The phylogeny of *Mycoplasma bovis* from pneumonic goats based on16S rRNA Sequences in El-Salam Canal at northern Sinai Governorate

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Abstract
Mycoplasma bovis (*Mb*) is significant ruminant respiratory pathogen of economic importance**.** This study determined the incidence of isolation of *Mb* in association with pneumonia based on respiratory manifestation in goats and examined the antigenic variability between same species of Mb in El-Salam Canal at northern Sinai Governorate. Three hundred and thirty five samples were collected from goats suffering from respiratory manifestations (274 nasal swabs and 61 lung tissue). The samples were subjected to *Mycoplasma* (*M*) and *Mb* isolation by culture and polymerase chain reaction (PCR) based on 16S rRNA gene specific primers. *Mb* was recorded in 8% and 9.8% and 11.4% and16.4% from nasal swabs and pneumonic lungs to the total no. of samples by culture and PCR respectively. Results revealed that, *Mb.* is one of the important pathogen causing pneumonia in goats. In additions, there was great dissimilarity of 16S rRNA gene between isolated strains and the isolates was harboring a novel genotype. Histopathological findings supported the mycoplasmic lesions. This research provides the first evidence of isolation and identification of *Mb* from the nasal swabs and lungs of goats with respiratory problems. The study showed for the first time the sequence analysis of 16S rRNA gene of this pathogen isolated from pneumonic goats in this area.
Keywords: *Mycoplasma bovis*, goats, respiratory disease, El-Salam Canal.

Introduction

The goats is an important commodity in many areas of the world, where it is kept as a source of meat, milk, and fiber. Mycoplasmal infections are considered from important goat diseases with great significant losses ***(DaMassa et al, 1992)***. *Mycoplasmas* (*M*) differ from other bacteria in their very small size, total absence of a cell wall and complete resistance to antibiotics. Many *M. species* are important pathogenic for caprine ***(Waseem et al, 2012)*** causing respiratory infection ***(Sharif and Muhammad, 2009)***. Studies on small ruminant mycoplasmas, have mainly focused on contagious agalactia and contagious caprine pleuropneumonia. Where, the *M.*

most commonly associated with these diseases in sheep and goats are *M. capricolum subsp. capripneumoniae, M. mycoides subsp. capri*, *M. agalactiae*, *M. putrefaciens* and *M. ovipneumoniae* ***(Babak et al, 2011; Moradi et al, 2011 and Zinka et al, 2013).*** Also, ***Sheehan et al (2007)*** and ***Gonçalves et al. (2010)*** focused on several species of *M*. rather than *Mb*. in small ruminant. On the other hand, only a few studies around the world concerning *Mb* in goats have been conducted. During the past decades *Mb* has spread to numerous countries with the global transport of animals ***(Miklós 2005).*** It is not host specific ***(Ongor et al., 2008).*** It can cross the species barrier and have been found in avian and human species ***(Ongor et al., 2009).*** It has occasionally been isolated from goats ***(Hassan et al., 2011),*** and this is not unusual ***(DaMassa, et al., 1992). Hassan et al. (2011)*** concluded that, significance of *Mb* colonization as a risk factor for the development of clinical disease in the individual animal is unknown and the prevalence of *Mb*-associated disease is also unknown. Furthermore, the economic impact of *Mb* can be severe. Meanwhile, ***Nicholas and Ayling (2003)*** estimated that, *Mb* accounted for one-quarter to one-third of all respiratory disease losses. Therefore, there is a paucity of information about the potential of this agent in goats. To work and perform culture, biochemical and serological tests would take long times at least two weeks while by PCR due to no need of purification and cloning, pathogenic *M* are easy to detected even within the mixed organisms in a sample, without spending long times (It requires at least 2 to 3h) ***(Awan et al., 2009).*** PCR with mycoplasmal 16S rRNA and specific primers have been applied for detection of a variety of *M species* ***(Zendulkova et al., 2007)*** and act as a significant improvement on current tests as diagnosis of *M*. infection in both early and chronic infections ***(Kumar et al., 2011)***. Recently sequencing methods have been introduced as a new approach for studying the molecular epidemiology of bacterial pathogens. Gene sequencing remains a popular typing method used among a wide range of *M species*. Many *M* devote a relatively large percentage of their genomes to mechanisms promoting their survival, including antigenic variation to evade the host ***(Nicolas et al., 2008),*** so, there is a paucity of information about genome sequences of *Mb* isolated from goats. The objectives of this study are the isolation and identification of *Mb* on the basis of biochemical and PCR based on 16S rRNA gene specific primers from the nasal swabs and lung cultures of goats with nasal discharges and determination the antigenic variability between the same species of this pathogen in El-Salam

Canal at northern Sinai Governorate.

