MYCOPLASMA ISOLATED FROM CATTEL LUNGS AND THEIR PATHOGENICITY STUDY

LAILA, M.EL-SHABINY[1], MANAL, M.ABOU EL-MAKAREM[1] AND H.S. NADA[2]

1Animal Health Research Institute, Agricultural Research, Centre, Giza, Egypt.
2 Faculty of Veterinary Medicine Kafr El-Sheikh, Tanta University.

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Abstract

A total of 170 lung samples, 85 of which from Balady cattle and 85 from Friesian cattle, revealed 30 mycoplasma isolates (17.6%). The Balady lung samples revealed 19 mycoplasma isolates, 5 of which were from normal lungs, typed as 2 *M.bovirhinis* and 3 were *M.arginini*, while 14 mycoplasma isolated pneumonic lungs were typed as 5 *M.bovis*, 3 *M.bovigenitalium*, 3 *M.bovirhinis* and 3 *M.arginini*.

The Friesian lung samples revealed 11 mycoplasma isolates, 3 of which were isolated from apparently normal lungs, typed as 1 *M.bovirhinis* and 2 were *M.arginini*, while 8 mycopasma isolated from pneumonic lungs were typed as 4 *M.bovis*, 2 *M.bovigenitalium*, 1 *M.bovirhinis* and 1 *M.arginini*.

Antibiogram was made for the isolated mycoplasma species against 17 antibiotics. They were highly sensitive to Trospectomycin sulphate, Enrofloxacin, Norfloxacin, Tiamulin and Ciprofloxacin.

Pathogenicity study was made by injecting the isolated mycoplasma strains intraperitoneally into mice. The test resulted in congestion of the lungs injected with *M.bovis* and *M.bovigenitalium* after one week of infection, and mycoplasmas could be reisolated from the lungs.

INTRODUCTION

Respiratory diseases, especially, pneumonia represent a serious economic important problem affecting calves due to morbidity and mortality (Sood *et al.*, 1986).

Mycoplasma is one of the causative agents of pneumonia in calves and in adult cattle and buffaloes.

So far, *M.mycoides* was known to be the cause of pneumonia in bovine

The culture medium was heart infusion broth and agar, and the culture procedure was as described by Sabry and Ahmed (1975) as follows:

0.5 g of lung tissue was aseptically placed into a sterile mortar, cut by sterile scissors and ground using sterile sand. Five ml of broth were added, then, from the mixture, direct plating was made, and 0.2 ml were transferred into broth which was incubated at 37°C for three days. Then, 0.2 ml broth were dispensed into 1 ml broth and plating was made. This step was repeated 3 times. The agar plates were incubated at 37°C under reduced oxygen tension in humidified candle jars. The plates were examined after 48 hours under a stereomicroscope for the presence of Mycoplasma colonies having typical fried egg appearance.

Purification and maintenance of the isolates

These were made according to Sabry and Ahmed (1975). Genus determination was carried out as described by Freundt *et al.* (1973) using digitonin sensitivity test in which the agar plate was inoculated with 0.2 ml of the test culture using the running drop technique. A disc impregnated in 1.5% ethanol was pressed in the middle of the inoculated area. The plate was incubated at 37°C to be examined for the presence of inhibition zone which indicated a positive test.

Biochemical characterization was adopted according to Erno and Stipkovits (1973) as follows.

Glucose fermentation test: 2.9 ml of glucose medium was inoculated with 0.1 ml of the suspected mycoplasma culture, incubated aerobically at 37°C and examined daily for seven days. A yellow colour indicates a positive test.

Arginine deamination test: 2.9 ml of arginine medium was inoculated with 0.1 ml of the suspected mycoplasma culture, and incubated aerobically at 37°C for 7 days. A red colour indicates a positive test.

Tetrazolium reduction test: 2.9 ml of tetrazolium medium was inoculated with 0.1 ml of the suspected mycoplasma culture and incubated aerobically at 37°C for 7 days. A pink colour 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°C. After 3 days, a glistening layer appeared owing to the presence of lipase, indicating a positive test.

