Advanced Study on Mycoplasmas Isolated From Sheep and Goats

Ammar, A.M.*; Eissa, S.I. **; Marwa, I. Abd El-Hamid*; Hanaa, A.Ahmed. ** and Abd El-Aziz, E. E. **

*Bacteriology, Mycology and Immunology Department, Fac. of Vet. Med., Zagazig University.
**Mycoplasma Research Department, Animal Health Research Institute, Dokki, Giza.

ABSTRACT

Sheep and goats are important farm animals in Egypt. In the present study, 172 samples (131 samples from sheep and 41 samples from goats) were obtained from animals from different areas in Sharkia governorate and El-Basatin abattoir in Cairo. Mycoplasma isolation revealed that *M. arginini* was isolated from sheep and goats as 21.11 % and 35.29 % respectively, *M. ovipneumoniae* was 37.78 % and 17.65 % and *M. agalactiae* was 41.11 % and 47.06 %. Only 2 samples were identified as *Acholeplasma* species. Polymerase chain reaction (PCR) confirmed the isolation of *M. agalactiae* by the presence of specific band at 360 bp and *M. ovipneumoniae* by the presence of specific amplicone at 1070 bp. Tilmicosin was found to be the antibiotic of choice by using broth indicator microdilution technique with MIC range of (0.0098 – 0.078 µg / ml). Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) revealed the presence of differences in the protein make up of mycoplasma isolates.

INTRODUCTION

Class mollicutes is distinguished from other prokaryotes by their total lack of cell wall. This feature explains many of the unique properties of mycoplasma as; their typical morphology and elasticity; their sensitivity to lysis by osmotic shock, alcohols, organic solvents, detergents, antibody and complement; their filterability through 0.45 µm pore diameter filters; their fried-egg colony shape on agar and their total resistance to penicillin and other antibacterial substances which degrade or inhibit peptidoglycan synthesis *(1)*. Mycoplasmal diseases of small ruminants are spread world-wide and constitute an important socio-economical problem, particularly in regions where small ruminants represent a substantial source of milk and meat provision for the population *(2)*. The most important mycoplasma infections affecting sheep and goats are contagious caprine pleuropneumonia (CCPP) and contagious agalactiae syndrome (CAS) *(3)*. *Mycoplasma arginini* was involved as a causative agent of mild pulmonary changes in sheep and goats *(4 - 6)*.

This study was aimed to isolate and identify mycoplasma from sheep and goats and also to assess the value of using the recent techniques in confirming the identification of mycoplasma isolated from sheep and goats with the classical methods and determine the most effective antimicrobials.

MATERIAL AND METHODS

1- Sampling

A total of 131 samples were collected from sheep (86 samples from apparently healthy sheep and 45 from diseased sheep), while 41 samples were collected from goats (25 samples from apparently healthy goats and 16 from diseased goats). These samples were obtained from different areas in Sharkia governorate and El-Basatin abattoir in Cairo in the form of Nasal, conjunctival and ear swabs and tracheal, lung and lymph nodes tissue.

2- Isolation of mycoplasma

The samples were propagated on PPLO broth and agar media *(7)* and Digitonin sensitivity was done *(8)*.

3-Biochemical identification and serological typing

Was carried out by glucose fermentation, arginine deamination and film and spot formation tests *(9)* and serological typing was done by growth inhibition test *(10, 11)* and growth precipitation test *(12)*.

4- Polymerase Chain Reaction

A-DNA extraction *(13)*

Five ml of 24 hours broth cultures of isolates were centrifuged for 10 minutes at 12000 r.p.m. The pellet was washed twice in 100 µl of PBS (pH 7.2) and suspended in 50 µl PBS. The cell suspension was heated directly at 100°C for 10 minutes in a heat block and then cooled on refrigerator for 10 minutes. Finally, the cell suspension was centrifuged for 5 minutes and the supernatant containing chromosomal DNA was collected and stored at -20°C until used.

B-Primer selection

B .1. *M. agalactiae* using the 16S rRNA gene *(13)*

5'- CCT TTT AGA TTG GGA TAG CGG ATG-3'.

5'- CCG TCA AGG TAG CGT CAT TTC CTA C-3',

B.2. *M. ovipneumoniae* using the 16rDNA gene *(14).*

5'- AAC AGC GGC TAA TAC CAG ATA C-3'.

5'- AGA CTT CAA TCC GAA CTG AGA C -3'.

