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MYCOPLASMA INFECTION IN GREATER FLAMINGO, GREY CHINESE GOOSE AND WHITE PELICAN

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SUMMARY

A total of 134 zoo birds were examined for mycoplasma. Four out of 23 Greater flamingo (Phoenicopterus ruber roseus) were found to be infected with *Mycoplasma gallisepticum* (MG). Mycoplasma gallinaceum could be isoalted from grey Chinese goose (Anser cygnoides) and White pelican (Pelecanus onocrotalus) in a rate of 8% and 13.8% respectively. the electrophoretic patterns of the isolated MG field strains proved that their major protein bands resembled that of Mycoplasma gallisepticum R-reference strain. A characteristic protein band at the level of 60 KD (approximately) could be detected in M.gallinaceum isolates.

INTRODUCTION

Mycolasma pullorum and M. gallinaceum were isolated from chickens in Egypt (Abd El-Rahman, 1986 and Ammar et al. 1989), El-Shater et al. (1990) isolated M.pullorum from Silver pheasant and Golden pheasant, while M.gallinaceum was isoalted from comon peafowl and white peafowl. Mycoplasma gallisepticum (MG) infection is a well documented disease of chickens and turkeys. Natural infections though rare, have also been described in pheasants chukar partridges, peafowl and quail (Wills, 1955; Wichman, 1957; Osborn and Pomeroy, 1958 and Reece et al. 1986). Birds typically display swollen infraorbital sinuses and conjunctivitis with excessive lacrimation and emaciation. Mortality can range from slight to more than 30%. Cookson and Shivaprasad (1994) diagnosed MG in a group of Chukar partridges, pheasants and peafowl.

The present study describes isolation, identification and the protein patterns of mycoplasma isolated from zoo birds compared with reference strains of MG by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

MATERIAL AND METHODS

Samples:

A total of 134 tracheal swabs were collected from Greater Flamingo, Grey Chinese goose and White palican, all of these birds were kept in the zoological garden at Giza.

Media and Cutural Procedures:

Tracheal swabs were cultured for mycoplasma isolation using Frey's broth and agar media (Frey et al. 1968). The plates were incubated at 37°C with 5%Co_2 and humidity. The plates were checked for typical mycoplasma colonies after 24-48 hours of incubation for 7-10 days.

Biochemical characterization and serological identification:

The isolates were checked for glucose fermentation, arginine splitting and film and spot formation as described by Erno and stipkovits (1973). Serological identification of the isolates was carried out using growth inhibition test

(Clyde, 1964).

Mycoplasma strains and antisera:
Mycoplasma gallisepticum (MG) reference strains and antisera were obtained from Dr. S. J. Geary, Department of Pathobiology University of Connecticut . Locally prepared antisera were also used.

Preparation of mycoplasma proteins:
The liquid and solid media used to propagate the mycoplasmas have been described by Frey et al. (1968). Mycoplasma strains were grown in 500 ml. liquid medium for 36-48 hours at 37°C.

The cells were sedimented by centrifugation at 12,000 r.p.m for 30 minutes and washed 3 times in normal saline. Protein concentration was estimated by the Bradford method (Bio-Rad Laboratories, Richmond, Calif.).

Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE):

It was performed in 10% acrylamide resolving gels with 3% stacking gel according to the discontinuous buffer system of Laemmli (1970). Twenty -five ug of mycoplasma cell protein was mixed in 50 ul of sample buffer (1M Tris-Hcl pH6-8, 10%glycerol, 2% SDS, 5% 2-mercaptoethanol and 0.2% bromophenol blue), which was boiled 5 minutes in water and then loaded onto the gel. Molecular mass standards (Bio-Rad Laboratories, Richmond, Calif.) was included in each run. Gels were run at 25 mA at room temperature for 4 hours, stained with 0.5% Coomassie Brilliant Blue, 25% methanol, 10% glacial acetic acid, destained overnight with two changes of 25% methanol, 10% glacial acetic acid and then photographed.

RESULTS

The isolation of mycoplasma from 3 species of zoo birds is recorded in table (1). The incidence of mycoplasma was 17.4% in Greater Flamingo, 13.8% in Grey Chinese goose and 13% in White pelican.

Table (2) shows the biochemical characterization and serological identification of the isolates. All the isolates fermented glucose but did not split arginine.

The strains isoalted from Greater Flamingo were serologically identified as *Mycoplasma gallisepticum* while that isolated from Grey Chinese goose and White pelican were identified as Mycoplasma gallinaceum.

Fig (1) shows the protein patters of *Mycoplasma gallisepticum* (MG) reference strains. The protein banding patterns in the upper part of the gel (approximately betwen 45-95 Kilodalton level) of all strains of MG resembled each other closely. On the other hand, minor but distinct variations among MG strains were detected, particulatly between 31-45 KD level.

The sodium dodecyl sulphate-polyacrylamide gel electrophoresis patterns of mycoplasma isolated from zoo birds are shown in Fig (2). The major bands of MG field isolates resembled those of R-Reference strains of MG, while M. gallinaceum isolates have a characteristic protein band at the level of 60 KD approximately.

