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Research Paper

Mycoplasma AS A MAJOR PATHOGEN OF RESPIRATORY DISEASE IN CATTLE

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Abstract

This study was carried out on 180 samples including nasal, buccal and ocular swabs from living apparently healthy and diseased cattle, and also 180 samples from lung, trachea and bronchial lymph nodes from slaughtered cattle. After culturing and serotyping identification of isolates obtained from living diseased cattle showed that *M.bovis* was the most prevalent *Mycoplasma* spp. in an incidence of 13.8% followed by *M. arginine* in an incidence of 9.2% then *M.bovirhinis* in an incidence of 3.7,4.6% from nasal and buccal swabs .Also the culturing identification and serotyping of isolates obtained from slaughtered cattle *M.bovis* was the most prevalent *Mycoplasma* spp. in an incidence of 16.9%, from pneumonic lung and 4.6% for both trachea and lymph node samples while *M. arginine* was 9.2%, then *M.bovirhinis* in an incidence of 6.15% from pneumonic lung samples.

INTRODUCTION

Mycoplasma represent the smallest self-replicating life forms on earth and phylogenetically are related to gram positive eubacteria. They are lacking a cell wall and intracytoplasmic membranes, have only one type of membrane, the plasma membrane (Razin, 1985).

The role of Mollicutes and specifically *Mycoplasma bovis* (MB) as an active agent in cases of bovine respiratory disease (BRD) is poorly defined. Multiple *Mycoplasma* species have been isolated from apparently healthy calves (Thomas A, *et al*. 2002: Wiggins MC, *et al*.2007) and the status of the organism as a primary or secondary pathogen is unclear. The economic impact of mycoplasmosis, however, has been estimated to be significant in the beef industry due to weight loss and health consequences (Nicholas RA, Ayling RD. 2003).

The most commonly encountered pathogenic *Mycoplasma* in cattle that causes significant commercial losses in the cattle industry is due to weight loss and health consequences is *Mycoplasma bovis* (*M. bovis)* (Francoz *et al*. 2005). Several studies have shown that *M. bovis* is the most common bacterium identified in feedlot cattle

affected by chronic pneumonia and in veal calves with fatal bronchopneumonia (Gerchman *et al*. 2009).*M. bovis* is also present in the respiratory tract of healthy cattle where it causes a subclinical upper respiratory infection and may provoke mastitis and metritis in cows and arthritis or tenosynovitis in fattening cattle (Gagea *et al*. 2006).
Mycoplasma firstly reported in Egypt by El-Ebeedy *et al*. (1985), spread of *Mycoplasma* infection was throughout the Egyptian farms and become endemic in some areas. Various types of *Mycoplasma* were isolated from dairy Friesian cows and buffaloes with mastitis. These *Mycoplasma* included *M.bovis, M bovigenitalium, M.dispar, M.bovirhinis and M. arginini.* Our study aimed for determine Mycoplasma occurrence in cattle suffering from respiratory disease.

MATERIAL AND METHODS

Samples

360 different samples including nasal, buccal, ocular swabs from living apparently healthy and diseased cattle . Lung, trachea and bronchial lymph node samples, from freshly slaughtered cattle.

Isolation and Identification

The culture medium , culture procedure was as Sabry and Ahmed (1975) ,purification and maintainace of the isolates were made according to Sabry and Ahmed (1975),genus determination (differentiation between *Mycoplasma* and *Acholeplasma spp*.) was carried out by Digitonin test as described by Freundt *et al*. (1973) in which the agar plate was inoculated with 0.2ml of the test culture using runing drop technique. A disc impregnated in 1.5% ethanol was pressed in the middle of inoculated area.the plate was incubated at 37^0C to be examined for the presence of inhibition which indicate positive test (*Mycoplasma*) while *Acholeplasma* are resistant to digitonin.Biochemical characterization was carried out according to Erno and Stipkovits (1973) as followsGlucose fermentation test: 2.9 ml of glucose medium was inoculated with 0.1 ml of the suspected *Mycoplasma* culture, incubated aerobically at 37^0C and examined daily for seven days. A yellow color indicate a positive test,Arginine hydrolysis test: 2.9 ml of arginine medium was inoculated with 0.1 ml of the suspected *Mycoplasma* culture, incubated aerobically at 37^0C and examined daily for seven days. A red color indicates a positive test,Film and spot formation test: 0.2 ml of *Mycoplasma* broth culture was cultivated on heart infusion agar plate which was incubated at 37^0C. After 3 days , a glistening layer appeared due to lipase , indicates a positive test.

