Animal Health Research, Dokki, Giza
(Mycoplasma section)
Head of Dept. Prof. Dr. A. Rashwan

COMPARATIVE STUDY BETWEEN REFERENCE AND FIELD STRAINS OF MYCOPLASMA BOVIS AND MYCOPLASMA BOVIGENITALIUM BY SDS-PAGE

(With 2 Fig.)

By

S.I. EISSA; A.M. ZAITOUN*; S.A. EL-SHATER** and N.M. AL-ZEFTAWI

(Received at 30/4/1994)

دراسة مقارنة بين عترات ضابطه وحقليه من ميكوبلازما بوفيس وميكوبلازما بوفيجينتليم باختبار التحليل الكهربائى الرأسى

صبرى عيسى ، احمد زيتون ، سعيد الشاطر
نبيل الزفتاوى

تم اجراء اختبار التحليل الكهربائى الرأسى (SDS – PACE) لاحدى عشر عتره حقليه من ميكوبلازمابوفيس وكذلك ثلاثة عشر عتره حقليه من ميكوبلازما بوفيجنيتليم عزلت من حالات التهاب الضرع – لم تستجب للعلاج – للأبقار والجاموس بمقارنة عترات معمليه ضابطه . ووجد درجة تشابه عاليه بين معظم عترات الميكوبلازمابوفيس . والاختلاف بين عتره وعتره كان متمركزا فى منطقة الوزن الجزئى ١٩ – ٢٧ كيلودالتون . وكان نموذج تحليل بروتين خلايا الميكوبلازما بوفيجنتليم أظهر تشابه كبير بين كل العترات المستخدمه . وبمقارنة صورة تحليل بروتين خلايا الميكوبلازمابوفيس والميكوبلازما بوفيجنتليم تم تحديد ثلاث أحزمه شائعه عنـــد وزن جزئـــى ٤ ر ٩٧ ، ٧٥ ، ٤٠ كيلودالتون .

*: Dept. of Vet. Med., Fac. Vet. Med. Assiut Univ.
**: Institute of serum and Vet. Vaccines, Abbassia, Cairo.

SUMMARY

Eleven strains of *Mycoplasma bovis (M.bovis)* and thirteen strains of *Mycoplasma bovigenitalium (M. bovigenitalium)* were isolated from drug resistant mastiti cases of cows and buffaloes. These strains were examined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAEG) in comparison with reference strains. A high degree of similarity was established among most *M.bovis* strains. Strain-to-strain differences of tested *M.bovis* strains were mainly confined to the molecular weight region 19-27 KDa. *M.bovigenitalium* whole-cell protein patterns showed the same set of major bands in all investigated strains. Comparing the protein profiles of *M.bovis and M.bovigenitalium,* three common bands were detected (molecular weights 97.4, 75 and 40 KDa).

Keyword: M.bovis, M.bovigenitalium.

INTRODUCTION

Mycoplasma mastitis of dairy cows was firstly recorded by *HALE* et al. *(1962)* in Connecticut, U.S.A. Thereafter, several outbreaks of mycoplasma mastitis of dairy cows were reported in different countries *(WEHNERT* et al., *1983; EL-EBEEDY* et al., *1985; EISSA, 1986; GIBB, 1986; AHMED, 1987; Zaitoun, 1990 and JACKSON and BOUGHTON, 1991).* This may refer to spread of the disease. In dairy buffaloes, mycoplasma mastitis was also reported by *PAL* et al. *(1984); ZAITOUN (1990) and ZAITOUN and EISSA (1994).*

Both *M.bovis* and *M.bovigenitalium* were the commonest isolated mycoplasmas from cows and buffaloes suffered from drug resistant mastitis *(EISSA, 1986; AHMED, 1987; ZAITOUN, 1990 and ZAITOUN and EISSA, 1994).* Identification of isolated mycoplasma depends mainly on serological basis *(FREUNDT and EDWARD, 1979)* whereas morphologic characteristic and biochemical reactions are of limited value.

RAZIN and ROTTEM (1967) noticed that the electrophoretic patterns of whole cell proteins in polyacrylamide gel electrophoresis were adequate for identification of mycoplasma. *RECENTLY, SACHSE* et al. *(1992)* compared 34 isolates of *Mycoplasma bovis* by SDS-PAGE, and they found a high degree of similarity among most of the strains.

Consequently, the aim of the present work was to evaluate the use of cell protein electrophoresis (as a rapid test) for characterization of mycoplasmas derived from mastitis in cows and buffaloes in comparison with reference strains.

MATERIAL and METHODS

1-Reference strains:

Mycoplasma bovis (Donetta strain) and *Mycoplasma bovigenitalium* (PG 11 strain) reference strains were obtained from Dr.S. Geary, Dept. of Pathobiology, Univ. of Connecticut, U.S.A. and subjected to SDS-APGE.

2- Field strains:

Eleven isolates of *M.bovis* and thirteen isolates of *M.bovigenitalium* were isolated from incurable mastitic cows and buffaloes *(EISSA, 1986; ZAITOUN and EISSA, 1994).* These isolates were identified serologically by growth precipitation *(KROGSGAARD-JENSEN, 1972)* and growth inhibition tests *(CLYDE, 1964),* and were subjected to SDS-PAGE.

3- Preparation of SDS-PAGE antigens *(THIRKELL et al., 1990):*

Normally, five hundred milliliter broth cultures were grown in PPLO medium *(LEACH, 1967),* incubated at 37°C for 2-3 days. Cells were thereafter harvested in MSE cooling centrifuge (14.000 t.p.m. for 30 min. at 4°C). The pellets (sediment) were washed three times in phosphate buffered saline (PBS, pH 7.2) and the final pellets were resuspended to be 2 mg protein per ml., then stored at -20°C till use.