Material and methods:

Samples:

Study area: El- Salam Canal was designed to supply water to 620.000 feddans of new reclaimed land using 2.340 Billion m3/ year of the agricultural drainage after mixing it with 2.11 Billion m3/ year of Nile water (Damietta branch). Drainage water is to be supplied through El-Serw and Hadous Drains. There are different animals drank from that water and graze on plants in the area. Three hundred and thirty five goats were examined for the clinical cases of pneumonia on the basis of respiratory manifestation collected from El-Salam Canal at northern Sinai Governorate in which Mycoplasma infection was suspected.

Three hundred and thirty five goats samples were collected, out of them 274 nasal swabs (194 apparently healthy and 80 diseased goats) and 61 lung tissues (50 slaughters and 11 dead goats). *Mb* reference strain was kindly provided from Prof. Dr. Ruhnke, Vet. Microbiol. Dept., Ontario Vet. Collage, Guelph, Ontario, Canada and used as a positive control. (Reference GenBank: AY780798.1)

Isolation and identification of *Mycoplasmas*: Isolation of *M.* species and propagation of reference strains was performed according to ***Ruhnke and Rosendal (1989).*** The isolation of *M* from Lung samples were cultured according to the method described by ***Ter Laak et al. (1992).*** Biochemical and serological examination of the isolates were carried out according to ***Clyde (1983).***

Histopathological Examination. The collected lung samples were rapidly fixed in 10% neutral buffered formalin solution, washed, dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate and embedded in paraffin wax. Paraffin sections of 3-5 microns thickness were obtained and stained with hematoxylin and eosin according to ***Bancroft and Gamble (2002).***

DNA extraction: Genomic DNA was extracted from *Mb* reference strain as described by ***Ghadersohi et al. (1997).*** From each swab of nasal and lung tissues, *Mycoplasma*-DNA was extracted according to ***Wit et al. (1993)*** with some modifications and 5 µl of the final DNA solution were used per PCR.

Oligonucleotides Primers:

The nucleotide sequences of primers were designed to detect 16S rRNA of *Mb* were as follows: Left primer (478-498) 5`ATGCCTTGGTCTGGAAGTCG 3` and Right primer (699- 719) 5`GCTCGCCGCTACTAAAGAA A3`. The selected primers were tested by using Blast software which estimates the specificity of selected primers against all published sequences in the gene

bank.

Polymerase chain reaction (PCR): The PCR assay conditions were performed as described by ***Hotzel et al. (1996).*** To avoid false positive PCR results, the precautions for PCR described by ***Kwok and Higuch (1989)*** were strictly followed. The amplified product size of *Mb* was equals to 229bp. Positive control (all the components for the PCR with *Mb* reference strain DNA) and controls (all the necessary components for the PCR except template DNA) were included in each set of amplifications.

Cycle: The sequences were performed by Jena bio-science, Germany. Using ABI prism 310 Genetic Analyzer.

Phylogenetic analysis: The DNA sequence was analyzed using Bioedite software for analysis of sequence analysis, graphical view, Sequence Identity Matrix and phylogenetic tree analysis for maximum likhood relation.

Results and discussion:

Respiratory disease problems have constituted serious handicaps to improved small ruminant production in different regions of the world ***(Egwu et al., 2000).*** Pneumonia occurring due to *M* is generally regarded as one of the most frequent and serious cause of morbidity, mortality and economic losses associated with respiratory diseases in goats. Generally, respiratory manifestations were the most prominent symptoms and have been reported as common owner's complaints in goats at the area of investigation. The examined goats showed, nasal discharge varied from watery to abundant mucoied purulent, coughing, fever, depression, loss of appetite, dyspnoea and hyperventilation. The infected animals were treated with Tylosin. ***Amit et al (2012),*** reported that, Tylosin was recommended as drug for treatment of *Mb* and flock recovered with in the period of 15 days.

Isolation and identification:

Results in Table 1 showed that, from 274 nasal swabs collected from apparently healthy and diseased goats, *Mycoplasma* was isolated in 65 cases. Out of these, 22 (8% and 33.8% to the total no. of samples and to the total no. of isolates respectively) was *Mb* by conventional culture. From 61 lung specimens collected from slaughters and dead goats, 17 *M* isolates were obtained. Out of these, 7 (11.4% and 41.1%) (to the total no. of samples and to the total no. of isolates respectively), was proven to be *Mb* with conventional culture The other isolates of *M* belonged to other species. This is in parallel with ***Hassan et al (2011) and Egwu et al (2012),*** who succeeded to isolate *Mb* from goats with varied in their percentage with our results as similar, high or low, it might be refereed to, immunostatus of the animals, site of isolation, methods of isolation and identification, and

consistent source of infection. Meanwhile, ***Nicholas et al (2008)*** found that, *Mb* occasionally associated with respiratory disease in goats.