Serotyping of Mycoplasma isolates:

was applied using growth inhibition test as described by Clyde (1964) using a disc saturated with anti serum that was placed in the middle of suspected agar culture. The presence of inhibition zone indicates a positive result.

RESULTS

From the results recorded in Table 1 it shows that the total number of *Mycoplasma* isolates obtained from both apparently healthy and diseased ,living and slaughtered animals in an incidence of 29.3% from apparently healthy and 40.9% from diseased cattle. *Mycoplasm*a were isolated in nasal swabs of diseased animals in an incidence of 62.5% followed by buccal and ocular swab in an incidence of 60% as showed in table (2). *Mycoplasma* isolates were recovered in an incidence of 62% from the examined diseased lung samples and bronchial lymph node while the higher incidence from

trachea in an incidence of 75% as showed in table (3). Result of serotyping showed in table 5 showed that *M.bovis* was the predominant isolated speices in an incidence of 41.5% (27 out of65) ,followed by *M. arginini* in an incidence of 32.3%(21 out of 65) while *M. bovirhinis* were 15.3% and 6.15% for *M. bovoculi* and *1.5%* for *M. bovigenitaliumM.bovis* was the predominant isolated species from lung tissue in an incidence of 16.9%,followed by nasal swabs 13.8%,m.arginine while *M.arginine isolation was the same for nasal swabs and lung tissue(9.2%)* for *M.bovirhinis* was isolated from lung samples in an incednce of 6.1%.4M. bovoculi were isolated 3from ocular swabs in an incidence of 4.6%, and one from buccal swabs

DISCUSSION

Respiratory disease in cattle is a complex syndrome and its etiology involves many different factors including stress factors, environmental, viral , bacterial and Mycoplasmal infections. Respiratory diseases occurring due to *Mycoplasma* is regarded as the most frequent and serious cause of morbidity, mortality and economic losses. So it is very essential to study the *Mycoplasma spp*. causing respiratory disease in diseased animals as well as from apparently healthy cattle.

In the present investigation regarding the primary isolation from apparently healthy and diseased cattle a total of (44 out of 150) examined samples from apparently healthy cattle with an incidence of 29.3% and (86 out of 210) 40.9% from slaughtered .this result agree with those obtained by Eissa *et al* (2007) who found that the incidence of *Mycoplasma* from private farm was 31.58%.

Applying digitonin test for genus determination and differentiation between *Mycoplasma* and Acheloplasma we found that *Mycoplasma* was isolated from both apparently healthy and diseased cattle in an incidence of (49% from living and 51.4% from slaughtered) this result nearly agree with those obtained by Elzahaby R.A.(2010)that isolate *Mycoplasma* from cattle in an incidence of 66.25%.

Biochemical and serotyping of the isolates revealed different types of *Mycoplasma* spp. Including *M.bovis* 41.5% , M.arginine 32.3%, *M.bovirhinis* 15.3%.several epidemiological studies Hashem (2008), Elzahaby R.(2010) indicated that M.bovis was the highly isolated *Mycoplasma* spp. From cattle in an incidence of 59.7%, 29.7%respectivelyIn the present study , *M.bovis* was the predominate isolated *Mycoplasma* speices 41.5%, this results agree with El-Shafey D. (2005), Elzahaby R.(2010) ,who identified *M. bovis* with highest incidence 29.24%*M.bovis* was the predominate isolated *Mycoplasma* speices isolated from lung of diseased cattle (16.9%)followed by nasal swabs (13.8%) while *M.arginine* isolation was the same for nasal swabs and lung tissue(9.2%). For *M.bovirhinis* 4 of which was isolated from lung samplesin an incidence of 6.15