C- DNA amplification *(13, 14)*

PCR amplification for *M. agalactiae* was performed in 50 µl reaction mixture consisting of 5 µl of 50 ng *M. agalactiae* genomic DNA, 5 µl of 10 x *Taq* buffer (10mM tris- HCl [pH 8.8], 50 mM KCl), 1 µl of 50 pmol of each primer, 1.5 mM $MgCl_2$, 0.5 µl (2U) of *Taq* thermostable DNA polymerase, 1 µl of 10 mM of d NTPs mixture and 35µl of DNase-RNase- free, deionized water. DNA amplification was performed by applying the following program, initial denaturation at 94 °C for 5 minutes then 30 or 35 cycles, consisting of denaturation at 94 °C for 45 seconds, primer annealing at 60°C for 1 minutes and extension at 72°C for 2 minutes, followed by a final extension step at 72°C for 3 minutes.

While PCR amplification for *M. ovipneumoniae* was performed in 50 µl reaction mixture consisting of 5 µl of *M. ovipneumoniae* genomic DNA, 5 µl of 10 x *Taq* buffer, 3 µl of 30 pmol of each primer, 1.5 µl of 25 mM $MgCl_2$, 0.5 µl (2U) of *Taq* thermostable DNA polymerase, 1 µl of 10 mM dNTPs mixture and 31µl of DNase- RNase-free, deionized water and DNA amplification was performed by applying the following program in the PROGENE thermal cycler, initial denaturation at 94 °C for 5 minutes then 35 cycles, consisting of denaturation at 94 °C for 1 minutes, primer annealing at 55°C for 1 minutes and extension at 72°C for 1.5 minutes, followed by a final extension step at 72°C for 10 minutes.

Following amplification, 5 µl of each amplicon was mixed with sample buffer and applied on agarose gel 1% (w/v) containing 0.5 µg of ethidium bromide. The samples were electrophoresed at 100 volts for 90 min on a horizontal electrophoresis unit. A 100 bp DNA ladder was used as a molecular weight standard (Pharmacia). After electrophoresis, the gel was visualized and photographed. Image analysis was made by Image QuantTL-V2003.03 (Amersham Biosciences).

5-*In vitro* antimicrobial susceptibility of mycoplasma field strains isolated from sheep and goats by broth indicator microdilution (MIC) technique

Used to determine the MIC values *(15)* of eight antibiotics (tilmicosin, tylosin, tiamulin, spiramycin, tulathromycin, doxycycline, erythromycin and florfenicol) against 24 mycoplasma strains.

6-Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis *(16)*

Propagated mycoplasma broth cultures were centrifuged at 12000 r.p.m. for 30 minutes in cooling centrifuge (4°C) for making a pellet of mycoplasma cells. This pellet was washed two or three times in phosphate buffer saline (pH 7.2). The suspension was kept at -20°C until used. Estimation of the protein concentration was determined *(17).* Samples were boiled for 5 min in sample buffer (50 mM Tris-HCl [pH 6.8], 10% SDS, 50% glycerol, 2% β-mercaptoethanol, 0.1% bromophenol blue) and proteins separated on 10% acrylamide gels by adjusting the current to provide 1.5 mA per gel for 4-5 hours in a Hoefer SE 400 electrophoresis unit (Hoefer Scientific Instrument, San Francisco, California, USA). Gels were stained with Coomassie brilliant blue. SDS-PAGE

Molecular Weight standard, Low range (Pharmacia) was used as marker. Image analysis was made by Image QuantTL-V2003.03 (Amersham Biosciences).

RESULTS

1- Incidence of positive samples

The incidence of positive samples from apparently healthy animals was 61.26 % (68 positive samples out of 111 samples), while that from diseased animals was 67.21% (41 positive samples out of 61 samples).

2- Primary isolation of mycoplasmas from sheep and goats

Is shown in Tables 1, 2.

3- Digitonin test for differentiation between *Mycoplasma* and *Acholeplasma* genera

Revealed that all the isolates from both sheep and goats were digitonin sensitive so they belonged to genus *Mycoplasma* except 2 samples from nasal swabs of apparently healthy sheep were acholeplasma.

4- Biochemical and serological identification of the obtained mycoplasma isolates

Is shown in Table 3.

5- PCR for mycoplasma isolates

Revealed that *M. ovipneumoniae* isolates recovered from sheep and goats gave a a characteristic band at 1070 bp which was specific for the used 16rDNA gene as shown in Fig. 1, while *M. agalactiae* isolates gave a specific band at 360 bp using the 16S rRNA gene as shown in Fig. 2.

6-Antibiotic sensitivity of mycoplasma isolates using broth indicator microdilution MIC technique

Revealed that tilmicosin was the most effective antibiotic against all the tested field isolates of mycoplasma which isolated from sheep and goats with MIC range of (0.0098 – 0.078 µg / ml) as shown in Table 4.