In the area of investigation, there were high levels of heavy metals in goats that hazardous for its health that reflect on its performance ***(Gehad 2010),*** besides**,** the goats were kept together with sheep and cattle and this mixing is a major factor favoring mycoplasmosis. The same conclusion was previously reported by ***Mohammad et al (2012)*** and ***Abo-Elnaga et al (2012).*** Clinically healthy animal play a very important role in the epidemiology of the disease, making unsuccessful both prophylaxis and eradication programs ***(Miklós 2005)*** and *Mb* once present in a herd it can be readily transmitted from infected to uninfected animal ***(Maunsell et al, 2011 and Moradi et al, 2011).*** These contribute a constant source of infection to the adjacent goats and probably explain the high rates of *M*b infection recorded in this study**.** This also represents a serious impact on pneumonia of the involved animals.

Histopathological finding: The presence of *Mb* in pneumonic lungs must be interpreted together with histopathology to confirm the isolation (Fig 4 and 5). This study, the main histopathological findings in the lung showed interstial pneumonia, catarrhal bronchitis (hyperplasia of epithelial lining , the lumen contained mucous inflammatory exudates (made from mucous inflammatory cells), peribronchiolar edema, lymphocytic infiltration, congestion of the B.V., alveolar emphysema and fibrinous pleurisy. These findings were similar to the findings reported by ***Amit et al (2012).***

Polymerase chain reaction (PCR): PCR has been accepted as a valuable method for diagnosis of a variety of Mycoplasma species and mycoplasmal infections using 16S rRNA ***(Zendulkova et al, 2007).*** Meanwhile, PCR can provide a rapid early warning system when carried out on clinical samples, enabling a full investigation to take place when results are positive and it is not influenced by antimicrobial therapy ***(Mohammad et al, 2012)*** and ***Krešimir et al (2013).*** In this study as showed in Table 2 and Fig. 1, we selected 35 clinical samples, (which were representative samples from the total no. of samples)11 out of them were bacteriologically positive and the remaining 24 samples were bacteriologically negative, were subjected to PCR based on 16S rRNA gene specific primer for *Mb,* the result revealed that, PCR based on 16S rRNA gene specific primer to *Mb* succeeded to detect and identify *Mb* in 19 out of 35 cases from the cases of positive and negative bacteriological culture by the presence of specific amplicon at 229 bp. These findings showed that, our assay based on the 16S rRNA gene is more specific

and sensitive for the detection of *Mb* in actual natural samples and, thus, can be a promising alternative tool for diagnosis and epidemiological studies of *Mb* infection. These results parallel with ***Kumar et al (2011).*** The lower percentage of the isolation than that from PCR might be attributed to the fact that, isolation from chronically affected animals is sometimes difficult, because of the over growing of bacteria of secondary infections or the inhibitory effect of the administrated antibiotics ***(Nicolas and Ayling, 2003)*** or low numbers of organisms in the sample and or the fragility of *M* itself.

In the present study as showed in Tables 1 and 3 out of 61 lungs examined, 17 (27.8%) (to the total no. of samples)were pneumonic . Seven cases (11.4%) (to the total no. of samples) by culture and 10(16.4%)(to the total no. of samples) cases by PCR were positive to *Mb*. So, the result suggested that *Mb* is one of the important pathogens causing pneumonia in goats**.** ***Passchyn et al (2012)*** stated that, *Mb* is a highly infectious pathogen of animal causing pneumonia and many other diseases. *Mb* can cause chronic pneumonia in the face of an immune response and prolonged antibiotic therapy ***(Mihai et al, 2006).*** Also, ***Ikheloa et al (2004***) recovered *Mb* from pneumonic lung of goats.

With all due respect to the literature available, this is the first isolation report of *Mb* from nasal discharge and lungs of goats. These are in accordance with data obtained by ***Nicolas and Ayling (2003); Momani et al (2006) and Kumar et al (2011)*,** who recorded that, there is as such no survey available for the economic losses due to *Mb* infection and little is known about *M species* in sheep and goat. Meanwhile, ***Hassan et al (2011),*** concluded that, significance of *Mb* colonization as a risk factor for the development of clinical disease in the individual animal is unknown and the prevalence of *Mb*-associated disease is also unknown. Besides, only a few studies around the world concerning *Mb* in goats have been conducted.

