Assiut University web-site: www.aun.edu.eg

ISOLATION AND SEQUENCING OF MYCOPLASMA ARGININI GENOME ASSOCIATED WITH RESPIRATORY DISORDER IN SMALL RUMINANTS

ELSHAFAY, DINA [1]; NERMIN AWAD [2]; ELNAKER F. YASSER [3] and YOUNIS E. EMAD [4]
[1] Mycoplasma Department, Animal Health Research Institute, Dokii, Giza.
[2] Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Mansoura University.
[3] Department of Animal Medicine, Fac. Vet. Med. New Valley, Assiut University.
[4] Department of Internal Medicine and Infectious Diseases, Fac. Vet. Med, Mansoura Univ.

Received: 31 December 2017; **Accepted:** 31 January 2018

ABSTRACT

The purpose of this study was to evaluate the role and variety of *Mycoplasma arginini* (*M. arginini*) in respiratory manifestation in sheep and goat flocks. *M. arginine*, were isolated from 40 nasal swabs out of 160 nasal swabs examined which obtained from (79) sheep and (81) goat with respiratory manifestations. In sheep, the percent of isolation was (25.32%), 20 isolate out of 79 nasal swabs, while the percent of isolation in goat was (24.69%) 20 isolate out of 81 nasal swabs examined. Higher percent of isolation was found in age group (1- 2) year old sheep (50%) as 7 isolates out of 14 nasal swabs examined, while in goat the higher percent of isolation was in age group less than one year by 44% (11 isolates out of 25 nasal swabs examined. The lower isolation rates were found in adult sheep and goat (3 - 4 years old) by 15.38% in sheep and 6.45% in goat. *Mycoplasma* isolates was molecularly characterized by PCR and DNA sequencing as *M. arginini* and submitted to Gen Bank. An accession number has been assigned to each nucleotide sequence and was KP972458-KP972459.

Key words: Goat – *Mycoplasma* - PCR – sheep pneumonia.

INTRODUCTION

Respiratory diseases can cause prolonged illness or even sudden death in sheep and goat. Pneumonia appear in all ages of sheep, which not only has a direct effect on their growth but also plays important role in lamb mortality (Thonney *et al.*, 2008), more over contagious caprine pleuropneumonia (CCPP) from the pneumonic diseases which exaggerate economic losses due to the risk of death in goat flocks (Ruragirwa and McGuire, 2003). Many infecting agent incriminated, it may be bacteria, Chlamydia, Mycoplasma, or viruses. Pasteurellosis or mannheimiosis, the most predominant causes especially in sheep while *Mycoplasma pneumonia* is often unnoticed. Other bacteria infrequently present with respiratory disease in small ruminants are *Arcanobacterium pyogenes*, *Staphylococcus spp*., *Streptococcus spp*., *Haemophilus spp.*, and *Klebsiella pneumonia* (Nicholas *et al.*, 2008a).

Although *M. arginini* in sheep and goat is an important pathogen by itself but co infection by M. haemolytica exaggerates the pathologic injury of pneumonia (Lin *et al.*, 2008; Nicholas *et al.*, 2008b). *Mycoplasma arginini* is frequently present in lungs, mouth, and esophagus of pneumonic sheep (Cottew, 1979), and (Ayşe Kılıc *et al.*, 2013), Also *M.* arginini isolated from cases of ovine keratoconjunctivitis. (Leach, 1970).

M. arginini is a mammalian parasite, which found in many animal species. Accumulating signs in the case reports suggests the pathogenic role of M. arginini as a new human zoonosis. (Yechouron *et al.*, 1992). Therefore the aim of this work is to investigate more about the role of *M. arginini* in the respiratory affections in sheep and goat beside the phylogenic analysis of native strains.

MATERIALS AND METHODS

1- Animals

This study was performed in winter months on four flocks of sheep and goats containing 712 sheep and 305 goats in Sherbin and Dikrins cities, follows the governorate of Dakahlia, Egypt. These animals were classified according to their ages into 3 groups (Table 3), the flocks examined clinically according to Kelly (1990) for respiratory manifestation, and data and type of housing of examined animals were recorded.

Corresponding author: Dr. ELNAKER F. YASSER
E-mail address: yasserelnaker@yahoo.com
Present address: Department of Animal Medicine, Fac. Vet. Med. New Valley, Assiut University

2- Samples
One hundred and sixty nasal swabs were collected on PPLO broth from sheep and goats with signs of respiratory manifestation. The swabs were preserved at – 20°c until used.

3- Isolation of *Mycoplasma*:
a) Media used for cultivation and isolation of *Mycoplasma*:
a.1) Liquid and solid media prepared as described by Sabry and Ahmed (1975).
a.2) Digitonin sensitivity test was done for the obtained isolates according to Erno and Stipkovits (1973).
a.3) Biochemical characterization was carried out by glucose fermentation and arginine deamination tests as described by Erno and Stipkovits (1973). Film and spot formation medium (Fabricant and Freundt, 1967).

