY

Mycoplasma organisms were isolated for the first time from naturally infected Nile carp (L. niloticus) in Egypt. The isolated organisms were investigated microbiologically, where they showed the typical characters of the genus Mycoplasma. The serological typing of the isolated organisms from L. niloticus revealed an antigenical difference from mycoplasma of farm animals and poultry. The experimental inoculation of healthy L. niloticus through gil scrafication resulted in mild gill damage with signs of asphyxia and low mortality. The In-Vitro antibiotic sensitivity testing for the isolated mycoplasma revealed their high sensitivity to Enroflocin and low sensitivity to Colistin sulphate.

## INTRODUCTION

Mycoplasma have been described as a wide - host ranged pathogenic organisms with various clinical signs in man (Krause and Talyer, 1992), plant and animals (Lee and Davis, 1992). Recently, it appears to play an important role in fish as it was found to be a componenet of the water sediments in some fresh water lakes (Olah et al., 1972), detected by electron microscopy in the skin tumor of the Dover Sole (Matsuda and Chamberlain, 1976) and as fish cell culture contaminants (Kunze et al., 1972). Kirchhoff et al., (1983) succeeded to isolate mycoplasma for the first time from the gills of Tench (Tinca tinca) with signs of Red disease. The authors could identify the isolated organism as Mycoplasma mobile (Kirchhoff et al., 1987).

In Egypt, trials for the isolation of mycoplasma organisms from fishes have been undertaken. Other than the work of El-Shabiney et al., (1989 and 1996) where mycoplasma was isolated for the first time from some clinically normal fishes, namely T. niloticus, Bagrus bayad, Synodontis schall, Solea solea. and Clarias lazera, the literature concerning this point in Egypt is almost lacked.

The importance of mycoplasma as an erreger of serious disease problems with its periodical isolation from both apparently healthy, clinically diseased fishes especially at areas of organic

pollution and from contaminated fish cell cultures at different localities, enforced us to investigate the role played by this organism in conjunction with some ecological factors namely, high fish density on the pathogenicity of the organism to one member of the family Cyprinidae, the Nile and freshwater lake fish L. niloticus as well as its serological relationship to animals and poultry isolates.

## MATERIAL AND METHODS

### Fish:

### A. Naturally infected fish:

A total number of 20 Nile Carp (L. niloticus) were collected from Giza fish market. They showed the general signs of bacterial infections manifested with skin hameorrahges and fin rot. The fish were examined clinically as described by Amlacker (1970) and bacteriologically after Stadtlander and Kirchhoff, (1989).

### B. Experimental fish:

A total number of 40 apparently normal, living Nile Carp (L. niloticus) were collected alive from River Nile. They were kept in full glass aquaria, supplied with chlorine-free tap water in the Fish Disease lab., Animal Health Research Institute, Dokki, during which acclimatization and bacteriological examination for random sample of 5 fish was carried out to ensure that these experimental fish are free from the risk of natural infection.

### Mycoplasma antisera:

Different standard mycoplasma antisera namely M. agalactia, M. arginini, M. bovigenitalium, M. bovis M. gallinarum and m. Gallisepticum (National Institute of Health, Betheda, Maryland, 20014 U. S. A) were kindly supplied by Prof. Dr. N. M. Ai-Zeftawi, Prof. Dr. A. Rashwan, Animal Health Research Institute, Dokki and Prof. Dr. A. A. Ahmed, Animal Research Institute, Dokki and Prof. Dr. A. A. Ahmed, Animal Reproduction Research Instiute, to be used in the serological typing of the isolated mycoplasma organisms.

### Bacteriological examination:

The procedures for bacteriological isolation of mycoplasma from the gills of naturally as well as experimentally inoculated fishes was conducted according to Stadtlander and Kirchhoff, (1989) using the PPLO media supplemented with serum. The biochemical identification of the isolated organisms was carried out according to the method described by Alotle et al., (1970).

### Serological typing:

The gross inhibition test described by Clyde (1964) was performed on the isolated mycoplasma organisms against the previously mentioned, different standard mycoplasma antisera.

### In-vitro antibiotic sensitivity testing:

The In-vitro antibiotic sensitivity testing for mycoplasma isolates against various chemotherapeutic agents was performed and results were interpretated according to Oxoid Manual (1982).

**Experimental design:**

**1- Pathogenicity Test:**

A total number of 20 clinically normal L. niloticus with an average weight of 80-130g. were divided into four groups each of 5 fish . The fish in the first three groups were experimentally inoculated with 1 ml of 48 hour old mycoplasma broth culture ($3x10^8$ C. F. U.) / fish through gill scrafication (Eggbrecht, 1986). The five fish in the fourth group were kept as control inoculated with 1ml sterile saline / fish through gill scrafication. All fish groups were observed for one week for any abnormal clinical signs.

