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ENZYME LINKED IMMUNOSORBENT ASSAY FOR THE DIAGNOSIS OF MYCOPLASMAL MASTITIS IN COWS AND BUFFALOES

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SUMMARY

A total of 80 milk samples randomly selected from milk samples collected from cows and buffaloes ofadairy mastitic herd at Giza Governorate in Egypt, manifesting clinical mastitis. Milk samples were submitted to examination for the presence of Mycoplasma infection by the culture method and ELISA test.

Using the culture method, 40 cows milk samples harboured 10 Mycoplasma species, 7 of them were antigenically related to *M. bovis* and 3 were *M. arginini*, while 40 buffalo milk samples revealed 12 Mycoplasma isolates, 4 of them were *M. bovirhinis* 2 were *M. bovis* and 6 *M. bovigenit* alium.

ELISA test was applied for the detection of Mycoplasma antigen in the milk samples, it was found that 12 cows milk samples were positive for Mycoplasma, 8 were *M. bovis*, 4 were *M. arginini*, while 14 buffalo milk samples were positive for Mycoplasma, 7 of them were *M. bovig*, 3 were *M. bovis* and 4 were *M. bovirhinis*. It was concluded that the number of Mycoplasma positive milk samples was 5 % higher in ELISA method than in cultural method showing that it was more sensitive. Moreover, the same serotypes were detected by ELISA showing that no cross reactions were detected between the Mycoplasma species which emphasizes the specificity of ELISA test. ELISA also was proven to be rapid; decreasing the time of diagnosis from 10 days to 3 hours.

INTRODUCTION

Mycoplasma as a cause of mastitis in cows and buffaloes in Egypt is of considerable economic importance due to reduction of milk production El-Ebeedy *et al* (1985 & 1986). It was previously studied in Egypt by many investigators such as El-Ebeedy *et al.* (1985 & 1986). Eissa (1986). Ahmed and Sabry (1989) and El-Shabiny (1989).

Mycoplasma organisms were isolated from mastitic milk of buffaloes and cows by Pale et al. (1984) and El-Bably (1992).

Since there is no effective treatment for Mycoplasma mastitis, the control depends on isolation and segreghation of infected animals (Jasper, 1982), the speed of identifying infected animals is necessary to reduce the number of new infections and that is why ELISA test is applied to reduce time of diagnosis.

MATERIALS AND METHODS

The target population was 40 randomly selected milk samples from Friezian cows and 40 from dairy buffaloes of clinically mastitic dairy herd at Giza Governorate, Egypt. The milk samples were cultured according to Sabry and Ahmed (1975) using HN media. Genus determination and biochemical characterization were done as described by Erno and Stipkovits (1973) and serotyping was adopted using growth inhibition test according to Clyde (1984).

ELISA test was applied according to Ansari *et al.* (1983) and Boothby *et al.* (1986). Bovine Mycoplasma antigens, *M. bovis*, M. bovigenitalium, *M. bovirhinis* and *M. arginini* were previously prepared as described by Bois *et al.* (1984). 200 ul of a suspension of these antigens in PBS RPH 7.2 in 3 mg protein per ml was used for coating the 96-well microtitration plate (Linbro). The plates were left overnight at 4°C or 2 hours at 37°C. The

plates were washed 3 times in PBs. 100 ul of the milk samples or the milk incubated at 37°C for 48 hours with the Mycoplasma broth were put, the plates were incubated that 37°C on a horizontal shaker, washed 3 times then 200 ul of 1/100 goat antirabbit IgG peroxidase conjugate were dispensed then the plates were incubated 1 hour at 37°C, washed 3 times then 100 ul of the substrate ABTS (2,2 - azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) was added to each well), then the plates were incubated till the formation of green colour in the positive control well, the stopping solution of 50 ul of 0.45 % HCI was used and the plates were agitated 1-2 minutes on horizontal shaker. The positive samples were detected by green colour compared with the -ve control. The optical density was read at 405 nm wave length.

The following reference Mycoplasma type culture and antisera were obtained from Diagnostic Lab., Cornell University, Ithaca, New York:
M. Bovis 201
M. arginini ATCC 23938
M. alkalescens ACC 29103
M. bovirhinis ATCC 27748
M. Bovigenitalium ATT 19852
M. laidlawii ATCC 23206
ELISA test was adopted on homologous and heterologous cultures and antisera to test specificity.

RESULTS

From the result of Table (1). it is clear that the number of positive milk samples was higher by ELISA test; 14 out or 40 examined buffalo milk samples and 12 out of 40 cows milk samples, while using the culture method; 12 out of 40 buffalo milk samples were positive for Mycoplasma and 10 out of 40 cows milk samples wee positive. The types of Mycoplasma detected by both methods were the same. No cross reactions were found between the different species of Mycoplasma.

DISCUSSION

Several methods were previously used for the diagnosis of mycoplasmal mastitis such as the culture method (El-Ebeedy *et al.*, 1986; Ahmed, 1987), counter immunoelectrophoresis (Bois *et al.*, 1984; El-Shabiny, 1989 and El-Shabiny *et al*, 1989), immunofluorescent antibody test (Bass and Jasper, 1972).

In the present study, ELISA was used for the diagnosis of Mycoplasma mastitis, it was found to be accurate and rapid by Boothby *et al.* (1986). The present study proves the test also to be less time consuming; 3 hours compared with 10 days in culture method. It was also concluded that ELISA is more sensitive than the culture method showing higher number of Mycoplasma positive samples (35 %, 30 %) and (30 %, 25 %) in buffalo and cows milk respectively. Besides, ELISA is as specific as specific as the culture method showing

Table (1): Comparison between culture and ELISA methods for the diagnosis of Mycoplasma mastitis in buffalo and cows.

	Oulture method					ELISA method				
	No.+ve samples	Type of Mycoplasma				No.+ve samples	Type of Mycoplasma			
		M. arg.	M. bovis	M. bovis	M. bovis		M. arg.	M. bovis	M. bovis	M. bovis
Buffalo milk	12/40 30 %	0	2	6	4	12/40 35 %	0	3	7	4
Cows milk	10/40 25%	3	7	0	0	12/40 30 %	4	8	0	0

* There is a 5 % in crease in Mycoplasma positives in ELISA compared with the culture method.

no cross reactions between the different Mycoplasma species. So, it could lead to mass screening for bovine milk in a manner useful in maintaining surveillance program for Mycoplasma inmaintaining surveillance program infection. This study also confirms the presence of Mycoplasma as a cause of mastitis in buffaloes in Egypt.

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