7-SDS – PAGE of mycoplasma isolated from sheep and goats

A- SDS – PAGE of *M. arginini*

Revealed that field isolates of *M. arginini* from sheep gave 17-23 bands ranged from 359.41 – 16.01 kDa and that of goat gave 5-6 bands ranged from 200.16 – 22.14 kDa (Fig. 3). All sheep field isolates shared common antigenic bands at 63.07, 22.30, 17.77, 17.07 and 16.01 kDa, while goat field isolates shared common antigenic bands at 161.30, 67.05, 28.59 and 22.14 kDa. It was found that sheep and goat field isolates of *M. arginini* differed in many protein bands, but they shared four common antigenic bands at 200, 161, 96 and 22 kDa.

B- SDS – PAGE of *M. ovipneumoniae*

Revealed that field isolates of *M. ovipneumoniae* from sheep gave 20- 27 bands ranged from 343.24 – 15.25 kDa and that of goat gave 23-26 bands ranged from 362.14 – 15.61 kDa (Fig. 4). All sheep field isolates shared common antigenic bands at 39.87 and 19.34 kDa, while goat field isolates shared common antigenic bands at 66.03, 50.28, 41.12, 22.37, 19.16, 16.02 and 15.61 kDa.

C- SDS – PAGE of *M. agalactiae*

Revealed that *M. agalactiae* reference strain gave 39 protein bands ranged from 144.1-12.81 kDa, while field isolates of *M. agalactiae* from sheep gave 36- 45 bands ranged from 144.44 - 10.59 kDa and that of goat gave 34 - 40 bands ranged from 143.01-10.55 kDa (Fig. 5). Sheep field isolates of *M. agalactiae* shared with the reference strain in the protein profile from 99.01-33.23 kDa and from 29.72 -12.47 kDa Goat isolates of *M. agalactiae* nearly resembled the reference strain but lacked the protein band at 58.02 kDa and characterized by the presence of extra protein bands at 119.31, 84.80, 54.17, 37.99, 35.47, 31.16, 23.50, 16.58, 15.92, 14.95, 12.17, 11.69 and 10.55 kDa. The electrophoretic pattern of *M. agalactiae* field isolates from sheep and goat revealed that the presence of specific protein bands at 40, 48, 55 and 80 kDa was characteristic for *M. agalactiae*.

Table 1. Total recovery rate of mycoplasmas from apparently healthy and diseased sheep.

samples	Apparently Healthy			Diseased			Total		
	No. of exam. samples	No. of pos. samples	%	No. of exam. samples	No. of pos. samples	%	No. of exam. samples	No. of pos. samples	%
Nasal swabs	28	10	35.71	16	14	87.50	44	24	54.55
Conjunctival swabs	5	2	40	19	19	100	24	21	87.50
Ear swabs	22	22	100	-	-	-	22	22	100
Tracheal samples	5	5	100	-	-	-	5	5	100
Lymph node samples	2	-	-	3	-	-	5	-	-
Lung samples	24	16	36.36	7	4	23.53	31	20	32.79
Total	86	55	63.95	45	37	82.22	131	92	70.23

Table 2. Total recovery rate of mycoplasmas from apparently healthy and diseased goats.

samples	Apparently Healthy			Diseased			Total		
	No. of exam. samples	No. of pos. samples	%	No. of exam. samples	No. of pos. samples	%	No. of exam. samples	No. of pos. samples	%
Nasal swabs	9	4	44.44	12	2	16.67	21	6	28.57
Conjunctival swabs	2	1	50	-	-	-	2	1	50
Ear swabs	3	2	66.67	-	-	-	3	2	66.67
Tracheal samples	3	2	66.67	-	-	-	3	2	66.67
Lymph node samples	4	-	-	2	1	50	6	1	16.67
Lung samples	4	4	100	2	1	50	6	5	83.33
Total	25	13	52	16	4	25	41	17	41.46

Table 3. Biochemical and serological identification of the obtained mycoplasma isolates.

Animal	Sheep									Goats								
Bio. Identif.	Group1			Group2			Group3			Group1			Group2			Group3		
	G -	A +	F&S -	G +	A -	F&S -	G -	A -	F&S +	G -	A +	F&S -	G +	A -	F&S -	G -	A -	F&S +
Sero. Identif.	*M. arginini*			*M. ovipneumoniae*			*M. agalactiae*			*M. arginini*			*M. ovipneumoniae*			*M. agalactiae*		
Nasal swabs	8 (36.36%)			12 (54.55%)			2 (9.09%)			4 (66.67%)			1 (16.67%)			1 (16.67%)		
Conj. swabs	1 (4.76%)			11 (52.38%)			9 (42.86%)			-			1 (100%)			-		
Ear swabs	4 (18.18%)			5 (22.73%)			13 (59.09%)			-			-			2 (100%)		
Tracheal samples	2 (40%)			-			3 (60%)			-			-			2 (100%)		
Lung samples	4 (20%)			6 (30%)			10 (50%)			1 (100%)			1 (20%)			3 (60%)		
L.N samples	-			-			-			1 (20%)			-			-		
Total	19 (21.11%)			34 (37.78%)			37 (41.11%)			6 (35.29%)			3 (17.65%)			8 (47.06%)		