Phylogenetic analysis: To evaluate sequence variations among *Mb* isolated from pneumonic goats, the nearly complete 16S rRNA gene from 4 selected strains were sequenced. The graphical view of the sequences of it is illustrated in Fig. (2). It is obvious in that, the number (no.) of nucleotide differences of the 4 *Mb* strains varied from 0 to 12. Furthermore, the no. of polymorphic positions between them varied from 0 to 46 polymorphisms. ***Malin et al (2002),*** mentioned that, the no. of nucleotide differences of 8 *Mb* isolates varied from 0 to 10 and the no. of polymorphic positions varied from 0 to 12. Regarding, the similarity differences due to different reasons; these include, isolate purity, DNA extraction

methods, and possible chimeric molecule formation ***(Heikens, 2005).*** All of these problems to some extent affect final identifications. As shown in Table (4) and Figs. (2) and (3) the sequence data for the 4 *Mb* illustrate that, the similarity matrix between, strains no.1 and no. 2 was 98.6% and strains no. 3 and no. 4 were completely identical to each other, the similarity matrix between them was 100%. this does not means that each of two identical strains are the same species but considered different species and these findings confirmed the result which was mentioned in previous literatures, ***Malin et al (1998)*** concluded that, when two 16S rRNA sequences are very similar, it is always difficult to know if the corresponding organisms should be grouped within the same or within different species. This problem has also been observed for other *M*. Furthermore, *M mycoides subspecies capri* and *M mycoides subspecies mycoides* LC type have only 3 nucleotide differences in their 16S rRNA sequences but are still regarded as two different species. Besides, the polymorphisms and sequences differences between isolates of the same species are referred to as intraspecific variations. On the other hand, there are great phylogenetic difference among the both strains no.1 and no.2 varied from both strains no.3 and no.4, they have 46 polymorphisms in positions 69, from 165 to 169, from 172 to 176, from 178 to 180, from 181 to 184, from 186 to188, 190, from 194 to 195, from 199 to 200, from 203 to 204, from 206 to 209, from 211 to 213, from 216 to 222 and from 224 to 228. Meanwhile, the reference strain is slightly identical with both strains no. 1 and no. 2 in which they had 4 polymorphisms in the positions 76 to 78 and 137. Regarding, there are great dissimilarity between reference strain and strains no.3 and no.4 in positions 69, from 76 to 78, 137 and the same 46 polymorphisms in between both strains no. 1 and 2 and both strains no. 3 and no. 4 which was mentioned before. The sequences information was used for the construction of phylogenetic tree. The variations between the 4 strains have resulted in the phylogenic tree in Fig. (3) which shows the close relation between strains no. 1 and no. 2 (in one cluster) and between strains no. 3 and no. 4 (in one cluster), while there are great variations between both strains no. 1 and no.2 and both strains no.3 and no.4. Besides, the reference strain is far from both strains no.3 and no.4 and slightly identical with strains no. 1 and no. 2. These findings were confirmed by the identity matrix in Table 3 where the similarity percentage was 98.2 % between strains no. 1 and no. 2 and was 100 % between strains no. 3 and no. 4. Also, there are great variations between both strains no.

1 and no.2 from both strains no. 3 and no. 4 in which the similarity percentage was 77.7 % for each and there was great similarity difference between the reference strains and strain no. 3 and no. 4 where the similarity percentage was 79.7 for each, and there was slightly identical between the reference strain and both strains no. 1 and no. 2 where the similarity percentage was 98.2%. This means that, these species differ from each other. This came in agreement with ***Malin et al (1998)*** who reported that, however, if the 16S rRNA sequence identity for two species is below 97%, they are likely to represent different species. Therefore, *Mb* was found to have high intraspecific variation in their 16S rRNA gene and the polymorphisms pattern indicated that gene conversion takes place.

From this result it is clear that, 16S rRNA gene sequencing is highly useful for phylogenetic identification of the same strains of *Mb* and this go in hand with ***Gee et al (2003).*** On the other hand, ***Malin et al (1998)*** showed that, the classification of microorganisms is partly based on 16S rRNA gene data because they are supposed to reflect phylogeny gene and it is a preferred choice as diagnostic target for many bacteria. More recently PCR and sequence analysis are becoming more available and affordable, and their applications in the investigation of African *Mycoplasma* isolates have begun to elucidate more information on the epidemiology of these ***agents (Wade et al., 2010).*** They should therefore be used to monitor new epidemics and enhance the surveillance and control of the disease in endemic countries.

The strain-specific polymorphism patterns of the 16S rRNA genes of *Mb* may be used as epidemiological markers for pneumonia in goats due to *Mb*. Similarity with ***Pettersson et al (1998),*** who detected the same similarity among other species of *M*. In this study, an unexpected finding was phylogenetic difference between the 4 strains; this may be due to the individual variation in the nucleotide sequences between the different isolates of the same origin. This fact proved that, 16S rRNA gene is phylogenetic marker for *Mb* taxonomy, and it is one of the most powerful and precise method for determining phylogenetic interrelationships, also, this phylogeny-based identification of this pathogen provides not only a powerful tool for rapid diagnosis but also the basis for etiological studies of this pathogen. These findings confirmed the result which was mentioned in previous literature, ***(Petti et al, 2005 and Rong et al, 2009).***