4 - Polymerase chain reaction (PCR):
A- Preparation of samples for DNA extraction (Yleana *et al.*, 1995).
B- Primer selection.

Table 1: Primers of *Mycoplasma* as shown in table below.

***Mycoplasma* Species**	**Forwarded primer**	**Reverse primer**	**product size (bp)**	**Thermal cycle**	**Reference**
Sequence of 16S common gene for ruminant *Mycoplasma*	MunivF 5- AGA TC CTA CGG GAG CA GCA -3	MunivR 5' ACT GC GAT TCC GAC TTC ATG 3'	1000	Initial denaturation step at 94 °C for 5 min., followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55°C for 1 min., and extension at 72°C for 1.5 min. A final extention step at 72°C for 10 min	**Alberto *et al.* (2006)**
Mycoplasma mycoides Subspecies capri	5-ACTGAGCAATT CCTCTT-3	5-TTAATAAGTCTC TATATGAAT-3	195	Initial denaturation 94 ºC for 5 min, Then 35 cycles of Denaturation 94 ºC for 1min , Annealing 46 ºC for 90sec, Extension 72 ºC for 1min, final Extension 94 ºC for 10 min.	**Vijay *et al.* (2013)**
Mycoplasma agalactiae	MAGAUVRC1-L 5' - CTC AAA AAT ACA TCA ACA AGC - 3'	MAGAUVRC1-R 5' - CTT CAA CTG ATG CAT CAT AA - 3'	1624	initial sample denaturation, 5 min at 95 °C 35 cycles: denaturation, 30 s at 94 °C; annealing, 30 s at 50 °C; extension, 1 min at 72 °C - completion of the amplification process by final extension at 72 °C for 10 min	**Zendulkova *et al.* (2007)**
Mycoplasma ovipneumoniae (16S–23S intergenic spacer)	MoIGSF GGAACACCTCC TTTCTACGG	MoIGSR CCAAGGCATCC ACCAAATAC	390	initial denaturation step at 95°C (15 min) 30 cycles at 95°C for 30 s, at 58°C for 30 s, and at 72°C for 30 final extension step at 72°C (5 min)	**Thomas *et al.* (2012)**

PCR amplification for *Mycoplasma* was performed in 50 µl reaction mixture consisting of 5 µl of 50 ng *Mycoplasma* genomic DNA, 25 µl of 2 x Master mix (Multiplex gen) VIVANTIS , 1 µl of 50 pmol of each primer, 0.5 mM MgCl , and 35µl of DNase- RNase-free deionized water. DNA amplification was performed as shown in Table (2), 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 50 volts for 20 min on a horizontal electrophoresis unit. A 100 bp DNA ladder was used as molecular weight standard (VIVANTIS). After electrophoresis, the gel was visualized photographed.

5- Selected published sequences of 16S rRNA genes, which used in sequence analysis and phylogeny:
>M.arginini.ATCC23243 gb|JN935883.1|:319-1256
>M.arginini.D7 gb|HQ661822.1|:269-1206
>M.arginini.E3.5 gb|HQ661820.1|:269-1206
>M.arginini.787 gb|HQ661826.1|:269-1207
>M.arginini.HAZ145_1 dbj|AP014657.1|:140970-141907
>M.arginini.C2-Ass-11 gb|JN543264.1|:1-932
>M.arginini.E2.5 gb|HQ661827.1|:350-1287
>M.arginini.C1-Beh-10 gb|HM635904.1|:1-932
>M.phocicerebrale.1049 gb|JN935885.1|:320-1257
>M.auris.UIA ref|NR_026035.1|
>M.neophronis.G.A ref|NR_108494.1|
>M.cloacale.383 ref|NR_024985.1|:308-1244
>M.salivarium.PG20 ref|NR_041745.1|:310-1247
>M.hyosynoviae.S-16 ref|NR_029183.1|:341-1277

6- Sequence and phylogenetic analysis:-

Sequencing of the PCR products of 16S rRNA gene were done in both directions then 943 bp sequences was used for nucleotide analysis. The original sequence was trimmed to remove unclear nucleotide sequences that appear usually in the beginning of the sequencing reaction. The nucleotide sequences of the M. arginini were compared with others published on Gen Bank using BLAST 2.1 and PSI- BLAST search programs, (National Center for Biotechnology Information "NCBI" http:// www.ncbi.nlm.nih.gov/). The obtained nucleotide sequences comparisons and their multiple alignments with reference *M. arginini* and other *Mycoplasma*s sequences were done using the BioEdit sequence alignment editor, (Hall, 1999) and MegAlign™ (DNASTAR, Lasergene®, and Version 7.1.0, USA). The phylogenetic trees were raised using MegAlign™ for tree reconstruction of sequences by Neighbor-joining method based on Crustal. Random seeding value of 111 was used for Bootstrapping values calculation. (Thompson *et al.*, 1994) For end gaps Clustal V was used. Sequence divergence and identity percent's were calculated by MegAlign™. The obtained sequences were submitted to Gen Bank (after trimming and correction) and retrieved accession numbers; KP972458 and KP972459 for samples sequences which designated Dak-1/M.arg/EG014 and Dak-2/M.arg/EG014 respectively.