DISCUSSION

The isoaltion of mycoplasma from gallinaceaus birds in Egypt was recorded for the first time by El-Shater et al. (1990).

In the present study, *M.gallinceum* was isolated from Grey Chinese goose and White pelican. Mycoplasma gallisepticum could be isolated from Greater Flamingo. Cookson and Shivaprasad

Table (1) Incidence of Mycoplasma in Zoo birds

Bird	No. of Examined bi	No. of positive	%
Greater Flamingo	23	4	17.4
Grey Chinese Goose	65	9	13.8
White Pelican	46	6	13
Total	134	19	14.2

Table (2) Serological Identification of isolates using Growth Inhibition Test

Bird	No. of isolates	Biochemical Pattern				Positive Identification
		Glucose	Arginine	Film & spot		
Greater Flamingo	4	+	–	-	-	M.gallisepticum
Grey Chinese Goose	9	+	–	-	-	M.gallinaceum
White Pelican	6	+	–	-	-	M.gallinaceum

Fig. (1) Coomassie blue stained Sodium dodecyl sulphate-polyacrylamide gel electrophoresis protein profile of M.gallisepticum reference strains

Lanes from left to right

Lanes 1-3 : M.gallisepticum R-strain

Lanes 4-6 : M.gallisepticum A5969strain

Lanes 7 Molecular weight standard (Low range)

Lanes 8-10 : M.gallisepticum S-strain

Lanes 11-13 : M.gallisepticum F-strain

Electrophoresis current from up (-) to down (+)

K.D = Kilodalton

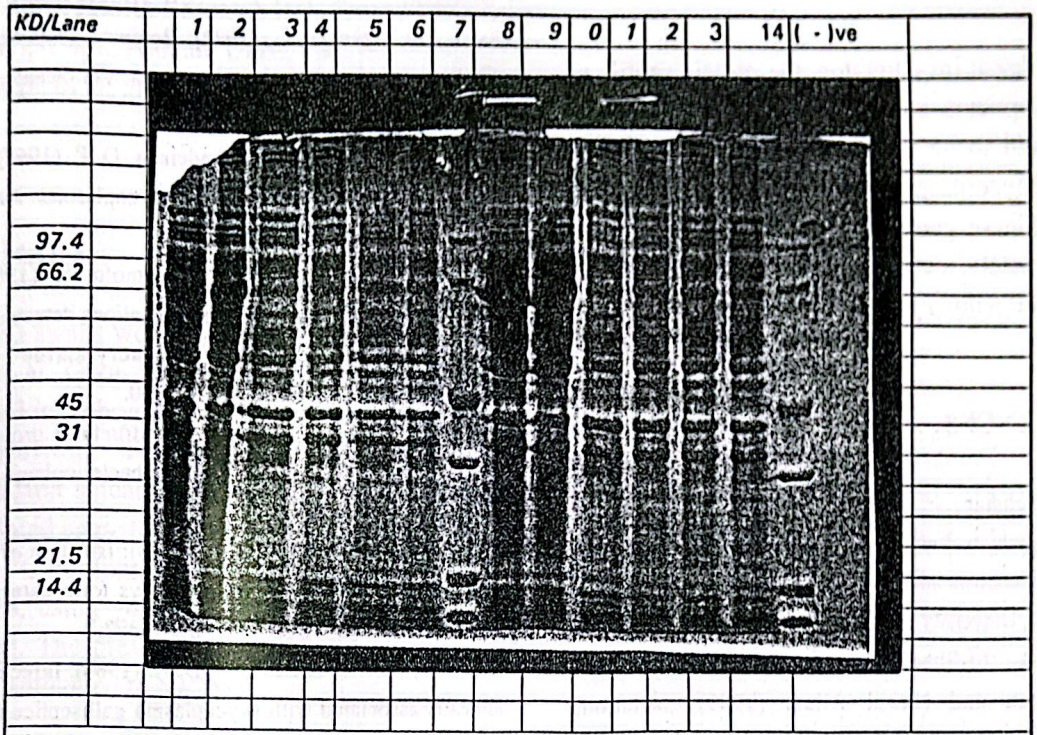

Fig. (2) Coomassie blue stained Sodium dodecyl sulphate-polyacrylamide gel electrophoresis protein profile of Mycoplasma isolated from Zoo birds.

Lanes from left to right

Lanes 1-2 & 8-9 : M.gallisepticum

Lanes 3-4 & 10-11 : M.gallinceum

Lanes 5-6 & 12-13 : M. gallinaceum.

Lanes 7 &14 : Molecular weight mass (low range)

K.D = Kilodalton

(1994) diagnosed M.gallisepticum infection in group of partridges, pheasants and peafowl.

The sodium dodecyl sulphate -polyacrylamide gel electrophoresis (SDS-PAGE) of *M.gallisepticum* strains showed differences in protein patterns (Khan et al. 1987).

In the present investigation, the protein profile of M.gallisepticum isolated from Greater Flamingo was found to be similar to that of R-reference strain. Mycoplasma gallinaceum isolated from Grey Chinese goose and White pelican showed approximately, a characteristic protein band at the level of 60 Kilodalton.

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