M.arginine and *M.bovirhinis* were isolated from both apparently healthy and diseased animals ,this agree with (El Shabiny *et al* 1999) who mentioned that *M.bovirhinis* has its normal habitat in the respiratory tract , and with Mosherf (1997) who could isolate *M.bovirhinis* and *M.arginine* from apparently normal calves lung Our obtained biochemical results was similar to the results of biochemical characterization that were recorded by several authors as, Hashem (2008) and Elzahaby(2010) that divided *Mycoplasma* into 3 biochemical groups based on their biochemical characterization and found that group(3) that indicate the possibility of being *M. bovis* or *M. bovigenitalium* was 42.45%.The results of serological identification agree with El-Shafey (2000) who identified *M. bovis* with highest incidence followed by *M. arginini and* Mohamed (2002), Hashem (2008) who found that *M. bovis* was the highly isolated *Mycoplasma spp*.from

Egyptian cattle. Also the result agree with Tenk *et al* (2004)who found that *M. bovis* was isolated from 59.9% of pneumonic lung samples and 25.2%from lung with normal appearance ,and Eissa *et al* (1998)who isolated *M. bovirhinis* from cattle in an incidence of 14.13%.

Table (1): Incidence of Positive Isolates From Diseased and Apparently Healthy Living and Slaughtered Primary Isolation

Animal status	Apparently healthy	diseased	total
Total No .of the examined samples	150	210	360
No of positive	44	86	130
incidence	29.3	40.9	3601

Table (2) recovery rate of *Mycoplasma* from apparently healthy and diseased living cattle.

Sites	Apparently healthy		diseased		total	
	N=*Mycoplasma* isolates	%	N= *Mycoplasma* isolates	%	N= *Mycoplasma* isolates	%
Nasal swabs	2	16.6	15	62.5	17	47.2
Buccal swabs	2	40	6	60	8	53
Ocular	1	25-	4	66	5	50
Total	5	23.8	25	62.5	30	49

Table (3) Recovery rate of *Mycoplasma* from apparently healthy and diseased slaughtered cattle

sites	Apparently healthy		diseased		Total	
	N=*Mycoplasma* isolates	%	N= *Mycoplasma* isolates	%	N=*Mycoplasma* isolates	%
Lung	3	27.2	18	62	21	52.5
Trachea	2	33.3	6	75	8	57
Bronchial lymph node	1	16.7	5	62.5	6	42.8
Total	6	26	29	64	35	51.4

Table (4): Serotyping of *Mycoplasma* isolates

Biochemical group	No. of isolates	Corresponding antisera	Results	
			positive	%
Group1	14	*M. bovirhinis*	10	15.3
		M. bovoculi	4	6.15
Group2	23	*M. arginini*	21	32.3
		untyped	2	
Group3	28	*M. bovis*	27	41.5
		M. bovigenitalium	1	1.5
Total	65			

Table (5): distribution of *Mycoplasma* isolated from cattle

Animal Status	Type of sample	N= sample examined	N= *Mycoplasma* isolated		*Mycoplasma* serotype									
			N=	%	*M. bovis*		*M. arginini*		*M. bovirhinis*		*M. bovoculi*		*M. bovigenitalium*	
					N=	%	N=	%	N=	%	N=	%	no	%
Living	Nasal swab	101	17	4.72	9	13.8	6	9.2	2	3.7	-	0	-	0
	Buccal swab	43	8	2.2	1	1.5	3	4.6	3	4.6	1	1.5	-	0
	Ocular swab	36	5	1.38	-	0	1	1.5	-	0	3	4.6	-	0
Slaughtered	Lung samples	100	21	5.83	11	16.9	6	9.2	4	6.1	-	0	1	1.5
	trachea	40	8	2.2	3	4.6	4	6.15	1	1.5	-	0	-	0
	Bronchial LN	40	6	1.67	3	4.6	1	1.5	-	0	-	0	-	0
Total		360	65	18	27	41.5	21	32.3	10	15.3	4	6.15	1	1.5

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