RESULTS

Clinical examination

Percent of respiratory manifestations in examined 4 flocks were 11.1% (79 /712) and 26.56% (81/305) in sheep and goats respectively, the clinical manifestations varies from nasal discharge, cough to dyspnea. (Table 2 and fig 1, 2).

Fig. (1): Sheep with nasal discharge

Table 2: Percent of sheep clinically affected with respiratory manifestations.

Flock No.	Sheep	Clinically affected	%	Goat	Clinically affected	%
Flock 1	**170**	**17**	**10**	**55**	**20**	**36.36**
Flock 2	**184**	**16**	**8.7**	**90**	**26**	**28.88**
Flock 3	**150**	**22**	**14.67**	**82**	**19**	**23.17**
Flock 4	**208**	**24**	**11.54**	**78**	**16**	**20.51**
Total	**712**	**79**	**11.1**	**305**	**81**	**26.56**

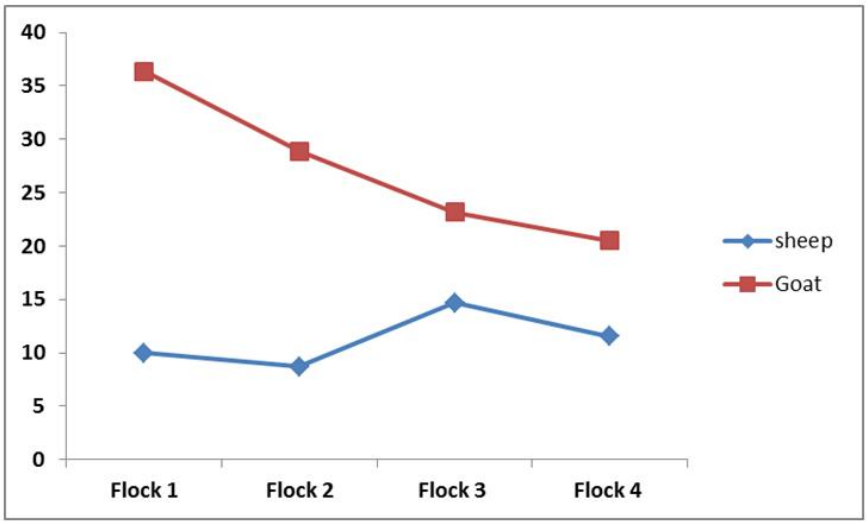


Fig. (2): Percent of clinically affected sheep with respiratory manifestations.

Isolation of *Mycoplasma spp*.

Table 3: *Mycoplasma* isolation from nasal swabs of sheep and goat with respiratory manifestation in relation to age and species of the animals.

Species / Age	Sheep			Goat		
	Total	+ve	%	Total	+ve	%
< 1 year	39	9	23.07	25	11	44
1 – 2 year	14	7	50	25	7	28
3 - 4 year	26	4	15.38	31	2	6.45
Total	**79**	**20**	**25.32**	**81**	**20**	**24.69**

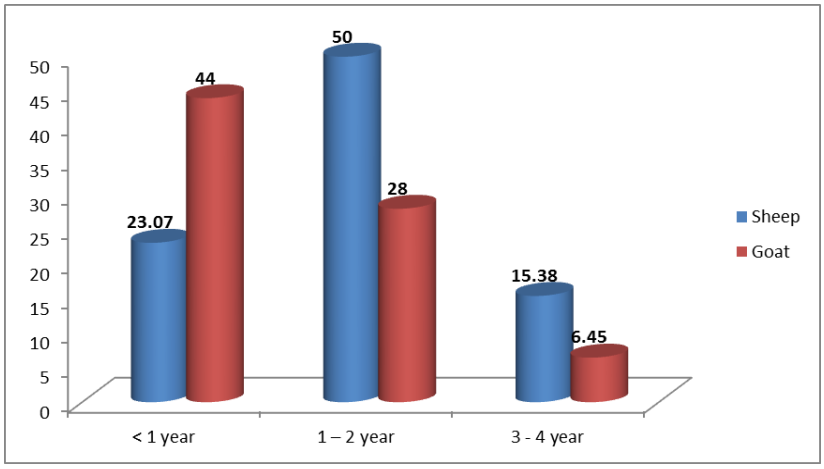
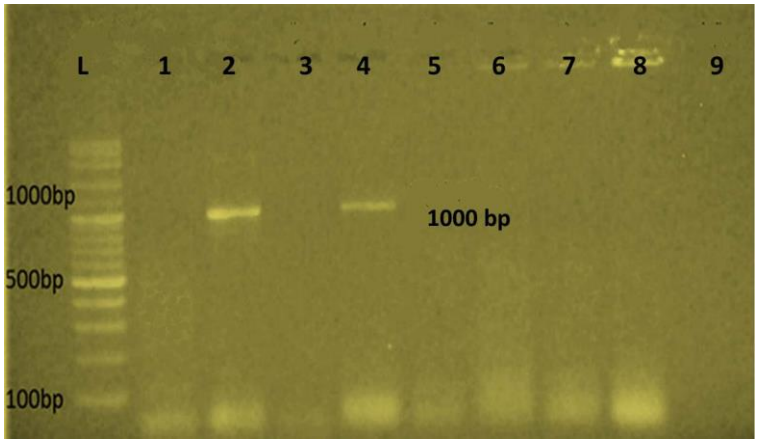