**Effect of high fish density on pathogenicity of mycoplasma to L. niloticus:**

A total number of 10 L. niloticus with an average weight of 80-130 g. and total length of 27 ± 3 cm. were overstocked in glass aquria at a rate of liter of water / 2.5 cm of fish length accoding to Mohamed and El-Sadawy (1997). All fish were inoculated with 1 ml. of 48 hours old, mycoplasma broth culture containing 3x108 C. F. U. / fish was rubbed on scarified gill filaments. Another 5 fish were infected in the same manner but kept in another aquarium of the same size without overcrowdness.

## RESULTS AND DISCUSSION

Three bacterial strains were isolated from 3 out of 20 naturally infected L. niloticus with general signs of gill damage, external haemorrhages and fin rot (Table 1). The isolated organisms were identified microbiologically and proved to have the full criteria of Class Mollicutes, genus Mycoplasma having the characteristic properties of typical fried egg colonies on Hayflick media containing horse serum (Fig. 1), with the appearance of daughter colonies around the mother colonies 3-5 days later (Fig. 2). Biochemical identification for the isolated mycoplasma species revealed their ability for glucose fermentation, Tetrazolium reduction, formation of film and spot on solid media containing 20 % heat-inactivated horse serum (Fig. 3), having phosphatase activity and their failure to hydrolyse arginin. These results indicated the first report on isolation of mycoplasma spieces from naturally infected L. niloticus in Egypt. This finding supported those of Kirchhoff and Rosengarten (1984), who could detect mycoplasma organisms for the first time on modified Hayflick medium under aerobic conditions at 25°C from the gills of a tench (Tinca tinca). Also these findings ae similar to those reported by El-Shabiney et al., (1989 and 1996) who could isolate mycoplasma organisms for the first time in Egypt from clinically normal fishes.

The isolated mycoplasmas did not show an inhibition zone when tested by gross inhibition test against the aforementioned available reference mycoplasma antisera, indicating that these mycoplasma are antigenically different from those of animal and poultry strains with their specific priority to fish as indicated by their isolation at an optimum incubation temperature of 25°C.

Concerning the In-vitro, antibiotic sensitivity test to the fish mycoplasma isolates, it appeared that

Table 1: Incidence and biochemical identification of mycoplasma organisms from examined L. nilotius fish

| Bacterial isolation | | | | Biochemical properties | | | | |
|---|---|---|---|---|---|---|---|---|
| Fish species | No. of exam fish | No. of isolates | Fish organ | Glucose ferment ation | Phosph atae ac- tivity | Arginin e hydol- ysis | Film and Spot | Tetrazo lium re- duction |
| L. niloticus | 20 | 3 | gill | + | + | - | + | + |

Table 2: In-vitro antibiotic sensitivity testing for mycoplasma isolates from examined L. niloticus

| Disc | Concentratio | Zone | Interpretation* |
|---|---|---|---|
| Enroflocin | 5 ug | 1.5 | ++++ |
| Norfloxcin | 10 ug | 1.3 | +++ |
| Oflozacin | 5 ug | 1.2 | +++ |
| Nitrofurantoin | 300 ug | 0.7 | + |
| Colistin sulphate | 10 ug | 0.5 | + |

Table 3: Pathogenicity of mycoplasma isolates to L. niloticus fish

| Isolate No. | Route of inoc- ulation | No. of dead / No. of inoculated fish | Interpretation* |
|---|---|---|---|
| 1 | G/S* | 0/5 | 0 |
| 2 | G/S | 0/5 | 0 |
| 3 | G/S | 1/5 | 20 |
| Non-inoculated control | G/S | 0/5 | 0 |

*Interpretation of the results are done according to Oxoid Maneial (1982).

Table 4: Effect of overcrowdness on the pathogenicity of the isolated mycoplasma to L. niloticus fish

| Isolate No. | No. of inoculated fish | Route of inoculation | No. of dead fish | Mortality % |
|---|---|---|---|---|
| 3 | 10 | G/S* | 4 | 40 % |
| Inoculated control | 5 | G/S* | 0 | 0 |

* G/S : Gill Scarification.