Bio. Biochemical **Sero.** Serological **Identif.** Identification
G Glucose fermentation **A** Arginine deamination **F&s** Film &spot formation
Conj. Conjunctival ***M.*** *Mycoplasma* **+** Positive
- Negative **L.N.** Lymph node

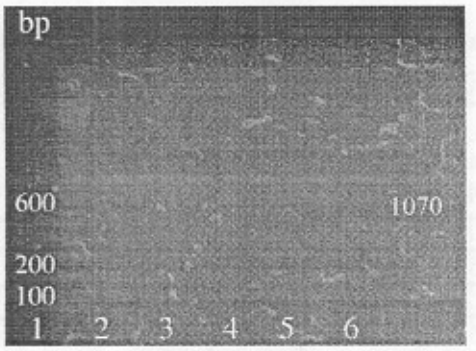

Fig. 1. Electrophoretic pattern of the PCR products of *M. ovipneumoniae* DNA isolated from sheep and goats using the 16rDNA gene.

Lane 1:- 100 bp DNA Ladder (Pharmacia) Lane 2:-*M. ovipneumoniae* sheep field isolate (1)
Lane 3:- *M. ovipneumoniae* sheep field isolate (2) Lane 4:- *M. ovipneumoniae* goat field isolate (3)
Lane 5:- *M. ovipneumoniae* sheep field isolate (4) Lane 6:- *M. ovipneumoniae* goat field isolate (5)

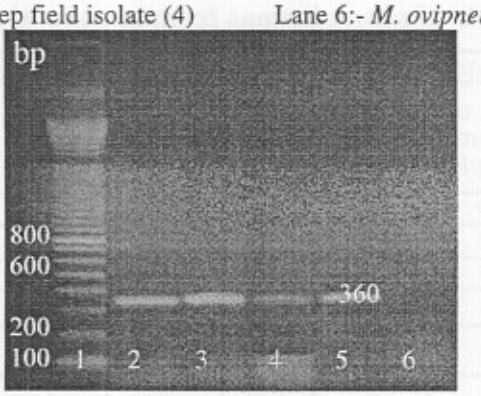

Fig. 2. Electrophoretic pattern of the PCR products of *Mycoplasma agalactiae* DNA isolated from sheep and goats using the 16S rRNA gene.

Lane 1:- 100 bp DNA Ladder (Pharmacia) Lane 2:-*M.agalactiae* reference strain
Lane 3:- *M.agalactiae* goat field (1) Lane 4:- *M.agalactiae* sheep field (2)
Lane 5:- *M.agalactiae* sheep field (3) Lane 6:- Control negative

Table 4. Ranges of MIC values of the eight antibiotics against all the tested field isolates of mycoplasma isolated from sheep and goats.

Antimicrobial drug	MIC. ranges for *M. arginini*	MIC. ranges for *M. ovipneumoniae*	MIC. ranges for *M. agalactiae*	Ranges of MIC.valuefor all tested field isolates
Doxycycline	1.25 – 5*	0.625 - 10	1.25 – 2.5	0.156 – 10
Tulathromycin	0.078 – 0.312	0.078 – 0.156	0.156 – 0.625	0.078 – 0.625
Erythromycin	0.625 -2.5	20	0.312 – 1.25	0.312 – 20
Florfenicol	1.25 - 5	2.5 - 5	1.25 – 2.5	1.25 – 5
Spiramycin	0.039 – 0.156	0.078 – 0.312	0.312 – 0.625	0.039 – 0.625
Tilmicosin	0.039 – 0.078	0.0098 – 0.0195	0.0098 – 0.0195	0.0098 –0.078
Tiamulin	0.078 – 0.156	0.078 – 0.156	0.039 – 0.078	0.156 –0.039
Tylosin	0.0195 – 0.039	0.078 – 0.156	0.156 – 0.625	0.625–0.0195

***All MIC values represented by µg / ml**

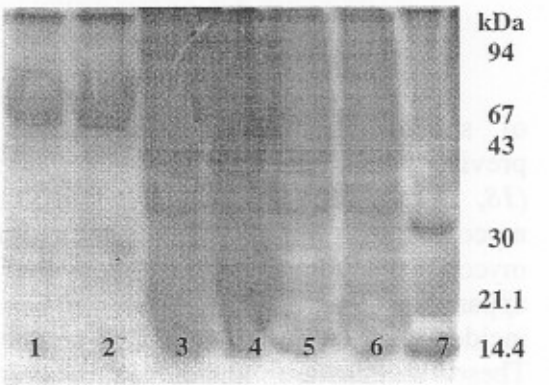

Fig. 3. SDS-PAGE protein profile of *M. arginini* field isolates from sheep and goats.