Fig. (3): *Mycoplasma* isolation from nasal swabs of sheep and goat with respiratory manifestation in relation to age and species of the animals

In Table (3) & Fig (3), percent of isolation of *Mycoplasma* from examined nasal swabs were 25.32% (20/79) and 24.69% (20/81) in sheep and goat respectively.

According to age, the higher percent of isolation was found in age group (1- 2) year old sheep (50%) as 7 isolates from examined 14 nasal swab were recorded, while in goat the higher percent of isolation were in age group less than one year old by 44% (11 isolates from examined 25 nasal swab). The lower isolation rate was found in adult sheep and goat (3- 4year-old) by 15.38% and 6.45% respectively.



Polymerase chain reaction (PCR).

Fig (4): Gel electrophoresis of PCR products of *Mycoplasma* 16s rRNA gene. L: VC100BP Puls DNA Ladder, Lane 2 and 4: The amplified products prepared from colony of positive nasal swabs of diseased sheep, Lane 1: negative control, Lanes 3 & 5-9 negative samples.

PCR results of the 40 isolates were found positive with genus-specific primers, 20 isolate from sheep and 20 isolates from goat were positive with species-specific primers for *Mycoplasma. arginini*. The bands obtained were 1000 bp with the Mycoplasma genus specific primer (Fig. 4).

In this study according to phylogenetic relatedness, the isolates classified into two unlike groups. Among the members of these two groups there were no changes in the nucleotide sequences of the 16s RNA gene. Phylogenetic distances among the strains are shown in Table (4) Figure (5). Among the isolates, two showed identical nucleotide sequence, meaning they represent the same strains. Isolates KP972458 and KP972459 for samples sequences, which designated Dak-1/M.arg/EG014 and Dak-2/M.arg/ EG014 respectively.

Table 4: The percentages of identities and diversities of nucleotide sequences of the *Mycoplasma* arginini 16s rRNA in the present study.

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
1		98.6	99.4	99.4	99.4	99.4	99.1	99.4	98.9	99.0	98.3	98.1	96.8	96.7	96.4	96.2	1
2	1.4		98.9	98.9	98.9	98.9	98.7	98.9	98.5	98.9	97.8	97.6	96.3	96.2	95.9	95.7	2
3	0.6	1.1		100.0	100.0	100.0	99.8	100.0	99.6	99.5	98.9	98.7	97.4	97.3	97.0	96.8	3
4	0.6	1.1	0.0		100.0	100.0	99.8	100.0	99.6	99.5	98.9	98.7	97.4	97.3	97.0	96.8	4
5	0.6	1.1	0.0	0.0		100.0	99.8	100.0	99.6	99.5	98.9	98.7	97.4	97.3	97.0	96.8	5
6	0.6	1.1	0.0	0.0	0.0		99.8	100.0	99.6	99.5	98.9	98.7	97.4	97.3	97.0	96.8	6
7	0.9	1.3	0.2	0.2	0.2	0.2		99.8	99.4	99.2	98.7	98.5	97.2	97.1	96.8	96.7	7
8	0.6	1.1	0.0	0.0	0.0	0.0	0.2		99.6	99.5	98.9	98.7	97.4	97.3	97.0	96.8	8
9	1.1	1.5	0.4	0.4	0.4	0.4	0.6	0.4		99.0	98.5	98.3	97.0	96.9	96.6	96.4	9
10	1.0	1.1	0.5	0.5	0.5	0.5	0.8	0.5	1.0		98.4	98.2	96.9	96.8	96.7	96.5	10
11	1.7	2.2	1.1	1.1	1.1	1.1	1.3	1.1	1.5	1.6		98.7	97.7	97.3	96.8	96.7	11
12	1.9	2.4	1.3	1.3	1.3	1.3	1.5	1.3	1.7	1.9	1.3		97.2	97.2	96.9	96.8	12
13	3.3	3.8	2.6	2.6	2.6	2.6	2.8	2.6	3.1	3.2	2.4	2.8		97.3	96.2	96.4	13
14	3.4	3.9	2.7	2.7	2.7	2.7	2.9	2.7	3.2	3.3	2.7	2.8	2.7		96.1	95.9	14
15	3.7	4.3	3.1	3.1	3.1	3.1	3.3	3.1	3.5	3.4	3.3	3.2	4.0	4.1		97.5	15
16	4.0	4.5	3.3	3.3	3.3	3.3	3.4	3.3	3.7	3.6	3.4	3.3	3.7	4.2	2.5		16
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	

