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ANTIGENIC STUDIES ON MYCOPLASMAS CAUSING RESPIRATORY TROUBLES IN TURKEYS, MAN, AND CATTLE

By

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SUMMARY

Turkeys sinus swabs, sputum swabs from human patients and lung samples from imported beef cattle with respiratory troubles were examined for mycoplasma. Mycoplasma gallisepticum was identified in 6 out of 36 sinus swabs. Five out of 37 patients were found to be infected with M. pneumoniae. Nine isolates of M. bovirhinis were isolated from the beef cattle.

The electrophoretic analysis of M. gallisepticum showed the characteristic 64 KDa protein band. The protein of M. pneumoniae showed high similarity and the 170 Kilodalton (KDa) protein band was detected in the investigated strains. Mycoplasma bovirhinis isolates showed some differences in the molecular weight region of 36 - 40 Kda.

Two common bands (40 and 76 KDa) were detected in M. pneumoniae and M. bovirhinis but M. gallisepticum have only one common band (76 KDa).

INTRODUCTION

Mycoplasma gallisepticum is the causative agent of chronic respiratory disease in chickens and infectious sinusitis in turkeys. The disease is characterized by nasal discharge, respiratory rales, coughing, and airsacculitis (Yoder, 1984).

Mycoplasma pneumoniae is the aetiologic agent of primary atypical pneumonia. The incidence of disease is highest among children and young adults (Geary et al., 1993). In Egypt, incidence of M. pneumoniae infection was 8.1 % (Korraa, 1991).

Mycplasma bovirhinis is a glucose-fermenting mycoplasma has only been reported from cattle. It appears to be the species most commonly isolated from the bovine respiratory tract (Cottew and Leach, 1969). Harbourne et. al. (1965) were the first to recognize it as a separate species when they isolated it from the lungs and nasal swabs of calves suffering from respiratory disease in England.

The purpose of the present study was to compare the antigenic components of mycoplasmas isolated from the respiratory tract of turkeys, man, and cattle.

MATERIAL AND METHODS

Samples: 1-Sinus swabs were collected from 36 turkeys with a history of mycoplasma infection, were grown in Frey,s medium (Frey et al., 1968).
2-Swabs from sputum samples of 37 patients (Ain-Shams University hospitals)with upper and/or lower respiratory tract infections were immediately placed in MaCartney bottles containing broth (Hayflick, 1965), after incubation 6- 12 hours at 37 C a loopful was inoculated on agar plates while 0.2 ml was transferred into broth. Three days after incubation, the inocula which did not show growth on the agar were blindly passaged twice. Culture was reported negative for M.pneumoniae after 15 days. The isolates were identified by growth inhibition test with specific antisera (Clyde, 1964)
3-Lung samples were collected from imported beef callte (Ireland)showed respiratory troubles, rise of temperature and mortality. The samples were cultured for isolation of mycoplasma as described by Sabry and Ahmed (1975).

Biochemical characterization of the isolated purified strains was carried out (Erno and Stipkovits, 1973).

The isolates were serologically identified by growth inhibition (Clyde, 1964).

Six isolates M gallisepticum from turkeys, 5 strains M. pneumoniae isolated from human patients and 9 isolates M. bovirhins recovered from beef cattle were examined using sodium dodecyl sulphate - polyacrylamide gel eleectrophoresis (SDS - PAGE).

The antigens were prepared as described by Thirkell et al. (1990). Using 10 % polyacrylamide slab gels, the SDS-PAGE technique was carried out using the discontinuous buffer as described by Laemmli (1970).

Electrophoresis was done at 25 mA for 3 hours in a Hoefer SE 400 electrophoresis unit (Hoefer Scientific Instruments, San Francisco, California, USA).

- Reference mycoplasma antisera:

Antisera against M. gallisepticum, M. pneumoniae and M. bovirhinis were obtained from Dr. S. J. Geary, Department of Pathobiology, University of Connecticut, USA.

- SDS-PAGE Molecular Weight Standards:

Low and High Range, Bio-Rad Laboratories, California, USA.

RESULTS

Mycoplasma gallisepticum could be detected in 6 out of 36 sinus swabs from turkeys with a history of mycoplasma infection (sinusitis, nasal discharge and respiratory rales). Sputum swabs from 37 patients with upper and/or lower respiratory tract infection were examined for mycoplasma. M. pneumoniae was isolated and identified from 5 cases.

Investigation of lung samples from imported beef cattle with respiratory troubles was carried out for detection of mycoplasma infection. Nine isolates were recovered and identified as M. bovirhinis.

Coomassie blue-stained sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) protein profiles of M. gallisepticum and M. pneumoniae showed similarity among the isolates (Fig. 1). The electrophoretic pattern of M. bovirhinis isolates on 10 % polyacrylamide gel with Coomassie stain was shown in Fig. 2.

