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INCIDENCE AND SDS -PAGE PROTEIN PROFILE ANALYSIS FOR MYCOPLASMA SPP. AND ACHOLEPLASMA SPP. ISOLATED FROM CAMELS IN EGYPT

KAMELIA M. OSMAN .*; MONA I. EL-ENBAAWY * and H.Amer.**

*Department of Microbiology, Faculty of Veterinary Medicine, Cairo University.

**Animal Health Research Institute, Dokki ,Cairo

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SUMMARY

Thirty-nine strains of *Mycoplasma spp.* (33) and *Acholeplasma spp.* (6) were isolated from 118 pneumonic lungs and 110 tracheal samples obtained from different abattoirs in Egypt. The percentages of their isolaion were 8.3 % from pneumonic lungs and 8.7 % from tracheal samples. Based on the biochemical profile and growth inhibition results, the camel isolates were identified as, *M. bovis*, glucose positive mycoplasma and *M. arginini*. The identity of these isolates was further confirmed by growth inhibition test using a panel of specific antisera against selected reference *Mycoplasma spp.* Whole-cell protein patterns generated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) were used to identify and classify field isolates of a chosen *Mycoplasma spp* (19) and *Acholeplasma spp* (6). A high degree of similarity between most of the strains was established with strain-to-strain differences.

INTRODUCTION

Camel numbers in the Middle East were estimated to be in millions. They deserve due consideration in research and development, as they are economically valued animals in their native countries. The literature are not abundant with information about either the Arabian camel (*Camelus dromedarius*) or the two-humped Bactrian camel (*Camelus bactrianus*) or their diseases. Respiratory affections in farm animals is a serious problem which hinders animal productivity and may result in great losses in animal husbandry (Seddek, 2002). Pneumonia in camel is multifactorial (Mahmoud et al., 1988). Several aetiological factors contribute to the occurrence of respiratory affection in camels which includes viruses, bacteria, fungi and parasites. In the present study, focusing was directed to *Mycoplasma species* from respiratory manifested camels. As very little is known about the role played by *Mycoplasma spp.* in diseases of camels in Egypt, the hereby

study was directed towards identification and characterization of *Mycoplasma and Acholeplasma spp.* recovered from the trachea and lungs of respiratory diseased camels with special attention to their protein profile patterns using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

MATERIALS AND METHODS

Samples:

110 trachea and 118 lungs of respiratory diseased camels were collected from different slaughter houses in Egypt. Isolation of *Mycoplasma spp.* was carried out using PPLO media (broth and agar) containing horse serum and 15% yeast extract (1%). The plates were fixed with adhesive tape and incubated at 37°C. These plates were observed microscopically (24x) for growth every two days up to 14 days (Sabry and Ahmed, 1975). For cloning and purification purposes, the broth cultures were filtered through 0.5 um (APD) Millipore filter. The filtrate was streaked on the solid media and incubated at 37°C as mentioned previously. The filtration and plating was repeated twice. The purified cultures were also incubated on nutrient agar as well as PPLO media without serum to know the sterol dependency of the isolates. For the characterization of the isolates, various biochemical parameters were recorded (Erno and Stipkovits,1973 and Sabry and Ahmed, 1975).

Purified isolates were examined by the disc growth inhibition test for serological characterization of mycoplasma isolates (Clyde,1994). Reference strains and antiserum of different *Mycoplasma spp.* were obtained from the National Institute of Health, Bethesda, Maryland 20014 (USA).

Mycoplasma antigen preparation (Thirkeill et al., 1990): Purified mycoplasma cultures were propagated gradually till the final volume of 500 ml broth culture. The propagated mycoplasma broth cultures were centrifuged at 18000 xg for 30 minutes in a cooling centrifuge. The pellets of mycoplasma cells were washed 3 times with physiological saline. The cells were resuspended in 5 ml of saline and kept at -20°C till used.

Protein banding by SDS-PAGE : It was carried out as described by Laemmli (1970). Separating and stacking gels were prepared. Protein pellets were diluted with sample buffer and placed in a water bath (100°C for 5 min), then cooled. Antigen samples as well as the marker (low molecular weight, Pharmacia) were loaded into the wells (20 ul each). The electrophoresis unit (Hoefer, USA) was filled with running buffer, and then the power supply (25mA per gel) was applied. The gel was stained by coomassie blue (R-250) stain, washed and finally destained in the destaining solution for 30-45 minutes 3-4 times. It was then inserted into 5% glycerol solution for 2 hours. Drying of the gel was conducted between 2 cellophane membranes. Quantitation of different mycoplasma an-

tigen fractions were performed using imaging densitometer by molecular analysis soft ware.