Serotyping of the mycoplasma isolate : Was applied using, (1) growth inhibition test as described by Clyde (1964) using a disc saturated with antiserum

that was placed in the middle of suspected agar culture. The presence of inhibition zone indicates a positive result, (2) Growth precipitation test according to Krogsgaard-Jensen (1972). The reference antisera were kindly supplied by Dr. Shin Diagnostic Lab. Cornell University, Ithaca. NY. USA.

Antibiogram : was performed according to Clyde (1964) using 17 different antibiotics, applying growth inhibition test as follows:

Each of the isolated mycoplasmas was cultivated on agar plate using single drop technique, then, a disc saturated with the standard concentration of antibiotic was pressed in the middle of the mycoplasma culture. The plates were placed in the candle jar and incubated at 37°C. The plates were examined daily under stereomicroscope. The inhibition zone was measured to indicate the degree of sensitivity to the used antibiotic.

Pathogenicity test : was made according to Singh and Prasad (1994) as follows:

Ten albino mice, 1 month old, proved to be free from mycoplasma by culture method were divided into 5 groups, each of 2 mice. Each group was inoculated intraperitoneally with one of the isolated mycoplasma species, *M.bovis*, *M.bovigenitalium*, *M.bovirhinis* and *M.arginini*, and group 5 was considered as a control group. All the 4 mycoplasma isolates $1x10^9$ C.F.U were grown in heart infusion agar at 37°C for 4 days under reduced oxygen tension. One typical colony of each isolate was picked up and grown in 10 ml heart infusion broth and incubated at 37°C for 48 h in a lighted candle jar. A mixture was prepared using 1 ml of inoculum and 4 ml of 5% sterile mucin. 0.5 ml of this mixture was used for intraperitoneal inoculation in mice.

One mouse of the control group was inoculated with 0.5 sterile heart infusion broth, and the second was inoculated with 0..5 ml of 1:4 mixture (v/v) of broth and 5% mucin.

The inoculated mice were kept under observation for 6 days, then, sacrificed and mycoplasma was reisolated from lungs and confirmed.

RESULTS

Mycoplasma isolation and serotyping

From the results recorded in Table 1, it is clear that, 5 mycoplasmas (2.9%) could be isolated from 5 Balady cattle normal lung samples, 2 of which were

antigenically related to *M.bovirhinis* (6.6%), and 3 were *M.arginini* (10%). On other hand, 14 mycoplasmas could be isolated from pneumonic lungs (8.2%), 5 of which typed as *M.bovis* (16.6%), 3 were *M.bovigenitalium* (10%), 3 were *M.bovirhinis* (10%), and 3 were typed as *M.arginini* (10%).

It was also found that, 3 mycoplasmas (1.7%) were isolated from 55 normal Friesian cattle lung samples, one of which was typed as *M.bovirhinis* (3.3%), and 2 were *M.arginini* (6.6%), while, 8 mycoplasmas (4.7%) were recovered out of 30 pneumonic lungs, 4 of which were typed as *M.bovis* (13.3%), 2 were *M.bovigenitalium* (6.6%), 1 was *M.bovirhinis* and 1 was *M.arginini* (3.3%).

Table 1. Mycoplasma isolated from cattle lungs.

animal species	Type of sample	No.of sample examd.	No.of Mycoplasma isolated		Mycoplasma serotypes							
					M.bovis		M.bovigen-italium		bovirhinis		M. arginini	
			No.	%	No.	%	No.	%	No	%	No.	%
Balady cattle	Normal lungs	50	5	2.9	-	0	-	0	2	6.6	3	10
	Pneumonic lungs	35	14	8.2	5	16.6	3	10	3	10	3	10
Friesian cattle	Normal lungs	55	3	1.7	-	0	-	0	1	3.3	2	6.6
	Pneumonic lungs	30	8	4.7	4	13.3	2	6.6	1	3.3	1	3.3
Total		170	30	17.6	9	30	5	16.6	7	23.3	9	30

Percentages were calculated from the total Mycoplasma isolated.

Antibiogram

The results of antibiogram recorded in Table 2 showed that the isolated mycoplasmas were highly sensitive to Enrofloxacin, Trospectomycin sulphate, Tiamulin, Norfloxacin and Ciprofloxacin. Oxytetracy clin and Gentamycin were of intermediate activity, while, the isolated Mycoplasmas were resistant to Amoxycillin and Erythromycin.