1 -*M. arginini* field isolate (1) from goat.
2- *M. arginini* field isolate (2) from goat.
3- *M. arginini* field isolate (1) from sheep.
4- *M. arginini* field isolate (2) from sheep.
5- *M. arginini* field isolate (3) from sheep.
6- *M. arginini* field isolate (4) from sheep.
7- Standard protein marker (Pharmacia)

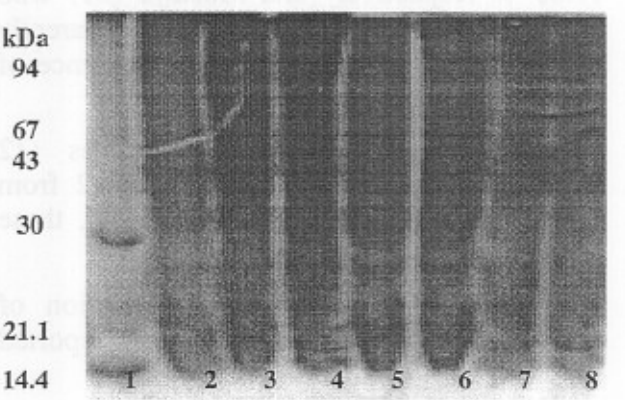

Fig. 4. SDS-PAGE protein profile of *M. ovipneumoniae* field isolates from sheep and goats.

1-Standard protein marker (Pharmacia).
2 *M. ovipneumoniae* field isolate (1) from goat.
3- *M. ovipneumoniae* field isolate (1) from sheep.
4- *M. ovipneumoniae* field isolate (2) from goat.
5- *M. ovipneumoniae* field isolate (2) from sheep.
6- *M. ovipneumoniae* field isolate (3) from sheep.
7- *M. ovipneumoniae* field isolate (4) from sheep.
8- *M. ovipneumoniae* field isolate (3) from goat.

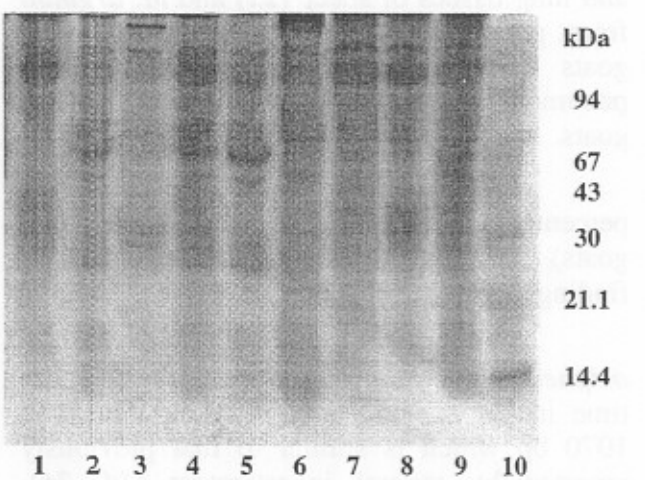

Fig. 5. SDS-PAGE protein profile of *M. agalactiae* field isolates from sheep and goats.

1- *M. agalactiae* field isolate (1) from goat.
2- *M. agalactiae* field isolate (2) from goat.
3- *M. agalactiae* field isolate (3) from goat.
4- *M. agalactiae* field isolate (1) from sheep.
5- *M. agalactiae* field isolate (2) from sheep.
6- *M. agalactiae* field isolate (3) from sheep.
7- *M. agalactiae* field isolate (4) from sheep.
8- *M. agalactiae* field isolate (5) from sheep.
9- *M. agalactiae* Reference strain.
10- Standard protein marker (Pharmacia).

DISCUSSION

The role of mycoplasma in the diseases of sheep and goats in Egypt has been previously studied as the first systematic study ***(18, 19)***. Regarding the primary isolation of mycoplasma from apparently healthy animals, mycoplasma isolates were recovered from apparently healthy sheep and goats with an incidence of 63.95 % and 52 % respectively. These results are much higher than that reported by ***Rania (20)*** who recovered mycoplasma from apparently healthy sheep and goats with an incidence of 13.40 % and 17.85 % respectively and ***Mostafa (21)*** who recovered mycoplasma from apparently healthy sheep and goats with an incidence of 14.67 % and 20.39 % respectively.