Divergence

Dak_1-M.arg-EG014
Dak_2-M.arg-EG014
M.arginini.ATCC23243
M.arginini.D7
M.arginini.E3.5
M.arginini.787
M.arginini.HAZ145_1
M.arginini.C2-Ass-11
M.arginini.E2.5
M.arginini.C1-Beh-10
M.phocicerebrale.1049
M.auris.UIA
M.neophronis.G.A
M.cloacale.383
M.salivarium.PG20
M.hyosynoviae.S-16

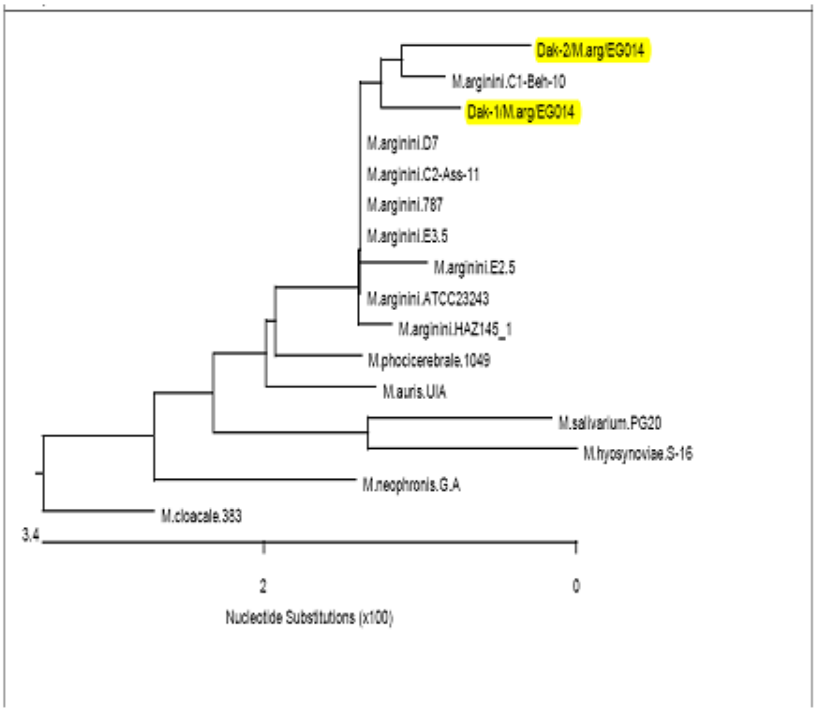


Fig. (5): Phylogenetic tree of *Mycoplasma arginini* 16s rRNA gene from sheep and goat with other *Mycoplasma* that were taken from the Gene Bank database 16s rRNA gene.

DISCUSSION

The respiratory diseases represent 5.6 % of all diseases in small ruminants Hindson and Winter (2002) Small ruminants are especially sensitive to respiratory infections, namely, viruses, bacteria, and fungi, mostly as a result of deficient management practices that make these animals more susceptible to infectious agents. The tendency of these animals to huddle and group rearing practices additional predispose small ruminants to infectious and contagious diseases Kumar *et al.* (2014), in the examined flocks, by clinical examination, the percent of respiratory manifestation was 11.1% as 79 were positive from examined 712 sheep while in goat was 26.56% (81 from 305) examined goats, clinical manifestation varies from nasal discharge, cough to dyspnea. Table (2) and fig (1, 2).