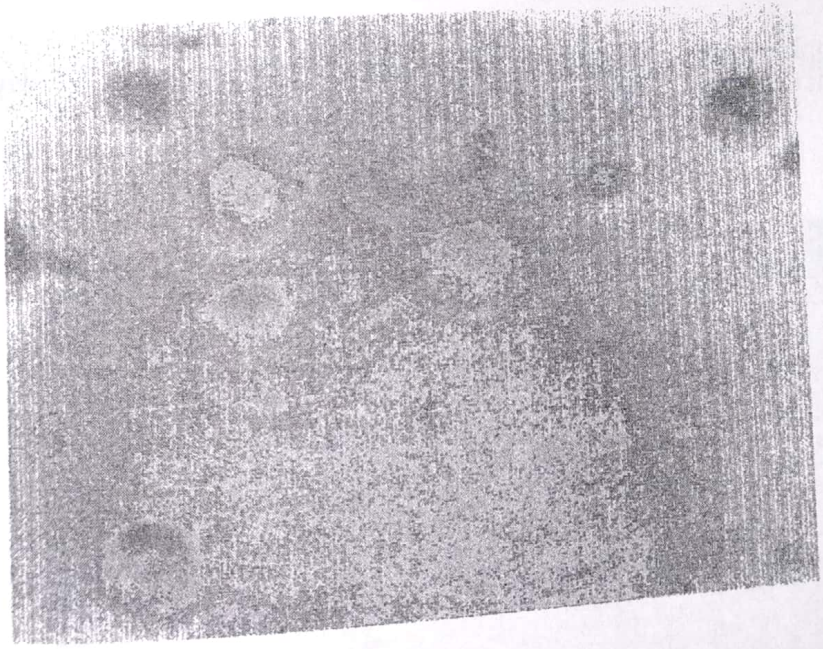

Fig. 1: Typical Fried - egg colonies of mycoplasma isolated from L. niloticus.

Fig. 2: Mycoplasma colonies surrounded by daughter cell colonies 3-5 days post-incubation at 25°C.

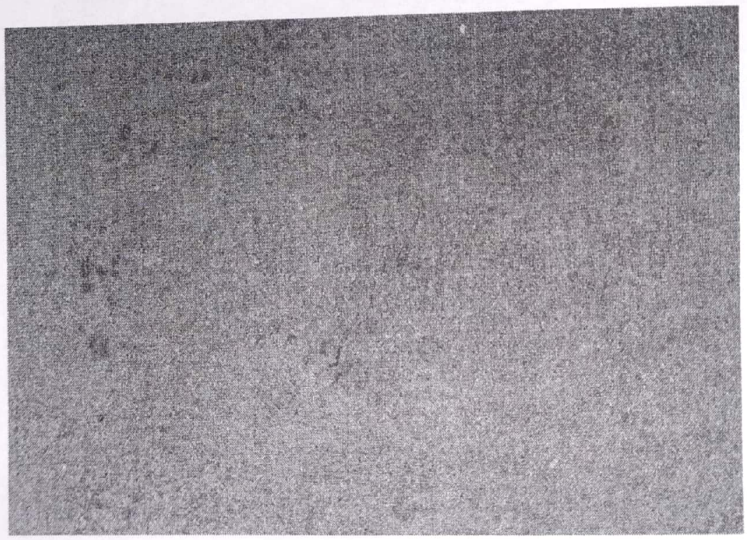

Fig. 3: A positive Film and Spots for the isolated mycoplasma from naturally infected L. niloticus.

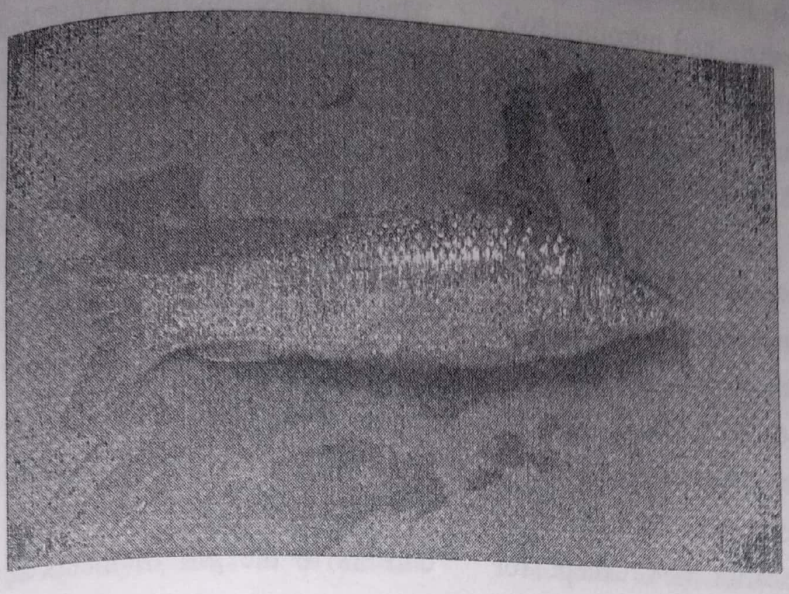

Fig.4: Experimentally inoculated L. niloticus showing slight fin haemorrhages.