RESULTS

Incidence of *Mycoplasma spp.* from camels with respiratory affection: Out of 118 lungs and 110 tracheal samples collected from camels showing respiratory affections, 19 and 20 respectively exhibited observable growth in PPLO broth (complete medium). Based on morphological, cultural and biochemical characteristics, these isolates were divided into 4 groups, each being distinct in its characteristics regarding digitonin inhibition, arginine decarboxylation, glucose fermentation and film and spot formation test. In addition to the serotyping the four groups were; Acholeplasma, *Mycoplasma bovis*, glucose positive mycoplasmas and *Mycoplasma arginini*. The incidence of the four groups from trachea and lungs are illustrated in Table (1).

Table (1):- The incidence of different isolated *Mycoplasma spp.* and *Acholeplasma spp.* from trachea and lungs of the examined respiratory affected camels.

Biotyping&serotyping / Parameter	Acholeplasma	*M.bovis*	Glecose positive	*M. arginin*
Digitonin inhibition	R	S	S	S
Arginin decarboxylation	-	-	-	+
Glucose.fermentation	+	-	+	-
Film& spot reaction test	-	+	+ -	-
Growth inhibition test against				
Acholeplasma	+	-	-	-
M. bovis	-	+	-	-
Glucose positive mycoplasmas	-	-	+	-
M. arginini	-	-	-	+
No of isolates from 110 trachea of camel (20)	4	6	3	7
% (8.7)*	1.754	2.63	1.315	3.07
No of isolates from 118 camel lungs(19)	2	8	4	5
% (8.3)*	0.877	3.50	1.754	2.19

* The %tage was calculated in relation to the total number of samples(228)

The results of SDS-PAGE of the selected field isolates of *M bovis* from trachea & lunge of camels:

It was clear from Fig. (1) that, the whole cell proteins of six *M. bovis* field isolates produced 23-24 protein bands corresponding to molecular weights of 18.675 and 130.45 KDa. Their comparison revealed a high degree of similarity between the isolates. It appears that, with very few exceptions, the relatively same protein bands were present in all strains patterns.

The results of the seven glucose positive mycoplasma indicated that there was a high degree of similarity between strain 1 & 2 in twelve common bands ranged from 18.268 and 114.7 KDa, while strains 3 and 4 also were common in 16 bands (18.879 -114.7 KDa). The remaining strains (5, 6, 7) had 15 common bands between 18.471 and 130.45 KDa.

M arginini showed a number of protein bands between 20 and 22. Field strains of *M. arginini* number 2 and 3 had a relatively high degree of similarity with 12 common bands of molecular weights between 20.674 and 125.58 KDa, while the isolates 4 and 5 showed a high degree of similarity with 12 common bands (19.662 -146.98 KDa). Eleven common bands (24.546 and 153.09 KDa) were detected between *M. arginini* strains number 6 and 7 (Fig. 2).