Sheep and goats appear to be close to each other in the *Mycoplasma* flora, which affect them with rare exceptions; this can be clearly explained by the close phylogenetic relationship of these two animal species. In small ruminants *Mycoplasma* infections are common although it more frequently in goats than sheep. Some of these *Mycoplasma*s induce severe and contagious diseases with massive economic influence. From the most important *M.* spp for the ruminants are the *M.* mycoides cluster Cottew *et al.* (1987). Pneumonia in sheep has been associated with *M. ovipneumoniae, M. capricolum subsp. capricolum, M. mycoides subsp. mcoides LC and M. arginini* (Chaturvedi *et al.,* 1992) and (Ikheloa *et al.,* 2004). *M. arginini* appears to be the second most common species isolated and it is often present in mixed culture with *M. ovipneumoniae*. *M. arginini* is less fastidious than *M. ovipneumoniae* and has the typical "fried-egg like" colony morphology unlike *M.ovipneumoniae* which lacks this phenomena (Goltz *et al.,* 1986). In this study, *M. arginini* is closely associated with sheep and goat pneumonia. One of the most characteristic features of *Mycoplasma*s is inability to grow easily in laboratory media in spite of the great improvement in medium formulation so their isolation appeared to be too hard for diagnostic laboratories (Waleed *et al.,* 2006), although isolation and identification of *Mycoplasma* still an efficient Toole in the diagnosis of *Mycoplasma* diseases. Multiple *Mycoplasma*s may well co-exist in goat lungs and, in that case, only the strains which grow well will be recovered. Sheep are less susceptible to pulmonary mycoplasmoses than goats (Thiaucourt and Bölske 1996), which is different with our results as the percentage of *Mycoplasma* isolation was nearly equal in sheep and goat but none of the isolates could be identified as one of the *Mycoplasma mycoides* cluster using PCR. Isolates were positive using 16S rRNA gene primer used for ruminant *Mycoplasma* species detection giving a clear band at 1000 bp.

In spite of *M. arginini* is of less importance in pneumonia of sheep and goats; it is frequently isolated from their respiratory tract. This result is agreed with that mentioned by (Zaitoun, 2001) who was able to isolate *M. arginini* and *M. agalactiae* from cases of bronchopneumonia in sheep due to

Mycoplasma alone or coupled with bacterial agents. In this study a total of 160 nasal swab were collected from sheep and goat suffer from respiratory manifestation for trails of *Mycoplasma* isolation and identification, our result in Table (3) & Fig (3) revealed that, *Mycoplasma* spp. was isolated in 40 (25%) nasal swab samples from 160 nasal swabs obtained from sheep and goats with respiratory manifestations. In sheep, percent of isolation was (25.32%) (20 isolate from 79 nasal swabs) while the percent in goat was (24.69%) (20 isolate from examined 81 nasal swabs), the obtained result were different from the result obtained by (Ammar *et al.*, 2008), who isolated *M. arginini* from sheep and goats by 21.11 % and 35.29 % respectively from examined (131 samples from sheep and 41 samples from goats) obtained from animals from different areas in Sharkia governorate and El-Basatin abattoir in Cairo, the type of samples and its number may explain the difference between this study and result of our study.

In regarding to age our result observed, higher percent of isolation were found in age group (1- 2) year old sheep (50%) as 7 isolates from examined 14 nasal swab, while in goat were detected higher percent of isolation in age group less than one year old by 44% (11 isolates from examined 25 nasal swab). The lower isolation rates were observed in adult sheep and goats 3 - 4 year old by 15.38% in sheep and 6.45% in goat, which may clarify that, adult sheep and goat are less susceptible to infection with *M. arginini.*

In this investigation, *M.* spp, *M. arginini*, were identified from nasal swabs of sheep and goat rising in some farms at Dakahlia governorate Egypt, by PCR technique and nucleotide sequences of the *M. arginini* 16s rRNA. Others mentioned that Polymerase chain reaction more sensitive and specific technique that allows direct detection of antigen and overcomes to cross-reactions of conventional tests. A comparison of the PCR technique with the microbiological culture, DNA fluorochrome staining, and hybridization techniques indicated that the PCR is a rapid, sensitive, and efficient method (Ball and Finlay, 1998, Van Kuppeveld *et al.*, 1994 and Gupta *et al.*, 2015).

CONCLUSIONS

Mycoplasma arginni may play a role as a cause of respiratory manifestations in sheep and goat but further studies are needed for studying epidemiology of *mycoplasma spp*. *Mycoplasma* infection in sheep and goats must be considered during dealing with respiratory infection. Genome sequencing for immediate identification of Mollicutes species beside the ability to perform accurate analysis of multiple samples in a relatively short period compared to the current routinely used protocols existing a great improvement for a Mollicutes identification scheme. *Mycoplasma arginini* isolates designated in gene bank Dak-1/M.arg/EG014 and Dak-2/M.arg/EG014 for samples sequences KP972458 and KP972459 respectively.

REFERENCES

Alberto, A.; Addis, M.F.; Chessa, B.; Cubaddu, T.; Profiti, M.; Rosati, S.; Ruiu, A. and Pitau, M. (2006): Molecular and antigenic characterization of a *Mycoplasma* bovis strain causing an outbreak of infectious keratoconjunctivitis. J. Vet. Diagn. Invest. 18: 41-51.

Ammar, A.M.; Eissa, S.I.; Marwa, I. Abd El-Hamid; Hanaa, A .Ahmed and Abd ElAziz, E. (2008): Advanced Study on *Mycoplasma*s Isolated From Sheep and Goats. Zag. Vet. J.; 36(2): 128-137.

Ayşe Kılıc; Hakan Kalender; Hatice Eroksuz; Adile Muz and Bülent Tasdemir (2013): Identification by culture, PCR, and mmunohistochemistry of *Mycoplasma*s and their molecular typing in sheep and lamb lungs with pneumonia in Eastern Turkey .Trop Animal Health Prod ; 45(7): 1525–1531.