Pathogenicity test

The results of pathogenicity test showed that the lungs of mice inoculated with *M.bovis* and *M.bovigenitalium* were congested compared with the lungs of mice infected with *M.bovirhinis* and *M.arginini*. Moreover, *M.bovis* and *M.bovigenitalium* could be reisolated from the lungs infected with these mycoplasmas.

DISCUSSION

From the data recorded in Table 1, it could be concluded that the rate of mycoplasma recovery was higher in pneumonic cattle lungs compared with apparently normal ones. It was also noticed that *M.arginini* and *M.bovirhinis* were the mycoplasma species that could be isolated from both Balady and Friesian cattle apparently normal lungs. This agreed with (Gourlay *et al.*, 1970) who mentioned that *M.bovirhinis* has its natural habitat in the respiratory tract, and with Mosherf (1997) who could isolate *M.bovirhinis* and *M.arginini* from apparently normal calve lungs.

In the present study, *M.bovis* was the predominating mycoplasma species isolated from pneumonic calf lungs (30%), followed by *M.bovigenitalium* (16.6%). Pfutzner *et al.* (1980), Shimizu *et al.* (1981) and Deverse *et al.* (1988) diagnosed calf pneumonia associated with *M.bovis*. Moreover, Liberal (1989) detected mycoplasmosis carriers in bovine herds by the isolation of *M.bovis* from calves nasal exudate, while, Bois *et al.* (1993) and Doherty *et al.* (1994) could isolate *M.bovis* from pneumonic lungs of calves.

The present study, also, coincides with that of Srivastava and Uppal (1985) who isolated *M.bovigenitalium* from lungs of calves showing signs of respiratory infection, and also, with Laak *et al.* (1992) who could isolate *M.bovigenitalium* from lungs of pneumonic calves.

In the present study, *M.arginini* was isolated from pneumonic Friesian cattle lungs at the rate of (3.3%). *M.arginini* could also be isolated from pneumonic lungs of calves by Muenster Matsuoka (1979).

The results of the antibiogram showed that the isolated mycoplasmas were highly sensittive to Trospectomycin sulphate, Enrofloxacin, Norfloxacin and Tiamulin. Our results agreed with those of Yancey and Klein (1988) who found that Trospectomycin sulphate was active in vitro against a variety of human and veterinary pathogens.

The present study, also, coincides with Devriese and Haesebrouck (1991) who found that *M.bovis* was sensitive to Oxytetracycline, Tylosin, Lincomycin, Gentamycin and Enrofloxacin. In addition, our results agreed with Allan and Pirie (1981) who stated, in vitro, the activity of Tiamulin against bovine respiratory tract mycoplasmas.

Regarding the pathogenicity study, it was found that the lungs of mice after

one week infection with *M.bovis* and *M.bovigenitalium* were congested, and the injected *Mycoplasmas* could be reisolated from the lungs, while, *M.arginini* and *M.bovirhinis* could not be reisolated from mice infected with these mycoplasmas. Singh and Prasad (1994) could not, also, reisolate *M.arginini* from infected mice.

Pathogenicity study was also previously applied by Tomas and Howard (1974) to Know the effect of different mycoplasmas including *M.bovirhinis* on explant cultures of bovine trachea which proved cytopathic effect represented by sloughing and flatness of the epithelial layer.

From the results of pathogenicity study of the present investigation, it could be concluded that *M.bovis* and *M.bovigenitalium* were more pathogenic to the lungs than *M.bovirhinis* and *M.arginini*. It is known that *M.bovirhinis* and *M.arginini* have their natural habitat in the lungs, and when the animal becomes understress or with the aid of other pathogenic organisms, they become able to produce the disease.

Table 2. Antibiogram for Mycoplasma isolated from cattle lungs.

