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RAPID DIAGNOSIS OF MYCOPLASMA INFECTION IN BUFFALOES USING IMMUNOBINDING ASSAY

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SUMMARY

Twenty six nasal and oropharyngeal swabs were collected from fatty buffalo calves in a herd at Ismailia Governorate suffering from pneumoenteritis with no response to antibiotic treatment and 40 milk samples collected from bufalo dairy herd suffering from mastitis at Beni suef Governorate, Egypt. The samples were submitted to laboratory diagnosis for the detectionof mycoplasma infection.

Using the culture method, 13 oropharyngeal and nasal swabs were positive, 4 were antigenically related to *Mycoplasma bovirhinis,* 2 were *M. bovigenitalium*, 2 were *A. modicum* and 5 were *A. laidlawii.*

Regarding the milk samples, 10 were positive; 2 were *M. bovirhinis,*5 were *M. bovigenitalium* and 3 were *M. bovis.*

Examination of these samples with immunobinding assay (IBA) gave the same number of positive samples and the same species of mycoplasma. The IBA proved to be as sensitive and as specific as the culture. method. Moreover, IBA is more simple and have the advantage of utility and rapidity, 2 hours are needed to complete diagnosis instead of 10 days in case of the culture method.

INTRODUCTION

The economic importance of buffalo as a source of milk and meat in Egypt led to the careful inyestigation made by many authors such as Sabry et. al., (1972), Ahmed (1974), El-Shabiny et. al., (1992), they isolated mycoplasma from pneumonic lungs, the genitalia and nostrils and oropharynx, respectively.

The aim of this study is to apply a sensitive immunochemical technique, i. e. immunobinding assay (IBA) to reach an accurate and rapid diagnosis of mycoplasma infection in buffaloes.

MATERIALS AND METHODS

Animals:

26 buffalo calves in a herd at Ismailia Governorate suffering from pneumoenteritis and 40 buffaloses in a mastitic dairy herd at Beni Suef Governorate suffering mastitis represented by swollen firm udder with rapid onset of the disease and loss of milk.

Samples:

26 nasal and oropharyngeal swabs and 40 milk samples were collected for diagnosis of mycoplasma.

Methods:

1- The culture method: The culture media and culture procedure wer as described by Sabry and Ahmed (1975), genus determination and biochemical characterization were as recommended by Erno and Stipkovits (1973). Serological identification was according to Clyde (1964) using GI test.

2- Immunobinding assay: According to Poumarat et al., (1991) and Martinez et. al., (1990). The assay was performed with 96-well flat bottom plates in which we put nitrocellulose disc size 0.45 prewashed with tris buffered saline (TBS) pH 7.4 containing Tween 20, then with TBS only,

then fluids were removed and broth cultures or milk samples to be identified were pipetted in 500 ul aliquots per well. After drying, a blocking solutions consisted of 0.05%. Tween 20, 1% gelatin in PBS then in TBS put in 200 ml aliquots/well, left for 20 minutes after which fluids were removed, rabbit hyperimmune serum diluted in blocking solution 1-20 wer dispensed in 200 ul aliquots/well then washed 3 times in TBS with tween 20 and once with TBS only for 2 minutes per each wash, 200 ul/well peroxidase conjugated antibody to rabbit diluted 1/1000 were dispensed, after 30 minutes incubation with slow agitation on a horizontal shaker. The fluieds were removed with a Pasteur pipette attached to a vacuum pump. The wells were washed 4 times as before for 2 minutes per each wash. The developing solution consisting of 2 ml ice cold methanol with 60 ml 4-chloronaphthol solution and 100 ml TBS with 60 ul 3% hydrogen peroxide mixed before use was added in 200 ul aliquots per well. A purple colour appearing within 2 minutes indicating positive reaction compared with a positive and negative control.

The following mycoplasma cultures and antisera were obtained from Diagnostic Lab. Cornell Univ., U. S. A.: *M. bovis* 201, *M. arginini* ATCC 23838, *M. alkalescens* ATCC 29103, *M. bovirhinis* ATCC 27748, *M. bovigenitalium* ATCC 19825, *A. laidlawii* ATCC 23206.

RESULTS

From the results of Table (1) it is clear that out of 26 nasal and oropharyngeal swabs, 13 harboured mycoplasma using the culture and immunobinding methods, 4 were *M. bovirhinis,* 2 were *M. bovigenitalium,* 2 were *A. modicum* and 5 were A. laidlawii. Out of 40 milk samples examined by both tests, 10 were positive for mycoplasma; 2 were antigenically related to *M. bovirhinis,* 5 were *M. bovigenitalium* and 3 were *M. bovis*

DISCUSSION

Many investigators allover the world tried several mathods for the diagnosis of mycoplasma infection in buffaloes, e. g. the culture method was performed by Pale et. al., (1984) and Zaitoun et. al., (1991) for the detection of mycoplasma infection in milk and immunperoxidase test was performed by El-Shabiny et. al., (1992) for the detction of mycoplasma infection in buffalo sera, nasal and oropharyngeal swabs. present study proved that IBA is as sensitive and specific as the culture method. It does not require expensive equipment and rapid enough to reduce the time of diagnesis from 10 days in case of the culture method to 2 hours. So, it could be extremely useful in implementing effective strategies for

Table (1): Detection of mycoplasma by culture and immunobinding methods.

Sample	No. examined	No. +ve	Mycoplasma species				
			M. bovirhinis	M. vobig.	M. bovis	A. modicum	A. laidlawii
Oropharyngeal and nasal	26	13	4	2	--	2	5
Milk	40	10	2	5	3	--	--

controlling mycoplasma infection in buffaloes including mastitis.

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