SDS-PAGE technique:

It was carried out as the method described by *LAEMMLI (1970).* Samples for electrophoresis (20 ug per track on the gel) were boiled for 5 min. after adding an equal volume of a solution containing 0.625 M Tris HCL (pH 6.8), 10% SDS, 50% glycerol, 0.1 bromophenol blue and 3.5M2-Mercaptoethanol.

The samples were subjected to electrophoresis in 10% polyacrylamide gels, with 3% stacking gel using the discontinuous buffer system. Electrophoresis was carried out at 25 m A for 4 hours in a Hoefer SE 400 electrophoresis unit (Hoefer Scientific Instruments, San Francisco, California, USA).

The gels were stained over night in a solution containing 50% methanol, 7% glacial acetic acid and 0.2% coomassie brilliant blue. Destaining occurred in a solution containing 45% methanol and 10% glacial acetic acid. The apparent molecular weights were determined by comparison with protein

standard of known molecular weight (SDS-PAGE Molecular weight standard, Low Range, Bio-Rad Laboratories, California, USA).

RESULTS

SDS-PAGE was carried out to study the protein patterns of the isolated field strains. Fig.1 shows the electrophoretic patterns of eleven identified *M.bovis* (field isolates) and a reference strain (Donetta strain), on 10% polyacrylamide gel with coomassie stain. The majority of proteins were ranged between 37000-75000 molecular weight. A high degree of similarity between most of the strains was established with minor differences confined to the lower portion of the gel (molecular weight region of 19-27 KDa).

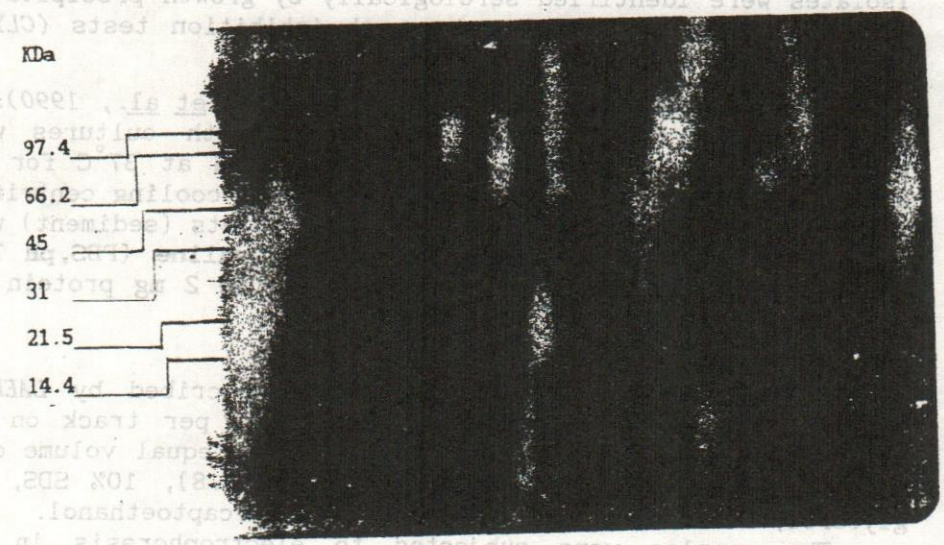

Fig.1: Electrophoretic patterns of proteins from eleven field isolates of *M.bovis* isolated from incurable mastitic cows. 10% polyacrylamide gel, coomassie stain, 20 ug protein loaded per track.
1- Molecular weight standard (Low Range)
2- *M.bovis* (Donetta strain)
3-13 *M.bovis* (field strains)

SDS-PAGE of whole-cell protein extracts of 13 field isolates and the refernce strain (PG 11) of *M.bovigenitalium* in a polyacrylamide gel are shown in Fig.2. The molecular weights of the proteins in the gel are shown in Fig. 2. The molecular weights of the proteins in the gel ranged from 29000 to 75000. All investigated strains showed the same set of major bands.

Fig. 2: Electrophoretic analysis of 13 field isolates of *M.bovigenitalium* isolated from drug resistant mastitic buffaloes. 10% polyacrylamide gel, coomassie stain, 20 ug protein loaded per track.
1- Molecular weight standard (Low Range).
2-14 *M.bovigenitalium* (field isolates).
15- *M.bovigenitalium* (PG 11 strain).

DISCUSSION

A satisfactory classification of unknown mycoplasma isolates was possible by comparing the patterns obtained by polyacrylamide gel electrophoresis of proteins of unknown strains with those of known bovine mycoplasma isolates *(DELLINGER and JASPER, 1972).*

In the present investigation, major electrophoretic lines with cultures of *M.bovis* from bovine mastitis were nearly identical. Such results were similar to those obtained previously by *RAZIN (1968) and SACHSE* et al. *(1992).*

The obtained results of the electrophoretic proteins analysis of 13 isolates of *M.bovigenitalium* isolated from drug resistant mastitic buffaloes and a reference strain (PG 11) showed a very close resemblance. *DELLINGER and JASPER (1972)* recorded that the electrophoretic pattern of *M.bovigentalium* isolated from the reproductive duct of cattle was not quite typical and the culture was found to be mixed.

Comparing the protein profiles of *M.bovis and M.bovigenitalium,* three common protein bands were detected (97.4, 75 and 40 KDa). It is concluded that the same set of major bands was presented in the investigated strains. This is in agreement with the results obtained by *SACHSE* et al. *(1992).*

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