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MYCOPLASMA AS A CAUSATIVE PATHOGENIC AGENT IN *CLARIAS LAZERA* IN EGYPT

By

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SUMMARY

A total of (16) cat fish, fresh water fish *Clarias lazera* caught from giza Governorate, egypt, were bacteriologically examined. Mycoplasma could be isolated from gills, branchial organs, liver, heart, spleen and gall bladder. The isolates belonged to genus Mycoplasma, they were glucose fermenters, exhibited phosphatase activity, hydrolized tetrazolium, produced film and spot. Moreover, the isolates hemolyzed and agglutinated RBCs, adhered to glass and plastic surfaces and aggregated in broth medium. When tested by growth inhibition test against some Mycoplasma available antisera, the Mycoplasma isolates are suggested to be species specific to fish.

Experimental infection of *Clarias lazera* with the isolated Mycoplasma 48 hour culture in the gills and subcutaneously resulted in several lesions including pitecheal hemorrhages on body surface, inflamed sloughed skin, congested spleen, destructive areas of fins especially tail. Mycoplasma could be also reisolated from kidney, heart, gills and site of inoculation.

The study is considered the second time for iaolation of fish Mycoplasma in Egypt. In addition it exceeds by being the first to speculate the pathogenic role played by the Mycoplasma in fish causing several lesions that renders fish unfit from human consumption.

INTRODUCTION

Fish are considered as a biological marker and an indicators of their environment due to exposure to chemicals, bacteria or other substances in the water. Moreover, fish are vulnerable to most types of infectious organisms which affect mammals (Gittino, 1972 and Brown and Gratzek, 1980).

Mycoplasma is of increasing importance causing diseases for man, animals, birds, plant and insects. Mycoplasma was isolated from different fish species, marine fish and brackish water fish *Tincatinca, Tilapia nilotica, Solea solea, Synodontis schall.* This was recorded by Rosengarten (1984), Kirchhoff et al. (1984) and El-Shabiny et al. (1989).

The properties and pathogenicity of Mycoplasma for fish was studied by Kirchhoff et al., (1984), they referred to the ability of producing a general haemorrhage and inflammation of skin. Morphological specialities were explored by Rosengarten and Kirchhoff (1984).

Antibody formation was investigated by Lotz (1983) and then Eggebrecht (1986). Gliding movement, ability to destroy epithelial cells and possession of chemotactic abilities were studied by Fisher et al. (1987). Fish Mycoplasma was considered as a surface parasitic agent causing damage of epithelium Stadtlander and Kirchhoff (1988 and 1990). The aim of this work is to study the pathogenicity of the isolated Mycoplasma for fish.

MATERIAL AND METHODS

A total of (16) fresh water cat fish, *Clarias lazera* were caught from Giza Governorate in fresh alive state. They were sent to the laboratory aquarium in original water in plastic bage and all accurate methods of transportation and cultivation were applied as described by Klinke and Marsha (1973) and Brown and Gratzek (1980).

Samples were taken from the gills, suprabranchial organs, liver, spleen, heart, kidney and gall bladder.

The sampels were cultivated on modified Hayflick medium (Chanock et al., 1962). The agar plates and broth cultures were incubated at 25°C.

Requirement for cholesterol, lack of reversion to bacterium and filterability through 450 nm membrane filter were tested according to kirchhoff and Rosengarten (1984). Genus determination was made using digitonin test according to Tully (1983). Biochemical characterization of the isolates was performed as described by Aluotto et al. (1970) including glucose fermentation, tetrazolium reduction, arginine and urea hydrolysis, film and spot formation and phosphatase activity. Agglutination and absorption for chicken RBCs was determined according to Sobeslavsky et al. (1968), hemolysis was tested as mentioned by Cole et al. (1968). The ability of adherence to glass or plastic surfaces were observed by dark field microscopy according to Kirchhoff et al. (1987).The Mycoplasma isolates were serologically examined by growth inhibition test (GI) according to Clyde (1964) against available reference antisera at the Mycoplasma Department such as *M. bovirhinis, M. fermentans, M. gallisepticum, M. galinacium, M. pullorim, M. Mycoides* and *M. aqalactia.*

Pathogenicity Study: Eggebrecht (1986):

First experiment 1: Twenty fresh water cat fish, *Clarias lazera* were kept in laboratory glass aquaria. Ten fish were infected by scratching gill lamella and inoculation of 0.5 ml of 48 hour Mycoplasma broth culture $2X10^9$ C.F.U. without thallium acetate, while 10 other fish were included in this experiment as control.

Second experiment 2: Ten fish were injected subcutaneously with 0.5 ml Mycoplasma broth culture, $2x10^9$ CFU.