Fourteen acholeplasma isolates (12 from apparently healthy animals and 2 from diseased animals) were recovered ***(20)***, these isolates were from nasal swabs.

Similar biochemical classification of mycoplasma isolates was previously reported ***(20)***.

M. agalactiae was isolated from nasal and lung tissues of sheep ***(22)*** and *M. arginini* from pneumonic lungs and nasal swabs of goats ***(23)***, while *M. ovipneumoniae* from pneumonic lungs ***(23)*** and nasal swabs ***(24)*** of goats.

M. agalactiae was isolated by higher percentage in small ruminants (sheep and goats) ***(25)***, which is consistent with our findings.

PCR confirmation of *M. ovipneumoniae* strains was done for the first time in Egypt and gave a specific band at 1070 bp which is similar to that previously reported by several investigators ***(14, 26)***, while *M. agalactiae* strains gave a characteristic band at 360 bp which is the same as previously cited in previous investigation ***(13, 27)***.

Tilmicosin was effective against *M. agalactiae* isolates when it was administered in early infection ***(28)*** which is confirmed by our findings. On the other hand, tylosin was effective against all the tested field isolates of mycoplasma isolated from sheep and goats and is used in the current treatment of mycoplasma in Egypt ***(29)***.

Our investigation showed that the field isolates of *M. arginini* differed in many protein bands which confirm previous findings ***(27)***.

The whole cell proteins of *M. ovipneumoniae* isolates from sheep and goats were studied by SDS-PAGE and showed that there were a degree of heterogeneity among all sheep field isolates. The degree of heterogeneity in protein pattern of *M. ovipneumoniae* was previously recorded ***(30-32)***.

In the present study, the electrophoretic profile of *M. agalactiae* isolates in sheep and goat are similar to that of the reference strain with few exceptions that reflect the genetic identity. Similar findings were previously reported ***(33)***.

It has been reported that *M. agalactiae* strains were characterized by the presence of surface protein (55 kDa) which was the most immunogenic ***(34)*** and is the surface exposed and accessible to antibodies developed during natural infection ***(35)***. The previous findings is confirmed during our investigation.

In the present study, *M. agalactiae* field strains gave membrane surface protein with molecular weight of 48 kDa which was previously reported as diagnostically relevant marker of *M. agalactiae* infection ***(36)***.

REFERENCES

1.Edward, D. G. F. F. and Freundt, E. A. (1967) : Proposal for Mollicutes as a name of the class established for order Mycoplasmatales. Int. J. Syst. Bacteriol., 17: 267 – 268.

2.Frey, J. (2002): PCR detection of microbial pathogens: methods and protocols, methods in molecular biology, vol. 216, chapter 4 (Mycoplasmas of Animals). Humana Press Inc., Totowa, NJ. Edited by: K. Sachse and J. Frey.

3. ***Egwu, G. O.; Ball, H. J.; Rodriguez, F. and Fernandez, A. (2000):*** *M. capricolum subsp. capricolum, M. mycoides subsp. mycoides LC* and *M. mycoides subsp. capri* in "Agalactia syndrome" of sheep and goats. Vet. Bull., 70 (4): 391 - 402.

4. ***Barile, M. F.; Delguidice, R. A.; Carsk, T. R.; Gibbs, C. J. and Morris, T. A. (1968):*** Isolation and characterization of *Mycoplasma arginini*. Proc. Soc. Exp. Biolo. Med., 129 (2): 489 - 494.

5. ***Foggie, A. and Angus, K. W. (1972):*** Observations on the distribution of *M. arginini* as a respiratory tract infection in sheep and its pathogenicity for specific pathogen free lambs. J. Vet. Rec., 90: 312 - 313.

6. ***Hassan, N. R. A. (1973):*** Pathological studies on pneumonia with particular reference to those associated with mycoplasma in some farm animals. M. V. Sc., Fac. of Vet. Med., Cairo University.

7. ***Sabry, M. Z. and Ahmed, A. A. (1975):*** Evaluation of culture procedure for primary isolation of mycoplasmas from female genitalia of farm animals. J. Egypt. Vet. Med. Ass., 35: 18 - 34.

8. ***Freundt, E. A.; Andrews, B. E.; Erno, H.; Kunze, M. and Black, F. T. (1973):*** The sensitivity of Mycoplasmatales to sodium –polyanethol sulphonate and digitonin. Zbl. Bakt. Hgy. In. Abt. Orig. A, 225: 104 - 112.