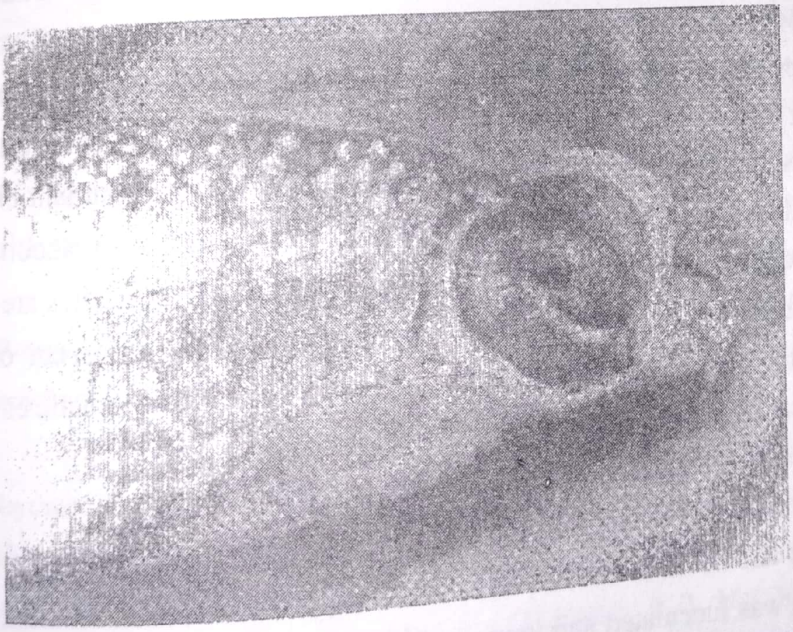

Fig. 5: Gills of experimentally inoculated L. niloticus showing gill haemorrhages and erosion of gill filaments.

the three isolates were highly sensitive to enroflocin, sensitive to norfloxacin, but less sensitive to colistin sulphate and nitrofurantoin. These results agreed with those of Frerich (1996) who added Ciproloxacin as a supplement to cell lines contamination with mycoplasma.

The results of the pathogenicity of the isolated mycoplasma to L. niloticus are to be seen in table (3). The failure of the isolated fish mycoplasma to reproduce clinical systemic disease in the same fish species (L. niloticus) after experimental inoculation of the three isolates through gill scarification except for some localized gill damage, fin haemorrhages (Fig. 4-5) with some sluggish swimming behaviour and only 20 % mortality in only one of the three inoculated fish groups and their reisolation from gills of experimentally inoculated fish, let us to suppose that the most predilection seat of mycoplasma organisms could be considered to be the external surface of gills and fins, but not the systemic organs, on the emphasis of their presence in the water sediments of freshwater lakes (Olah et al., 1972). These findings supported those of El-Shabiney et al (1989), who could isolate mycoplasma organisms from the internal organs of clinically normal fish and also those of Kirchhoff et al., (1984) who isolated Mycoplasma mobile 163K from gills of Tinca tinca. On the other hand, the recorded low mortality percent in the overcrowded low mortality percent in the overcrowded fish group and not in the other fish group (Table 4), which was inoculated and kept in normal fish density, may be attributed to the presence of different fish mycoplasmas with different degrees of virulence, some sort of immunity of fish against mycoplasma organisms which are occasionally present as opportunistic organisms in their environment from human and animals organic pollution as well as, the effect of over-crowdness stress factor which is usually reflected drastically on water oxygen content, fish gills and respiration as well as due to the local effect of mycoplasma organisms in interference with the respiratory mechanism of gill. This explanation could support those findings of Stadtlander and Kirchhoff (1995), who reported that the attachment of mycoplasma clusters to the gill filaments severely impaired the respiratory mechanisms and the loss of gill epithelial cells may have serious pathophysiological consequences.

In conclusion, the obtained data indicated the susceptibility of freshwater fish in organic polluted water to the infection with fish-specific mycoplasma of variable virulence, that differ antigenically from those of man and animal origin which can be localized and adhere to the external surface of fish skin, either the intact or abraded one with the resultant external infection. Also, a full epizootiological and immuno-pathological studies are urgently needed to fulfill the different aspect of this important infectious agent in the aquatic environment.

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