Ball, H.J. and Finlay, D. (1998): Diagnostic application of monoclonal antibody (MAb)-based sandwich ELISAs. Methods Mol. Biol., 104, 127–132.

Chaturvedi, V.K.; Pathak, R.C. and Singh, P.P. (1992): Pathogenicity of *Mycoplasma* mycoides subsp. mycoides LC for sheep and lambs. Ind. J. Comp. Microbiol. Immunol. Infect. Dis., 13, 1–4.

Cottew, GS. (1979): Caprine-ovine *Mycoplasma*s. In: The *Mycoplasma*s. II. Human and animal *Mycoplasma*s, ed. Tully JG, Whitcomb RF, pp. 103-132. Academic Press, San Francisco, CA

Cottew, GS.; Breard, A.; Da Massa, AJ.; Ernø, H.; Leach, RH., Lefevre, PC., Rodwell, AW. and Smith, GR. (1987): Taxonomy of the *Mycoplasma* mycoides cluster. Israel Journal of Medical Sciences , 23(6): 632-635.

Erno, H. and Stipkovits, L. (1973): Bovine *Mycoplasma*: Cultural and biochemical studies. Act. Vet. Scan. , 14: 450 - 463.11.

Fabricant, J. and Freundt, EA. (1967): Importance of extension and standardization of laboratory tests for the identification and classification of *Mycoplasma*. Ann N Y Acad Sci. Jul 28; 143(1): 50-8.

Goltz, J.P.; Rosendal, S.; Mccraw, B.M. and Ruhnke, H.L. (1986): Experimental Studies on the pathogenicity of *Mycoplasma* ovipneumoniae and *Mycoplasma* arginini for the respiratory tract of goats. Can. J. Vet. Res., 50, 59-67.

Gupta S.; Chahota, R.; Bhardwaj, B.; Malik, P.; Verma, S. and sharma, M. (2015): Identification of chlamydiae and *Mycoplasma* species in ruminants with ocular infections. Letters in Applied Microbiology, Volume 60, February Issue 2, pages 135–139.

Hall, A. (1999): BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nuc. Acids Symp. Ser.41: 95-98.

Hindson J.C. and Winter, A.C. (2002): "Respiratory disease," in Manual of Sheep Diseases, pp. 196–209, Blackwell Science, Oxford, UK, 2nd edition.

Ikheloa, J.O.; Ajuwape, A.T.P. and Adetosoye, A.I. (2004): Biochemical characterization and serological identification of *Mycoplasma*s, isolated from pneumonic lungs of goats slaughtered in abattoirs in Northern Nigeria. Small Rumin. Res., 52, 93–97.

Kelly, W.R. (1990): Veterinary clinical diagnosis, 3rd Edition Bailliere Tidall, London.

Kumar, A.; Rahal A.; Chakraborty, S.; Verma A. K. and Dhama, K. (2014): "Mycoplasma agalactiae, an etiological agent of contagious agalactia in small ruminants-a Review," Veterinary Medicine International In Press, 2014.

Leach, RH. (1970): The occurrence of *Mycoplasma* arginini in several animal hosts. Vet Rec 87: 319-320.

Lin, Y.C.; Miles, R.J.; Nicholas, R.A.J.; Kelly, D.P. and Wood, A.P. (2008): Isolation and immunological detection of *Mycoplasma* ovipneumoniae in sheep with atypical pneumonia, and lack of a role for *Mycoplasma* arginini. Res. Vet. Sci., 84, 367–373.

Nicholas, R.; Ayling, R. and McAuliffe, L. (2008b): Respiratory diseases of small ruminants. In: Nicholas, R., Ayling, R., Mcauliffe, L., Eds. *Mycoplasma* Diseases of Ruminants. CABI, Wallingford, UK; 2008: 171–179

Nicholas, R.A.J.; Ayling, R.D. and Loria, G.R. (2008a): Ovine *Mycoplasma* infections. Small. Ruminant Res., 2008; 76: 92–98.

Ruragirwa, F.R. and McGuire, T.C. (2003): Contagious caprine pleuropneumonia: Diagnosis and control. Available online at http://file://A:/Contagious#caprine#pleuropneumonia#Diagnosis. and#control.htm, p. 8

Sabry, M.Z. and Ahmed, A.A. (1975): Evaluation of culture procedure for primary isolation of *Mycoplasma*s from female genitalia of farm animals. J. Egypt. Vet. Med. Ass., 35: 18 - 34.9

Thiaucourt, F. and Bölske, G. (1996): Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses of sheep and goats. Rev Sci ech. Dec; 15(4):1397-414.