According to the available literatures there is no data about the phylogenetic sequences on *Mb* isolated from pneumonic goats and this conceded with ***Yuan et al (2011),*** who mentioned that, Mb genome was not published until 2010 and reported the complete

genomic sequence of *Mb* isolated from calves. On the other hand, ***Azza et al (2013)*** reported the phylogenetic sequences of *Mb* isolated from aborted she camel. Also, ***Nicolas and Ayling (2003), and Kumar et al (2011),*** recorded that, there is as such no survey available for the economic losses due to *Mb* infection. Regarding, identification of alternative genes suitable for PCR has been hampered by the lack of sequenced animal mycoplasma genomes ***(Roger et al., 2003).*** Additionally, ***Jechlinger et al (2004)*** stated that, the current knowledge of the molecular basis of pathogenicity of *M* is limited, and their strategies of infection at the molecular and cellular level remain to be elucidated. However, the impression of a single or primary host for each *M species* may need to be modified since the host range of most species has not been fully examined ***(Neimark et al, 2001)*;** this means that, goats *Mb* has not been fully examined. So, there are no researches about the sequence analysis of 16S rRNA of *Mb* derived from pneumonic goats. So, this study represents the first attempt to assess the genetic variability and differ in phylogenecity of 16S rRNA gene of goats *Mb* causing pneumonia at North Sinai Governorate. These findings are concordant with ***Hassan et al (2011),*** who, isolated *M. bovis* from goats milk, in the first time according to previous literatures in Egypt. Meanwhile, they did not show any recovery of *Mb* from pneumonic goats and did not used 16S rRNA gene sequencing of *Mb.*

Regarding, the genetic variability and differ in phylogenecity of 16S rRNA gene of *Mb* recorded in this work, means presence of a novel genotype of this pathogen. The same conclusion was previously reported by ***Michael and Sharon (2007) and Claudio et al (2010),*** who reported that, 16S rRNA can lead to the recognition of novel pathogen from clinical samples and many studies describing new species are solely based upon small subunit sequences or other polyphasic data.

Conclusions:

Overall, the high prevalence rate of *Mb* in nasal swabs and lung samples of goats is alarming in the present investigation, and stressing to further explore the pathogenic role of *Mb* in respiratory problems of goats and its prevalence. The pathogenic role should be investigated in goats in other parts of Egypt. Further investigation of complete genomic sequencing will lead to a better understanding of the physiology, potential pathogenicity and host specificity of *Mb*, and to the development of new prevention and treatment strategies.

Table (1): Incidence of *M. spp*. and *Mb* recovered from pneumonic goats

Samples	No. of samples	No. of *M.spp.* / *Mb*	% to the total No. of samples	% of *Mb* to the total No. of isolates
Nasal swabs from apparently healthy goats	194	40/**15**	20.6/**7.7**	37.5
Nasal swabs from diseased goats	80	25/**7**	31.2/**8.8**	28
Lungs from slaughters goats	50	12/**5**	24/**10**	41.6
Lungs from dead goats	11	5/**2**	45.4/**18.2**	40
Total	335	82/**29**	24.4/**8.7**	35.3

The bold numbers represent *Mb*.

Table (2): Comparison between bacteriological examination and PCR for detection of *Mb* (from selected representative samples no.35)

Samples	No. of samples	Culture		PCR	
		NO.	%	NO.	%
Nasal swabs from apparently healthy goats	10	3	30	5	50
Nasal swabs from diseased goats	10	3	30	6	60
Lungs from slaughters goats	10	3	30	5	60
Lungs from dead goats	5	2	40	3	60
Total	35	11	31.4	19	54.2

Table (3): Comparison between bacteriological examination and PCR according to the total no. of samples for detection of *Mb*.

Samples	No. of samples	No. of *Mb* by culture	% of *Mb* to the total No. of samples by culture	No. of *Mb* by PCR from representative samples (no.35) and from the total samples	% of *Mb* to the total No. of samples
Nasal swabs from apparently healthy and diseased goats	274	22	8	27 (5+22)	9.8
Lungs from slaughters and dead goats	61	7	11.4	10 (3+7)	16.4
Total	335	29	8.6	37	11