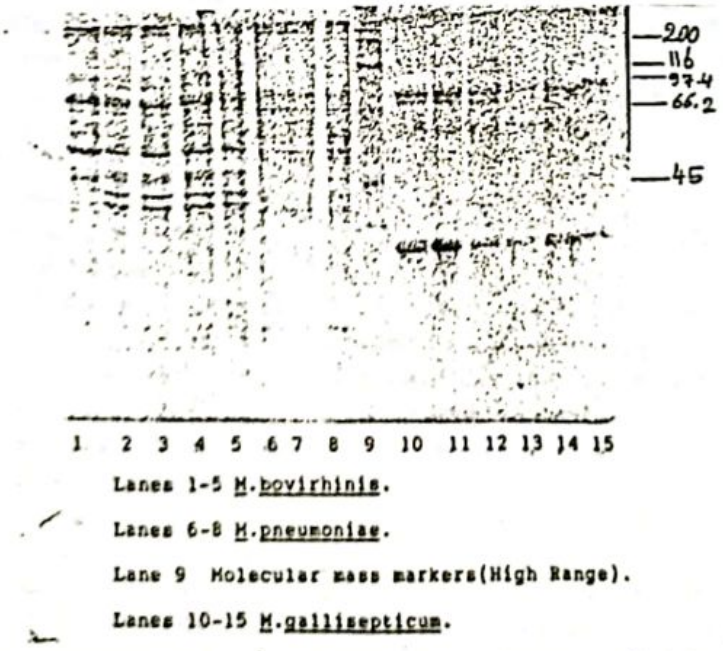
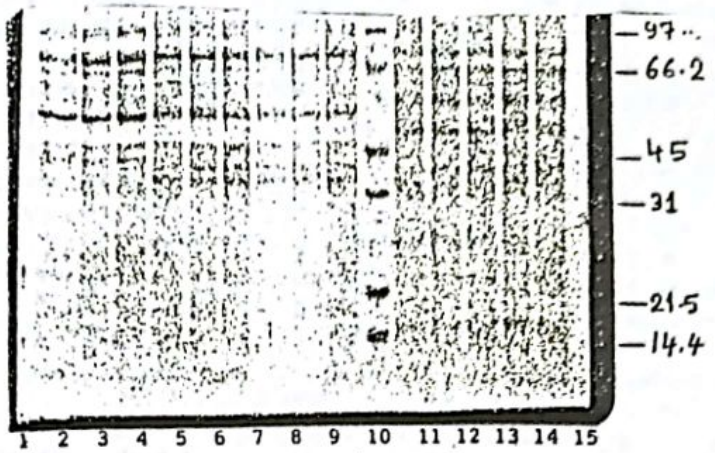


Fig. 1. Electrophoretic analysis of M. gallisepticum, M. pneumoniae, and M. bovirhinis. 10 % polyacrylamide gel, Coomassie stain, 20 Ug protein loaded per track.



Lanes 1-9 M.bovirhinis.

Lane 10 Molecular mass markers (Low Range) in Kilodaltons.

Lanes 11-15 M.pneumoniae.

Fig. 2. Coomassie blue - stained SDA - PAGE protein profiles of M. bovirhinis and M. pneumoniae.

Some differences among the isolates were detected in the molecular weight region of 36-40 KDa.

Comparing the protein patterns of M. bovirhinis and M. pneumoniae, two common bands could be detected (40 and 76 KDa). Mycoplasma gallisepticum have only one common band (76 KDa).

DISCUSSION

Some species of mycoplasma, such as M. gallisepticum and M. pneumoniae possess a differentiated structure Known as the terminal structure. Binding of mycoplasmas to host cells by means of this terminal structure has been well documented (Collier and Clyde, 1971).

The first reported cytadhesin in M. pneumoniae was the 168 KDa protein designated Pl (Hu et al., 1982). Pl was shown to bind to both respiratory epithelial cells and erythrocytes.

In the present study, the electrophoretic patterns of M. gallisepticum isolates showed no differences and have the 64 KDa protein band. Forsyth et al. (1992) isolated a 64 KDa lipoprotein haemagglutinin from M. gallisepticum..

The protein pattern of M. pneumoniae by polyacrylamide gel electrophoresis showed high similarity among the strains. The 170 KDa protein band was detected in the examined strains. Our results are in agreement with that of Geary et al. (1993) who proved that Pl protein (170 KDa) is surface exposed and not shared with any of the other mycoplasmas.

SDS-PAGE protein profiles of M. bovirhinis isolates showed some differences among isolates in the molecular weight region of 36-40 KDa. Comparing the protein patterns of M. gallisepticum. M. pneumoniae, and M. bovirhinis, we found that M. pneumoniae and M. bovirhinis have 2 common bands (40 and76 KDa), while only one common band could be detected in M. gallisepticum (76 KDa).

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