Antibiotic	Antibiotic concentration	M. bovis	M. bovig enitalium	M. bovirhinis	M. arginini
Amoxycillin	10 per g	△ $3mm^{xxx}$	$2mm^{xxx}$	$2mm^{xxx}$	$2mm^{xxx}$
Erythromycin	15 per g	4 m ^{xxx}	$3mm^{xxx}$	$2mm^{xxx}$	$2mm^{xxx}$
Oxytetracycline	30 per g	17^{xx}	18^{xx}	17^{xx}	17^{xx}
Tylosin	15 per g	19^{x}	20^{x}	20^{x}	20^{x}
Lincomycin	2 per g	20^{x}	20^{x}	21^{x}	21^{x}
Gentamycin	10 per g	17^{x}	18^{x}	18^{x}	18^{xx}
Enrofloxacin	10 per g	21^{x}	22^{x}	23^{x}	23^{x}
Ciprofloxacin	20 per g	21^{x}	22^{x}	23^{x}	23^{x}
Kanamycin	30 per g	20^{x}	21^{x}	21^{x}	21^{x}
Spiramycin	30 per g	20^{x}	21^{x}	21^{x}	21^{x}
Neomycin	30 per g	19^{x}	20^{x}	20^{x}	20^{x}
Vibramycin	30 per g	20^{x}	20^{x}	23^{x}	20^{x}
Trospectomycin	20 per g	23^{x}	23^{x}	21^{x}	23^{x}
Danofloxacin	30 per g	21^{x}	21^{x}	22^{x}	22^{x}
Tiamulin	30 per g	22^{x}	22^{x}	23^{x}	23^{x}
Norfloxacin	20 per g	22^{x}	22^{x}	22^{x}	23^{x}
Clindamycin	10 per g	16^{xx}	17^{xx}	17^{xx}	18^{xx}

△Zone of inhibition xxx resistant xx intermediate sensitivity x sensitive

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الميكوبلازما المعزولة من رئات الأبقار ودراسة تأثيرها المرضى

ليلى مصطفى الشعبينى[1]، منال محمد أبو المكارم[1]، هشام سعد ندا[2]

١ معهد بحوث صحة الحيوان – مركز البحوث الزراعيه – الدقى – جيزة – مصر .
٢ كلية الطب البيطري – كفر الشيخ – جامعة طنطا.

أجرى البحث على ١٧٠ عينة من رئات الابقار منها ٨٥ عينة من الابقار البلدى و ٨٥ عينة من الابقار الفريزيان. وقد أسفرت النتائج عن ٣٠ عترة ميكوبلازما (١٧,٦٪) وقد تم عزل ١٩ عترة ميكوبلازما من رئات الابقار البلدى كانت ٥ عترات منها من الرئات الطبيعية (السليمة ظاهريا) صنف اثنان ميكوبلازما بوڤيرينس، ٣ ميكوبلازما ارجنينى، بينما تم عزل ١٤ عترة ميكوبلازما من الرئات التى بها التهابات رئوية صنفت ٥ عترات منها ميكوبلازما بوفيس، ٣ ميكوبلازما بوفيجنتاليم، ٣ ميكوبلازما بوفيرينس و ٣ ميكوبلازما ارجنينى.

واسفر فحص عينات رئات الابقار الفريزيان عن ١١ عترة ميكوبلازما، عزل منها ٣ عترات من الرئات السليمة ظاهريا وصنفت واحدة ميكوبلازما بوفيرينس و ٢ ميكوبلازما ارجنينى. وتم عزل ٨ عترات ميكوبلازما من الرئات التى بها التهابات رئوية صنفت منها ٤ عترات ميكوبلازما بوفيس، ٢ ميكوبلازما بوفيجنتاليم، ١ ميكوبلازما بوفيرينس و ١ ميكوبلازما ارجنينى.

وتم عمل اختبار الحساسية للمضات الحيوية لعترات الميكوبلازما المعزولة باستعمال ١٧ نوع مضاد حيوى. وكانت عترات الميكوبلازما شديدة الحساسية لسلفات التروسيكتوميسن، الانروفلوكساسين، النورفلوكساسين، تيامولين والسيبروفلوكساسين.

وقد اجريت دراسة التأثير المرضى لعترات الميكوبلازما المعزولة بحقن هذه العترات فى الغشاء البريتونى للجرذان و نتج عن ذلك التهابات رئوية بالنسبة للجرذان التى تم حقنها بالميكوبلازما بوفيس والميكوبلازما بوفيجنتاليم بعد اسبوع من العدوى وتم اعادة عزل الميكوبلازما بوفيس والميكوبلازما بوفيجنتاليم.