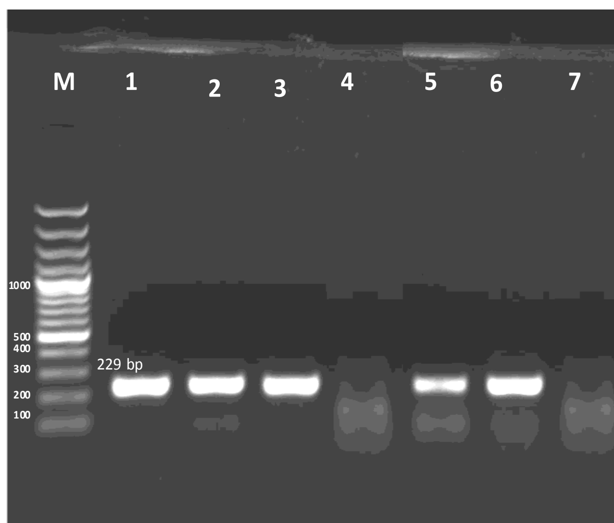

Fig (1): Agarose gel electrophoresis of 16S rRNA PCR product from *Mb*. M represents molecular weight marker showed 100bp-1000bp DNA ladder (Hae 111 digest); Lanes 1 to 3 and 5 represents the positive product of *Mb*, Lane 4 represents the negative product, Lane 6 represents positive control and Lane 7 represents negative control.

Fig. (2) Graphical view of four tested strains

Table (4) Sequence Identity Matrix.

	Refer.	1	2	3	4
refer.	1.000	0.982	0.995	0.794	0.794
1	---	1.000	0.986	0.777	0.777
2	---	---	1.000	0.790	0.790
3	---	---	---	1.000	1.000
4	---	---	---	---	1.000

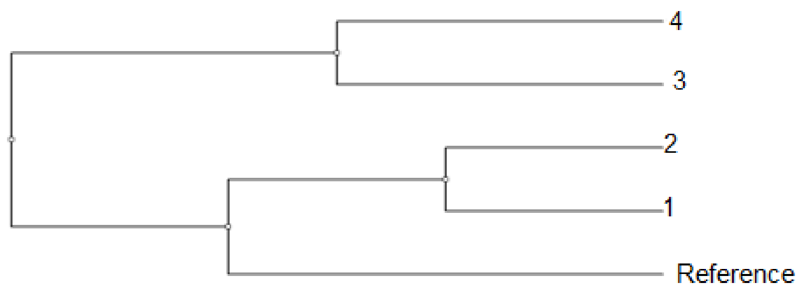

Fig. (3) Phylogenic tree of tested strain.

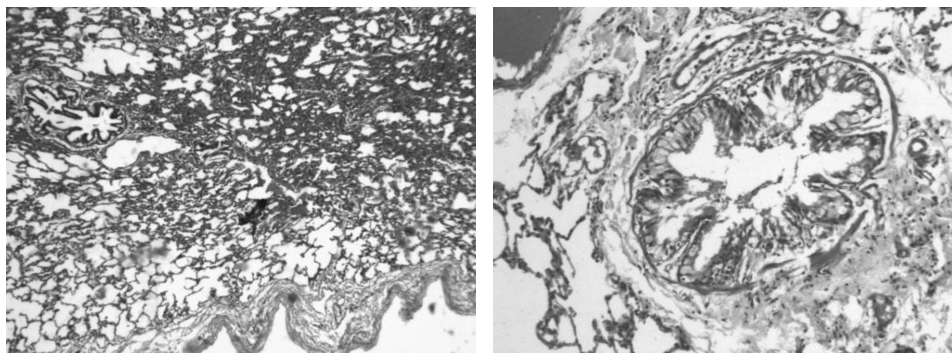

Fig. (4): light micrograph showing Interstial pneumonia, catarrhal bronchitis, alveolar emphysema, fibrinous pleurisy (H&E x4)

Fig. (5): light micrograph of lung showing catarrhal bronchitis (hyperplasia of epithelial lining , the lumen contain inflammatory exudates made from mucous, inflammatory cells). Peribronchiolar edema, lymphocytic infilteration. Congestion of the B.V.Alveolar emphysema (H&E x10).

References:

Abo-Elnaga, T. R., Azza S.A. Gouda, Kamel, Y. M., Mona A. Mahmoud and Wafaa A. Osman 2012: Application of the Polymerase Chain Reaction for Identification of mycoplasma Isolated from Aborted She Camel. Global Veterinaria 8 (4): 386 -392.

Amit Kumar, Amit K. Verma, Neeraj K. Gangwar and Anu Rahal, 2012: Isolation, Characterization and Antibiogram of *Mycoplasma bovis* in Sheep Pneumonia. Asian Journal of Animal and Veterinary Advances. V: 7: Issue: 2: P.: 149-157.

Awan M A., Abbas F., Yasinzai M., Nicholas R A J., Ayling M.A.A and Ahmed Z., 2009: Prevalence of Mycoplasma capricolum subspecies capricolum and *Mycoplasma putrefaciens* in goats in Pishin District of Balochistan Pakistan Vet. J., 29(4): 179-185.

Azza, S.A. Gouda; Wafaa, A. Osman; Kamel ,Y. M.; Abo - Elnaga T. R and Mona, A. Mahmoud, 2013: Sequence analysis of *Mycoplasma bovis* isolated from aborted she-camel with 16S rRNA specific primers. J. Egypt vet. med. Asso. vol. 73 No.3, p.383-400.