9. ***Erno, H. and Stipkovits, L. (1973):*** Bovine mycoplasma: Cultural and biochemical studies. Act. Vet. Scan., 14: 450 - 463.

10. ***Clyde, W. A. (1964):*** Mycoplasma spp. identification based upon growth inhibition by specific antisera. J. Immunol., 92: 958 - 965.

11. ***Sabry, M. Z; Erno, H. and Freundt, E. A. (1971):*** Manual of technical methods for the characterization and serotyping of mycoplasmas. Mycoplasma Research Department, Animal Health Research Institute, Ministry of Agriculture, Cairo, Egypt.

12. ***Kroggsgaard- Jensen, A. (1972):*** Mycoplasma: Growth precipitation as a sero-diagnostic method. Appl. Microbiol., 23: 553 – 558.

13. ***Yleana, R.C.J.; Bascunana, C.R., Bolski, K.G., Mattsson, J.G., Molina, C.F. and Johansson; K.E. (1995):*** In vitro amplification of the 16S rRNA genes from *M. bovis* and *M. agalactiae*. Vet. Microbiol., 47: 183 - 190.

14. ***Zhang, Li; Sun, Jicuo; Yan, Mingllua; Wang, Yingzhen and Yang, Zhuangsheng. (2004):*** Development of polymerase chain reaction for detection of *M. ovipneumoniae* in sheep. Chin. J. Vet. Sci. and Technology, 34 (11): 7 – 10.

15. ***Hannan, P. C. (2000):*** Guidelines and recommendations for antimicrobial minimum inhibitory concentration (MIC) testing against veterinary *Mycoplasma* species. Vet. Res., 31 (2000): 373 – 395.

16. ***Laemmli, U. K. (1970):*** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680 - 685.

17. ***Bradford, M. M. (1976):*** A rapid and sensitive method for quantification of microgram quantities of protein using the principle of protein dye binding. Analytical Biochemistry, 72: 248 - 254.

18. ***Al-Zeftawi, N. M. (1973):*** Some studies on mycoplasma associated with pneumonia in farm animals in A.R.E. M. V. Sc. Thesis, Department of Infectious Dis., Fac. of Vet. Med., Cairo University.

19. ***Al-Zeftawi, N. M. (1979):*** The role of mycoplasmatales in disease of sheep and goats in Egypt.Ph.D. Thesis, Department of Infectious Diseases, Fac. of Vet. Med., Cairo University.

20. ***Rania, A. E. E. (2006):*** Some bacteriological and mycoplasmological studies on Respiratory tract infection in sheep and goats. M. V. Sc. Thesis, Bacteriology, Fac. of Vet. Med., Zagazig University.

21.Mostafa, A. A. E. (2003): Some studies on isolation and characterization of mycoplasma in small ruminants in south east of Egypt. M. V. Sc. Thesis Bacteriology, Fac. of Vet. Med. Zagazig University.

22.Aly, A. A. and Dardeer, M. A. A. (2003): Pathological and mycoplasmal studies on sheep suffering from respiratory tract affection in one of Alexandria governorate farm. Egypt J. Comp. Path. and Clinic. Path. , 16 (2): 84 - 99.

23.Nevine, M. Sobhy ; Manal, Abou El-Makarem; Gehan, G. Shehab and Gehan, A. Hosny (2001): Bacteria and mycoplasma as causes of pneumonia in goats with special references to the associated histopathological changes.J. Egypt. Vet. Med. Ass. , 61 (3): 25 - 44.

24.Kusiluka, L. J. M.; Ojeniyi, B.; Firiis, N. F.; Kazwala, R. R. and Kototovic, B. (2000): Mycoplasmas isolated from the respiratory tract of cattle and goats in Tanzania.Act. Vet. Scand., 41 (3): 299 - 309.

25. Al-Momani, W.; Nicholas, R.A. and Abo –Shehada, M.N. (2008): Risk factors associated with *Mycoplasma agalactiae* infection of small ruminants in northern Jordan.Prev. Vet. Med., 83 (1): 1-10.

26. Ayling, R.D.; Mcauliffe, L. and Nicholas, R. A. J. (2003): Mycoplasma ovipneumoniae: a re – emerging pathogen of sheep and the development of improved diagnostic tests. Res.Vet. Sci., 74: 24 (supp A).

27.Dardeer, M.A.; Hanaa, A.A.; Sahar, A.O. and Hassan, A.M. (2006): Antigenic and Genetic Variability among Mycoplasmas Isolated From Sheep and Goat.Egypt J. Comp. Path. and Clinic. Path., 19 (3): 313 – 331.

28.Nicholas, R. A. J. (2002): Improvements in the diagnostic and control of disease of small ruminants caused by mycoplasmas. Small Rumin. Res., 45 (2): 145 - 149.

