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RAPID DIAGNOSIS OF MYCOPLASMAL MASTITIS

(With One Table)

By

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التشخيص السريع لميكوبلازما التهاب الضرع

ليلى الشعيبيني ، عبدالفتاح جاد ، صبرى عيسى ،
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وجد أن اختبار التحليل الكهربائي هو أكثر الطرق حساسية وسرعة واتقان وذلك في تشخيص حالات ميكوبلازما التهاب الضرع. وقد وجد بطريقة العزل الأولى ل ١٨٠ عينة لبن منها ٦٢ ايجابي بنسبة ٣٤٪ بينما وجد باستخدام اختبار التحليل الكهربي ٦٥ حالة ميكوبلازما بوفس و ٣٧ بو فيجنتاليم. وأثبتت نتائج فحص عينات نسيج الضرع بالزرع ٦ حالات بوفيجنتاليم وهي نفس النتائج التي وجدت باستخدام اختبار التحليل الكهربائي . وبالاضافة الى ذلك تميزت طريقة استخدام التحليل الكهربائي بالسرعة بالمقارنة بطرق الزرع الأولى .

SUMMARY

Counterimmunoelectrophoresis (CIEP) is a reliable specific technique for the diagnosis of mycoplasmal mastitis and more sensitive than other methods. It was found that the isolation procedures of 180 examined milk samples could reveal 62 (34.44%) strains of M.bovis and 34 (18.89%) of M.bovigenitalium, while CIEP technique revealed 65 (36.1%) as M.bovis and 37 (20.5%) as M.bovigenitalium. However the results obtained from the examination of tissue samples by either method were the same. In addition, the CIEP is more rapid as compared to the laborious and time consuming standard cultural method.

INTRODUCTION

Identification of mycoplasmal mastitis could be achieved by the isolation of mycoplasma species. HALE et al. (1962) isolated M.bovis from an outbreak of mastitis. M.bovis was also isolated from mastitic cows by GOURLAY et al. (1974) and RUHNKE and ONOVIRAN (1975).

Mycoplasma bovigenitalium was identified in milk samples from mastitic cows by JURMANOVA et al. (1983).

Rapid identification of mycoplasma species requires the use of sero-identification tests. Recently, the counterimmuno-electrophoresis is used for detection of mycoplasma antigens (CHO and LANGFORD; 1974); serotyping of animal mycoplasmas (EL-SHABINY, 1980) and for diagnosis of bovine mycoplasma (Bois et al., 1984).

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The aim of the present study is to evaluate counterimmuno-electrophoresis for the detection and identification of mycoplasmal mastitis as compared with the conventional methods of isolation, biochemical characterization and serological identification.

MATERIAL and METHODS

A Friesian dairy herd suffering from mastitis at Shobra Shehab was included in this investigation.

Field specimens :

1) Milk samples were obtained from 180 dairy cows.
2) Necropsy specimens were collected (9 udder tissues and 5 lungs).

I- Standard culture methods for isolation of mycoplasma were those adopted by SABRY (1868) by making indirect culture 0.1 ml milk was inoculated into broth and broth to broth passages and subcultures to agar plates were made twice at 48 hours intervals while incubation at 37°C. Daily examination of agar plates was carried out and the final reading was made on the 7th day; samples were accepted as negative after five transfers that did not show growth.

Mycoplasma cultures were maintained in the form of agar blocks in broth (SABRY et al., 1971) after 48 hours incubation at 37°C, then purification of the isolates was applied according to SABRY (1968) which needs at least four days. Digitonin sensitivity test was also applied as described by ERNO and STIPKOVITS (1973) which needed 48 hours to differentiate between mycoplasma and acholeplasma.

Biochemical characterization was screened and it took a duration of 48 hours and for sero-identification growth inhibition test was applied (CLYDE, 1964).

II- Counter-immunoelectrophoresis (CIEP): (BOIS et al., 1984).

Milk samples were prepared by centrifugation at 2.000 xg for 10 minutes, 0.5 ml of skim milk was inoculated in 4.5 ml of broth culture medium, filtered through 0.45 u millipore membrane. A 0.2 ml aliquot of filtered broth was inoculated on agar plate for control and broth were incubated at 37°C for 48 hours then centrifuged at 20.000 xg for 30 minutes, the sediments were used after washing in distilled water. The prepared samples were considered as antigens and were put on the wells made on agarose while the reference antisera were put on the wells for antibodies and the electric current was applied for 45 minutes till the formation of precipitation lines of homologous antigens and antibodies, and all gel slides were examined immediately after the run for the presence of precipitatione lines by indirect lighting.

RESULTS

The results in Table (1) showed that out of 180 examined milk samples 62 (34.44%) were positive and identified as M.bovis and 34 (18.89%) as M. bovigenitalium when submitted to standard cultural method while counter-immunoelectrophoresis (CIEP) revealed 65 (36.1%) positive milk samples identified as M. bovis and 37 (20.5%) as M. bovigenitalium.

It was found that out of 9 udder tissue samples 5 (55.56%) harboured M. bovigenitalium by culture method while 6 (66.67%) samples were shown to be M. bovigenitalium by CIEP technique. Of 5 lung samples 2 (40%) were identified as M. bovigenitalium by standard culture procedure and the same results were obtained by CIEP.

DISCUSSION

Diagnosis of mycoplasmal mastitis caused by M. bovis and M. bovigenitalium in Egypt was previously made by EL-EBEEDY et al. (1985 & 1986) and ESSA (1986).

Since there is no effective treatment for mycoplasmal mastitis, therefore, adequate and rapid diagnosis is considered to be one of the most important factors necessary for enhancing the control of the spread of the disease.

In this investigation, a comparison was made between the isolation method and CIEP technique for the diagnosis of mycoplasmal mastitis.

It was found that isolation procedures identified 34.44% of the milk samples as M. bovis and 18.89% as M. bovigenitalium while CIEP technique identified 36.1% of the milk samples as M. bovis and 20.5% as M. bovigenitalium. As regards the udder tissue and lung samples it was found that 55.56% and 40% of them respectively revealed M. bovigenitalium by the culture method while 66.67% and 40% of them respectively, were identified as M. bovigenitalium by CIEP technique.

From the results of the present study it is clear that not only there is a high correlation between CIEP and isolation but also the CIEP is more sensitive than the cultural method. Another good advantage is the rapid detection nature of CIEP as compared to the laborious and time consuming standard cultural method since the time needed for CIEP is 48 hours for incubation and 45 minutes for the test proper, while the isolation method needs about 2 weeks.

The results of this study is conventional to those reported by BOIS et al. (1984), for evaluation of CIEP for detection of mycoplasmas from milk and necropsy specimens. It is concluded that CIEP may be a tool for detection and identification of bovine mycoplasmas.

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Table (1)

Comparison between CIEP technique and isolation method for diagnosis of mycoplasmal mastitis.

Samples	No. Exam.	Isolation				CIEP			
		M.bovis		M.bovigenitalium		M.bovis		M.bovigenitalium	
		No. positive	%	No. positive	%	No. positive	%	No. positive	%
Milk	180	62	34.44	34	18:89	65	36.1	37	20.5
Udder	9	-	-	5	55.56	-	-	6	66.67
Lung	5	-	-	2	40.00	-	-	2	40.00
Total	194	62	31.9	41	21.1	65	33.5	45	23.19