One week post infection fish were killed, pathologic lesions were observed and also postmortem examination was made according to Reichenbach-Klink's (1973). A trial for reisolation of Mycoplasma from experimentally infected fish and the control ones were made by taking samples from gills, suprabranchial organs, heart, gall bladder, kidney, intestine, liver and spleen.

RESULTS

Mycoplasma could be isoalted from 3 out of 16 examined cat fish from spleen, gills, suprabranchial organs, heart, liver and gall bladder and revealed 8 isolates. They were sensitive to digitonin i.e. they belonged to genus Mycoplasma. they all were glucose fermenters, reduced tetrazolium, formed film and spot and had phosphatase activity as recorded in Table (1).

The isolated Mycoplasma showed no inhibition zone when tested by growth inhibition against the available antisera. They hemolyzed and and were absorbed to RBCs and adhered to glass and plastic surfaces.

Fish experimentally infected with the isolated Mycoplasma in gills revealed several pathologic signs including pitecheal haemorrhages on the body surface, pale gills, off food, destructive arease of fins especially the tail and skin with excessive mucous. Postmortem examination showed congested spleen, pink to yellowish discoloured liver and distended gall bladder with greenish fluid. Fish infected subcutaneously with Mycoplasma revealed inflamed and sloughed skin, pitecheal haemorrhages, off food and glance off the bottom.

Table (1): Biochemical properties of Mycoplasma.

Fish species	No.exam.	No.isolates	Dig	G	A	TTC	U	F&Sp	ph
Clarias lazera	16	8	+	+	-	+	-	+	+

Dig = Digitonin
TTC = tetrazolium
U = Urea
ph = phosphatase

G = Glucose
A = Arginine
F&Sp = Film and Spot

The post mortem examination showed pale gills, congested spleen, pink to yellowish dicolouration of liver, large distended gall bladder with greenish fluid. On contrary the control groups showed no skin damage, no inflammatory changes and no sloughed areas. The postmortem examiantion showed no pathogenic changes.

Mycoplasma could be reisolated from the infected fish from gills, heart and site of inoculation (subcut.).

DISCUSSION

The present study is the first approach in Egypt to the stages of relationship between Mycoplasma as a pathogen and fish as a host represented by experimental infection, observing the resulting lesions and reisolation of Mycoplasma.

Mycoplasma could be isolated from different organs such as spleen, gills, heart, liver, gall bladder and branchial organs as was previously done by El-Shabiny et al. (1989) who isolated Mycoplasma from *Tilapia nilotica, Solea solea* and *Synodontis schall.* The Mycoplasma isolates fulfilled the criteria of class Mollicutes, related to genus Mycoplasma beside having characteristic properties including 25°C as an optimum temperature for growth, fermentation of glucose, reduction of tetrazolium, film and spot formation and having phosphatase activity. these findings agreed with Kirchhoff et al., (1984) who isolated strain 163K from gills of a tench, *Tinca tinca.* Moreover, the isolates agglutinate and absorbed RBCs, adhered to glass and plastic surfaces was investigated by Kirchhoff et al., (1987) ar Rosengarten et al., (1988). Furthermore, th interaction of the isolates and erythrocytes we studied and were in agreement with th mentioned by Fisher and Kirchhoff (1987).

All the above mentioned criteria beside negativ testing of the isoaltes against available antiser suggested them as species specific for fish.

Regarding pathogenicity studies represented b Experiment 1 and 2, the inoculated Mycoplasm resulted in pitecheal haemorrhages on bod surface, inflamed, sloughed skin, destructive area of fins especially the tail, congested spleen yellowish discolouration of liver, distended gal bladder. All these lesions were mentioned by Reichenbach-Klinke's (1973) as pathologic signs Moreover, the properties of the isolated organism such as hemolysis ad absorption of RBCs, adherence to surfaces (attachment) and special structure are further indication of pathogenic potential of this Mycoplasma. Pathogenicity studies were previously performed by Kirchhoff et al., (1984) who inoculated strain 163K isolated from fish intraperitoneally or rubbed into scarified skin, resulted in general hemorrhages and skin necrosis. They considered Mycoplasma as a possible causative agent of red disease.

Stadtlander and Kirchhoff (1988) and (1990) characterized Mycoplasma mobile 163K as a surface parasite by applying experimental infection in gnotobiotic rat tracheal rings, they noticed the attachment of this Mycoplasma to tracheal ring cell causing damage to epithelium.

Attachment was also observed by Fisher et al. (1987), Fisher and Kirchhoff (1987) and Rosengarten et al., (1988a).

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