Thomas E. Besser; Margaret A. Highland; Katherine Baker; E. Frances Cassirer; Neil J. Anderson; Jennifer M. Ramsey; Kristin Mansfi Eld; Darren L. Bruning; Peregrine Wolff; Joshua B. Smith and Jonathan A. Jenks (2012): Causes of Pneumonia Epizootics among Bighorn Sheep, Western United States, 2008–2010. Emerging Infectious Diseases• www.cdc.gov/eid • Vol. 18, No. 3, March 406-414.

Thompson, D.; Higgins, G. and Gibson, J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nuc. Acids Res. 22: 4673-4680.

Thonney, M.L.; Smith, M.C.; Mateescu R.G. and Heuer, C. (2008): Vaccination of ewes and lambs against parainfluenza3 to prevent lamb pneumonia. Small. Ruminant Res., 2008; 74: 30–36.

Vankuppeveld, F.J.M.; Johansson, K.E.; Galama, J.M.D.; Kissing, J.; Bolske, G.; Vanderlogt, J.T.M. and Melchers, W.J.G. (1994): Detection of *Mycoplasma* contamination in cell cultures by a *Mycoplasma* Group-Specific PCR. Appl. Environ. Microbiol., 60, 149-152.

Vijay Kumar; Rajneesh Rana; Somya Mehra and Pramod Kumar (2013): Isolation and Characterization of *Mycoplasma* mycoides Subspecies capri from Milk of Natural Goat Mastitis Cases. Veterinary Science, Article ID 593029, 1-5 pages.

Waleed Al-Momani; Mahmoud A. Halablab; Mahmoud N. Abo-Shehada; Katie Miles; Laura McAuliffe and Robin A.J. Nicholas (2006): Isolation and molecular identification of small ruminant *Mycoplasma*s in Jordan. Volume 65, Issues 1–2, September 2006, Pages 106–112.

Yechouron, A.; Lefebvre, J.; Robson, HG.; Rose, DL. and Tully, JG. (1992): Fatal septicemia due to *Mycoplasma* arginini: a new human zoonosis. Clin Infect Dis. Sep; 15(3): 434-8.

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.

Zaitoun, A.M. (2001): Clinical study of pneumonic Mycoplasmosis and pasteurellosis (concurrent infection) in a commercial sheep flock. Assiut Vet. Med. J., 45 (89): 162-180.

Zendulkova, D.; Madanat, A.; Lany, P.; Rosenbergov, K. and Pospi, A.Z. (2007): Detection of *Mycoplasma* agalactiae by Polymerase Chain Reaction in Jordanian Sheep and Goat Herds. ACTA VET. BRNO, 76: 71–77; doi: 10.2754/avb200776010071.

عزل وتسلسل جينوم الميكوبلازما أرجينيني المرتبط بالاضطراب التنفسي في المجترات الصغيرة

دينا الشافعي ، نيرمين عوض ، ياسر الناقر ، عماد يونس

E-mail: yasserelnaker@yahoo.com Assiut University web-site: www.aun.edu.eg

اجريت هذة الدراسة علي اربع قطعان من الاغنام والماعز بمركزي شربين ودكرنس بمحافظة الدقهلية. حيث تم فحص عدد ٧١٢ راس اغنام و٣٠٥ راس ماعز من اعمار مختلفة وتبين اصابة عدد ٨١ ماعز و ٧٩ اغنام باعراض تنفسية وكان الغرض من هذه الدراسة هو تقييم دور وتحديد نوع الميكوبلازما أرجينيني. المرتبطة باعراض الجهاز التنفسي في قطعان الأغنام والماعز حيث تم اخذ مسحات انفية لعزل الميكوبلازما. وتم عزل الميكوبلازما من ٤٠ مسحة من الأنف من أصل ١٦٠ مسحة من الأنف. في الأغنام كانت نسبة العزل (٢٥.٣٢٪) (٢٠ معزول من أصل ٧٩ مسحة أنفية)، في حين كانت نسبة العزل في الماعز (٢٤.٦٩٪) (٢٠ معزول من ٨١ مسحة من الأنف تم فحصها). وتبين ان أعلى نسبة من العزل كانت في الفئة العمرية (١ - ٢) من الأغنام (٥٠٪) حيث تم عزل ٧ عزلات من أصل ١٤ مسحة أنفية، في حين كانت نسبة العزل أعلى في الفئة العمرية أقل من سنة واحدة في الماعز بنسبة ٤٤٪ (١١ عزلة من أصل ٢٥ مسحة من الأنف تم فحصها) وكانت اقل نسبة لعزل الميكوبلازما في الأغنام البالغة والماعز (٣ - ٤ سنوات) بنسبة ١٥.٣٨٪ في الأغنام و ٦.٤٥٪ في الماعز. وجين ميكوبلازما أرجينيني المعزول من العينات KP972458 و KP972459 سجلت في بنك الجينات تحت Dak-1/M.arg/EG014 and Dak-2/M.arg/EG014 على التوالي.