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THE APPLICATION OF IMMUNOPEROXIDASE TEST FOR THE DIAGNOSIS OF MYCOPLASMA INFECTION IN BUFFALOES

BY

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INTRODUCTION

Buffaloes are of great economic importance in Egypt due to provision of good yield of milk, high quality veal and beef. Mycoplasmas have been isolated from buffaloes in Egypt by several authors such as Sabry et al. (1972), Alzeftawi (1973) Ahmed (1974), Alaam and Sabry (1978) and Zaitoun et al. (1991),. The investigated the possible role of mycoplasma in causing diseases in buffaloes. They isolated mycoplasmas from pneumonic lung and trachea, oropharynx and nasal cavities and found them to be almost in association with respiratory diseases.

Mycoplasmas were also isolated from the genitalia of buffaloes suffering from reproductive disorders and also from mastitic buffaloes.

The aim of this study was the rapid and accurate diagnosis of mycoplasma infection in bufflaoes using imuno peroxidase test for detecting the antibodies in the sera of these animals.

MATERIALS AND METHODS

Animals: 26 buffaloe calves in a herd ate Ismailia Governorate.

Samples: 10 oropharyngeal swabs, 16 nasal swabs and 26 serum smaples.

Diagnosis:

1. **Isolation mehod**:

a) The culture media and culture procedure were used as described by **Sabry and Ahmed (1975)**.

b) Genus determination was done using digitonin test according to **Erno and Stipkovits (1973)**.

c) Biochemical characterization: glucose fermentaion test, arginine deamination test, totrazolium reduction test and film and spot test were used. The media and method were recommended by Erno and **Stipkovits (1973)**.

d) Serological identification using growth inhibition test (GI) as described by **(Clyde, 1964)**.

Immuno peroxidase test:

According to Polak-Vogelzang and Hagenaars (1976) as follows:

Agar block of 48 hours mycoplasma cultures were put on slides 4 X 6. All slides were placed in 0.5% H_2O_2 in methanol for 30 min. to destroy peroxidase present in the cells. After 5-10 min, in phosphate buffered saline (PBS), pH 7.6 with shaking, the Petri dishes containing the slides were put on a horizontal shaker, then the sera were placed for 30 min. Slides were washed in two changes of buffer from 10-15 min. then the agar blocks were treated with diluted peroxidase conjugated antibody to rabbit IgG in 0.5% bovine serum albumin in saline with 0.01% tween 80 for 30 min.

Slides were washed in three changes of buffer for 10-15 min. then agar blocks were treated with 0.05% (1-2 drops) 3,3-diaminobenzidine tetrahydrochloride and 0.01% H_2O_2 in tris buffer pH 7.6 for 5 min. to stain the peroxidase, then were washed in tap water. The colonies were examined under a lowpower microscope.

The positive samples were indicated when the colonies were stained. deep brown.

N.B.: Mycoplasma type cultures antisera and conjugated peroxidase were obtained from Research products Miles laboratories Lted, Slough, England.

RESULTS

From the results recorded in table (1) and on the bais of biochemical testing of the recovered cultures it is clear that 4 oropharyngeal isolates were recovered from a total of 10 swabs and identified as M. bovirhinis. From 16 nasal swabs 2 were M. bovigenitalium, 2 were A. modicum and 5 were A. laidlawii i.e. A. Laidlawii represented the majority of isolates.

Table 2 shows that application of immunoperoxidase test on the isolates fro the diagnosis revealed 4 M. bovirhinis, 2 M. bovigenitalium, 2 A. modicum and 5 A. laidlawii.

DISCUSSION

In the present study M. bovirhinis was isolated agreed with **Allam and Sabry (1978).**

M. bovigenitalium, A. modicum and A. Laidlawii were isolated from the nasal cavity as was previously found by **Davis 1967 and Gourlay et al. (1970) and Jurmanova and Krejci (1971).**

Mycoplasma bovigenitalium may cause reproductive disorders and arthritis as was reported by **Ahmed and Sabry (1982).**

Mycoplasma bovirhinis and A. modicum may be accused in the causation of pneumonia and pneu-

Table(1): Biochemical characterization and digitonin testing of Mycoplasma isolates.

Type of isolates	No. of isolates	Glucose	Arginin	Tetrazolium	Film & Spot	Digitonin
M.bovirhinis	4	+	-	-	-	+
M.bovigenitalium	2	-	-	+	+	+
A.modicum	2	+	-	+	-	-
A.laidlawii	5	+	-	+	-	-

Table(2): Immunoperoxidase reaction to Mycoplasma.

	No.Exam.	Mycoplasma species			
		M.bovr.	M.bovig.	A.modic.	A.Laidl.
Isolates from oropharynx	4	4	-	-	-
Isolate from nasal swab	9	-	2	2	5
	13				

moentritis as it was isolated from calves suffering pneumoentritis and this was also thought by Dawson et al. (1966).

Biochemical methods always prove to be efficient, rapid and accurate in the diagnosis of animal Mycoplasmas. This was investigated by El-Shabiny et al. (1989) and (El-Shabiny, 1989) by application of counter immunoelectrophoresis (CIEP) for rapid diagnosis of animal mycoplasms especially those causing mastitis.

In the present study the role of Immunochemistry is once more ascertained in the field of Mycoplasma study by the application of immunoperoxidase test for the diagnosis of Mycoplasma infection in fatty calves.

This test proved to be sensitive, rapid and accurate as compared with the conventional isolation method e.g. the immunoperoxidase test is not time consuming it needs 2 hours after preparation of 48 hours culture agar blocks while the isolation methods needs at least 12 days.

Immunoperoxidase test was also applied by Polak-Vogelzang and Hagenaars (1976) for the diagno-

sis of animal mycoplasma in sera and by Hill (1978) for the diagnosis of Mycoplasma directly from the tissue.

SUMMARY

Twenty six nasal and oropharyngeal swabs and 26 serum samples were collected from fatty buffaloe calves in a herd at Ismaillia Governorate suffering from pneumoenenitis with no response to antibiotic treatment. They were submitted to laboratory diagnosis using:

1. Isolation method including different subcultures on Mycoplasma culture media and according to certain culture procedure then genus determination was made, biochemical characterization was applied and serological identification was performed using growth inhibition test (GIT)

2. Immunoperoxidase test method was applied. Using the isolation method, it was found that out of 26 nasal and oropharyngeal swabs 13 Mycoplasma species were isolated, 4 were antigenically related to M. bovirhinis, 2 were M. bovigenitalium, 2 were A. modicum and 5 were A. laidlawii.

Immunoperoxidase test was applied on the isolated mycoplasmas agar blocks, and gave the same results as the identification using GIT.

It was concluded that immunoperoxidase test is rapid, it needs 2 hours after the preparation of 48 hours culture's agar block, while the isolation method is time consuming, it needs 12 days at least. The immunoperoxidase test is also more sensitive and accurate. It is able to detect the antibodies in every Mycoplasma positive cases while in case of the isoaltion method some Mycoplasma having fastidious growth or due to some inhibitory facors are missed in the diagnosis.

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