29.Nadra, M. I. A. (2002): Some studies on Mycoplaasmosis in some animals.Ph.D. Thesis, Department of Infectious Dis., Fac. of Vet. Med., Cairo University.

30.Parham, K.; Churchward, C.P.; McAuliffe, L.; Nicholas, R.A. and Ayling, R.D. (2006): A high level of strain variation within the *M. ovipneumoniae* population of the UK has implications for disease diagnosis and management.Vet. Microbiol., 118 (1-2): 83 - 90.

31.Alison, J. M.; Ionas, G.; Clarke, J.K.; Robinson, A. J. and Marshall, R.B. (2002): Comparison of *Mycoplasma ovipneumoniae* isolates using bacterial restriction endonuclease DNA analysis and SDS-PAGE. Vet. Microbiol. , 10 (6): 541 - 548.

32.Thirkell, D.; Spooner, R.K.; Jones, G.E. and Russell, W.C. (2002): Polypeptide and antigenic variability among strains of *M. ovipneumoniae* demonstrated by SDS-PAGE and immunoblotting. Vet. Microbiol. , 21 (3): 241 - 254.

33.Solsona, M.; Lambert, M. and Poumarat, F. (1996): Genomic, protein homogeneity and antigenic variability of *M. agalactiae.* Vet. Microbiol., 50 : 45 – 58.

34.Santona, A.; Carta, F.; Fraghi, P. and Turrini, F. (2002): Mapping Antigenic Sites of an Immunodominant Surface Lipoprotein of *Mycoplasma agalactiae*, AvgC, with the Use of Synthetic Peptides. Infect. Immun., 70: 171 - 176.

35.Tola, S.; Manunta, D.; Cocco, M.; Turrini, F.; Rocchigiani, A.M.; Idini, G.; Angioi, A.and Leori, G., (1997): Characterization of membrane surface proteins of *M.agalactiae* during natural infection. FEMS Microbiol. Lett., 154 : 355 - 362.

36.Rosati, S.; Robino, P.; Fadda, M.; Pozzi, S.; Mannelli, A. and Pittau, M. (2000):

Expression and antigenic characterization of recombinant *Mycoplasma agalactiae* P48 major surface protein. Vet. Microbiol., 71:201 – 210.

الملخص العربى

دراسة متقدمة على ميكروبات الميكوبلازما في الأغنام والماعز

أحمد محمد أحمد عمار * - صبري إسماعيل عيسى - مروة إبراهيم إبراهيم عبد الحميد***
هناء عبد القادر أحمد- عيد السعيدعبد العزيز****
* قسم البكتريولوجيا والفطريات والمناعة – كلية الطب البيطري – جامعة الزقازيق
**قسم بحوث الميكوبلازما – معهد بحوث صحة الحيوان – الدقي - الجيزة

تعد الأغنام والماعز من حيوانات المزرعة المهمة في مصر. تم إجراء هذه الدراسة على ١٧٢ عينة (١٣١ عينة من الأغنام و٤١ عينة من الماعز) والتي جمعت من مناطق مختلفة من محافظة الشرقية ومن مجزر البساتين بالقاهرة. تم عزل الميكوبلازما أرجينينى من الأغنام والماعز بنسبة عزل ٢١,١١% و ٣٥,٢٩ على الترتيب وتم عزل الميكوبلازما أوڤينيمونى من الأغنام والماعز بنسبة عزل ٣٧,٧٨% ١٧,٦٥% على الترتيب وأيضا تم عزل الميكوبلازما اجالاكتيا من الأغنام والماعز بنسبة عزل ١٤,١١% و٤٧,٠٦% على الترتيب. تم التعرف على عينتان أكوليبلازما فقط. تم تأكيد عزل الميكوبلازما اجالاكتيا بواسطة اختبار تفاعل إنزيم البلمرة المتسلسل عن طريق تواجد الحزمة الخاصة بها عند ٣٦٠ قاعدة مزدوجة وأيضا ولأول مرة في مصر تم تأكيد عزل الميكوبلازما أوڤينيمونى بواسطة اختبار تفاعل إنزيم البلمرة المتسلسل عن طريق تواجد الحزمة الخاصة بها عند ١٠٧٠ قاعدة مزدوجة. لقد أوضحت نتائج اختبار الحساسية باستخدام تقنية التخفيف بالطريقة السائلة في وجود الكاشف أن التيلميكوزين هو الأكثر فاعلية. أسفرت نتائج اختبار التحليل الكهربي للبروتين الخلوي عن وجود اختلافات في التركيب البروتينى لعترات البميكوبلازما.