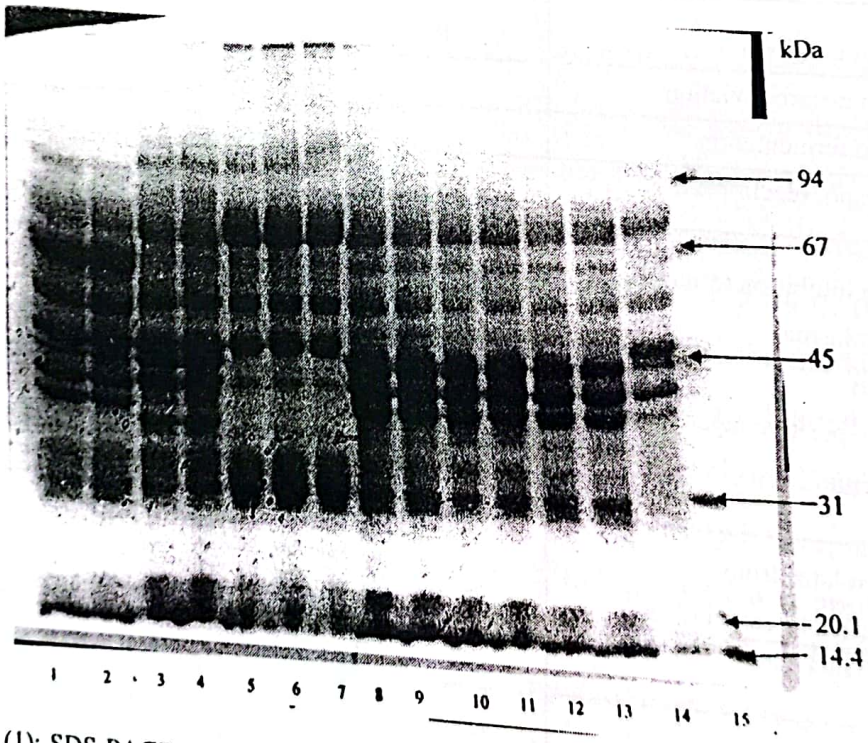

Fig (1): SDS-PAGE protein profiles analysis of Mycoplasma bovis and glucose positive mycoplasma isolated from respiratory affected camels.
Lanes (1, 2), (3,4), (5,6 and 7) : Glucose positive mycoplasmas.
Lanes (8,9), (10,11) and (12, 13) Mycoplasma bovis (field isolates).
Lane 14 Mycoplasma bovis (reference strain).
Lane 15 low molecular weight standard marker.

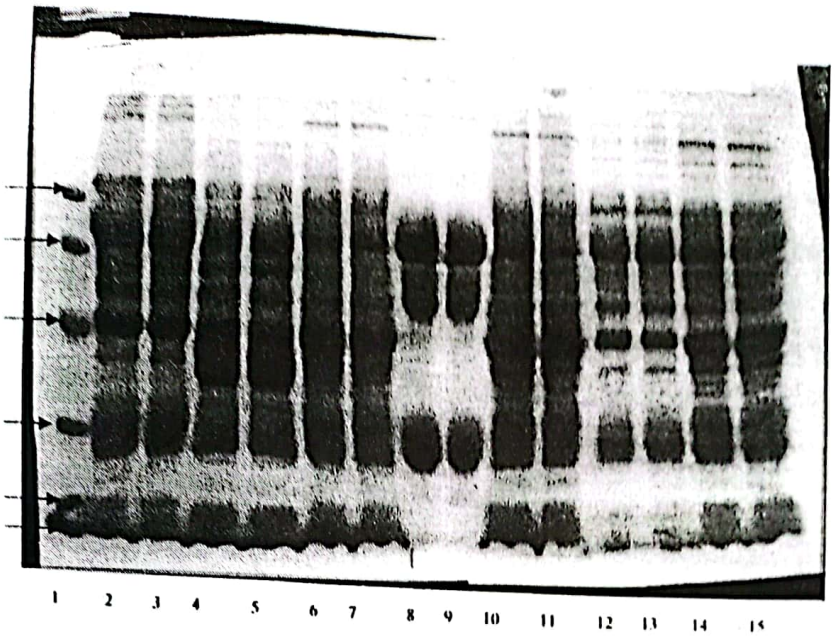

Fig (2): SDS-PAGE electrophoretic patterns of *Mycoplasma arginini* and Acholyplasma species isolated from pneumonic camels.
Lane 1: Low molecular weight standard marker.
Lanes : (2,3), (4,5) and (6,7): *Mycoplasma arginini* (field strains).
Lanes : (8,9), (10,11) and (12,13): Acholeplasma species (field isolates).
Lanes 14 and 15 : *Mycoplasma arginini* (reference strain).

The results of SDS-PAGE separation of whole cell protein of Acholeplasma species field isolates demonstrated that Acholeplasma had a number of bands between 9 to 22. Acholeplasma species strains number 8 and 9 had four common bands between 29.996 and 129.66 KDa and strains number 10 and 11 had four common bands between 33.772 and 141.89 KDa, while strains number 12 and 13 had thirteen common bands between 16.554 and 141.38 KDa (Fig. 2).

DISCUSSION

Mycoplasma spp. are wall-free prokaryotes, found in a variety of domestic animal hosts (Quinn, 2002). Animal mycoplasmas are considered as an infectious agents acquired by immunosuppressed hosts. (Yechouron et al.,1994). The available literature about the incidence of camel (*Camelus dromedarius*) Mycoplasmosis in Egypt is very scarce. In the present study, thirty nine strains of *Mycoplasma spp* (33n) and *Acholeplasma spp* (6n) were isolated from 118 pneumonic lungs and 110 tracheal samples of camel observed at different abattoirs in Egypt. The identity of these isolates were further confirmed by growth inhibition test using a panel of specific antisera against selected reference *Mycoplasma spp.* and *Acholeplasma spp.* Based on the biochemical profile and growth inhibition results, the camel isolates were identified as 14 *M. Bovis*, 5 glucose positive mycoplasmas and 14 *M. arginini.* The percentage of isolations were 8.3 % from pneumonic lungs and 8.7 % from tracheal samples. In