Babak K., Seyed A P., Mohammad G N., Mansour B. and Abbas A. 2011: Detection of *Mycoplasma agalactiae* by culture and polymerase chain reaction

(PCR) methods from Iranian goats African Journal of Microbiology Research Vol. 5(13), pp. 1668-1672, 4 July,

Bancroft, J.D. and Gamble, M. 2002: Theory and practice of histological techniques. In: Swisher, B. (Ed.), Microorganisms. Churchill Livingstone, Philadelphia: 325–344.

Claudio T. S., Anne M. W., Leonard W. M., and Tanja P., 2010: Sequencing of 16S rRNA Gene: A Rapid Tool for Identification of *Bacillus Anthracis*. Emerging Infectious Disease Journal.

Clyde, W.A. (1983): Growth inhibition test. Methods on Mycoplasmology Vol. 1,405. Academic Press, New York.

DaMassa Al J., Patricia S. Wakenell and Dale L. B. 1992: Mycoplasmas of Goats and Sheep. J. Vet. Diagn. Invest. 4: 101-113.

Egwu GO, Ball HJ, Rodriguez F, Fernadex A. 2000: *Mycoplasma capricolum* subspecies *capricolum, Mycoplasma mycoides* subspecies *mycoides* LC *and Mycoplasma mycoides* subspecies *capri* in agalactia syndrome of sheep and goats. Vet. Bull. 70: 391- 402.

Egwu G. O., Adamu M., Mshelia G. D. and Bukar-Kolo Y. M. 2012: Isolates of *Mycoplasma mycoides* subspecies *mycoides (SC)* in small ruminants in Sahel zone of Nigeria and its implications on disease control African Journal of Biotechnology Vol. 11(23), pp. 6396-6401.

Gee J.E., Sacchi C. T., Whitney A.M., Hoffmaster A. R., and Popovic T. 2003: Use of 16S rRNA gene sequencing for rapid identification and differentiation of *Burkholderia pseudomallei* and *B. mallei*. J. Clin. Microbiol. 41:4647-4654.

Gehad, 2010: Impact of El Salam canal on environment and some biological parameters of female goats in northern Sinai, Egypt. PhD thesis in Environmental Science Ain Shams University.

Ghadersohi, A.; Coelen, R.J. and Hirst, R.G. 1997: Development of a specific DNA probe and PCR for the detection of *Mycoplasma bovis.* Vet. Microbiol. 56:87-98.

Gonçalves R, I Mariano, A Núñez, S Branco, G Fairfoul and R Nicholas, 2010: Atypical non-progressive pneumonia in goats. Vet J, 183: 219–221.

Hassan, W.H., Mona, A. El-Shabrawy; Hakim, A.S., Azza, S.M. Abuelnaga, Samy A. A and Sadek E. G., (2011): Comparison between Molecular and Classical Techniques for Identification of *Mycoplasma* species Isolated from Mastitic Ruminants. Journal of American Science,;7(1): 506-513.

Heikens E., A., Fleer A., Paauw A., Florijn and Fluitt A.C., 2005: Comparison of genotypic and phenotypic methods for species-level identification of clinical isolates of coagulase-negative staphylococci. J. Clin. Microbiol. 2286-2290. - Fox G. E

Hotzel, H.; Sachse, K. and Pfutzner, H. 1996: Rapid detection of *Mycoplasma bovis* in milk samples and nasal swabs using the polymerase chain reaction. J. Appl. Bacteriol. 80: 505-510.

Ikheloa J.O., Ajuwape A.T.P. and Adetosoye A.I., 2004: Biochemical characterization and serological identification of mycoplasmas isolated from pneumonic lungs of goats slaughtered in abattoirs in Northern Nigeria. Small Ruminant Research. V. 52, Issue 1 , P. 93-97.

Jechlinger W., Chopra D R., Glew M., Citti C. and Rosengarten R. 2004: Molecular basis of *Mycoplasma agalactiae* pathogenicity. Berl Munch Tierarztl Wochenschr; 117(11-12):472-9.

Krešimir M., Selma P., Hywel B., Franjo M., Miljenko M., and Branka Š M., 2013: Comparison of antigen capture ELISA and PCR for detection of *Mycoplasma bovis* in milk samples**.** European Mycoplasma Meeting: Mycoplasmas – A Practical Approach 6th-7th June.

Kumar A., Verma, A.K. and Rahal, A. 2011: *Mycoplasma bovis* a multi disease producing pathogen: Asian J.of Animal and Veterinary Advanced 6 (6): 537-546.

Kwoks S. and Higuch, R. 1989: Avoiding false positive with PCR. Nature (London), 339: 237-238.