Egypt , relatively similar finding was obtained by Sabry et al (1976) who isolated untyped mycoplasma strains from the respiratory tract of camel. On the other hand, Sabry and Ahmed (1986) serotyped 303 isolates of mycoplasma from dromedary camels. Their results identified *M. bovis* and *Acholeplasma spp*. Also, El-Shabiny et al. (1998) isolated, biotyped and serotyped *Mycoplasma spp* and *Acholeplasma spp*. from the respiratory tract of the one-humped camels. They found that *M.arginini* was the predominant with isolation rate of 8,8%. These results suggested that the role of *M. arginini* in pneumonic camels should be explored in greater detail. *Although Mycoplasma mycoides* subsp. mycoides was detected in pneumonic Nigerian camels using the dot- enzyme- immunoassay and Western blot methods (Egwu and Aliyu,1997) yet, all of the examined samples in the present study were negative for this Mycoplasma spp by culture method.

Reports from other parts of the world (Radwan et al .,1985; Binder et al., 1990; Nicolet, 1994; Egwu et al., 1996 and Thomas et al., 2002) indicated that *M. Bovis* and *M arginini* were associated with other *Mycoplasma species*. They are important pathogenic agents responsible for severe pneumonia in cattle, sheep and goats, but their pathogenicity needs to be established experimentally (Radwan et al., 1985).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is adequate for identification of all strains of mycoplasma (Rasin and Rottem, 1967). Satisfactory classification of unkown mycoplasma isolates is possible by comparing the pattern obtained by SDS-PAGE of the protein of unknown strain with those of known mycoplasma isolates (Dellinger and Jasper, 1972).

The whole cell protein patterns generated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for six *M. bovis* field isolates of respiratory affected camels, produced 23-24 protein bands corresponding to molecular weights of 18.675 and 130.45 KDa. Their comparison revealed a high degree of similarity between the isolates. It appears that, with very few exceptions, the relatively same protein bands were present in all strains patterns (Fig. 1). The same technique was used by Sachse et al. (1992) who compared the whole-cell protein patterns for 34 isolates of *M. bovis*. A high degree of similarity between most of the strains was established . Strain-to-strain differences were mainly confined to quantitative variations of certain protein bands, particularly in the molecular weight regions of 64-68, 55 and 26 KDa. These results concluded that SDS-PAGE confirms some of the characteristic strain features met with whole-cell proteins of *M.bovis*. Brooks et al. (2004) applied the same technique but with the use of whole-cell lysate and proteinase K digest preparations of the *M. bo-*

vis. Coomassie blue staining for protein revealed approximately 50 bands for the lysate but only a single band for the digest. In Egypt, Eissa et al. (1994) and El-Shater et al. (1999) noticed a high degree of similarity among most *M.bovis* strains isolated from cattle and buffaloes.

The result of the whole cell protein patterns of the seven glucose positive mycoplasma from camels, indicated that there is a high degree of similarity between strain 1 & 2 in twelve common bands ranging from 18.268 and 114.7 KDa. While strains 3 and 4 also were common in 16 bands (18.879 KDa -114.7 KDa). The remaining strains (5, 6, 7) had 15 common bands between 18.47 KDa and 130.45 KDa. Fig (1). El-Shater et al. (1999) mentioned that SDS-PAGE and immunoblot can help in the identification of glucose positive mycoplasma isolated from camels. Also they added that glucose positive mycoplasma protein patterns had molecular weights between 22.7 and 96.1 KDa.

M. arginini field strains isolated from the camel, revealed a number of protein bands between 20 and 22. A high degree of similarity between most of the strains was established with 11-12 common bands of molecular weights between 19.662 KDa and153.09 KDa (Fig. 2). El-Shater et al. (1999) found that the molecular weight of the *M. arginini* bands which were isolated from cattle, ranged from 23 to 127 KDa. Three deeply stained protein bands at molecular weights of 30.5, 46.3 and 75.6 KDa were detected. Nearly similar results were obtained by Hussein (2003) who found two deeply stained protein bands of 33 and 40 KDa in sheep isolates while the goat isolates showed about 10 sharp protein bands at the region of 22-42 KDa.

The results of SDS-PAGE separation of whole-cell protein of Acholeplasma species field isolates demonstrated that Acholeplasma spp. had a number of bands between 9 to 22 (Fig. 2). No available data was found related to the protein profile of Acholeplasma spp. as they are considered to be commensal microorganisms with low medical importance (Quinn et al., 2002).

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