Malin H.; Joseph G. T., and Karl, E. J. 1998: Sequences of the 16S rRNA genes and phylogeny of the *goat Mycoplasmas Mycoplasma adleri, Mycoplasma auris, Mycoplasma cottewii* and *Mycoplasma yeatsii.* International J. of Systemic bacteriology. 48, p. 263-268.

Malin Heldtander and Karl-Erik Johansson , 2002: Intraspecific variation in the 16S rRNA gene sequences of Mycoplasma agalactiae and Mycoplasma bovis strains. Veterinary Microbiology Volume 85, Issue 3, P. 209**.**

Maunsell FP , Woolums AR., Francoz D., and Janzen E.D. 2011: *Mycoplasma bovis* Infections in Cattle .Journal of Veterinary Internal Medicine**.** Volume 25, Issue 20 4, p. 772.

Michael Janda and Sharon L. Abbott, 2007: 16S rRNA Gene Sequencing for Bacterial Identification in the Diagnostic Laboratory. J. clin. microbio. v. (45) 9: 2761.

Mihai Gagea, Kenneth G. Bateman, Rachel A. Shanahan, Tony van Dreumel, Beverly J. and McEwen, *et al.,* 2006: Naturally occurring *Mycoplasma bovis*–associated pneumonia and polyarthritis in feedlot beef calves J Vet Diagn Invest 18:29–40.

Miklós Tenk 2005: Examination of *Mycoplasma bovis* infection in cattle. Doctoral Thesis. Szent Istvan University Postgaduate School of Veterinary Science Budapest.

Mohammad K., Seyed A P., Abbass A., Babak R., and Homan K. 2012: Isolation and prevalence of *Mycoplasma agalactiae* in Kurdish sheep in Kurdistan, Iran.

Vet. World, 2012, Vol.5(12): 727-731.

Momani, A. W.; Halablab, M. A.; Abo-Shehada, M. N.; Miles, K. and McAuliffe, L. 2006: Isolation and molecular identification of small ruminant mycoplasmas in Jordan. Small Ruminant Research 65: 106–112.

Moradi Bidhend, S. Khaki, P. Pilehchian Langroudi, 2011: Isolation and identification of *Mycoplasma agalactiae* by culture and Polymerase Chain Reaction in Sheep and Goat Milk Samples in Kordestan province, Iran**:** Archives of Razi Institute, Vol. 66, No. 1, June 11-16.

Neimark, H., Johansson, K.E. Rikihisa, Y., and Tully, J.G., 2001: Proposal to transfer some members of the genera Haemobartonella and Eperythrozoon to the genus Mycoplasma with descriptions of' *Candidatus Mycoplasma haemofelis', 'Candidatus Mycoplasma haemomuris' 'Candidatus Mycoplasma haemosuis' and'Candidatus Mycoplasma wenyonii'*. International Journal of Systematic and Evolutionary Microbiology, 51, 891-899.

Nicolas, R.A.J. and D. Ayling, 2003: *Mycoplasma bovis* disease, diagnosis and control. Res. Vet. Sci., 74: 105-112.

Nicholas, R., Ayling, R., and McAuliffe, L., 2008: Respiratory diseases of small ruminants. In: Nicholas, R., Ayling, R., Mcauliffe, L., Eds. Mycoplasma Diseases of Ruminants. CABI, Wallingford, UK; 171–179. 37: 352-354.

Ongor H., Kalin R., Karahan M.,Cetinkaya B. and McAuliffe L. 2008: Isolation of *Mycoplasma bovis* from broiler chickens in Turkey. Avian Pathol., 18,1-2.

Ongor H., Kalin R., Karahan M., Çetinkaya B. and Akan M., 2009: Detection of mycoplasma species in turkeys by culture and polymerase chain reaction. Rev. sci. tech. Off. int. Epiz., 28 (3), 1103-1109.

Passchyn P, Piepers S, De Meulemeester L, Boyen F, Haesebrouck F.,and De Vliegher S. 2012: Between-herd prevalence of *Mycoplasma bovis* in bulk milk in Flanders, Belgium. Res Vet Sci. ;92(2):219-20.

Pettersson B, Bölske G, and Johansson K. E. (1998): Molecular evolution of *Mycoplasma capricolum subsp. capripneumoniae* strains based on polymorphisms in the 16S rRNA genes J. Bacteriol. 180(9):2350 - 8.

Petti C. A., Polage C. R. and Schreckenberger P., 2005: The Role of 16S rRNA Gene Sequencing in Identification of Microorganisms Misidentified by Conventional Methods. J. Clin Microbiol. December; 43(12): 6123 - 6125.

Roger D. Ayling, Richard J. Ellis, and Laura McAuliffe, 2003: Differentiation of mycoplasma Species by 16S Ribosomal DNA PCR and Denaturing Gradient Gel Electrophoresis Fingerprinting. J

Clin Microbiol. October; 41(10): 4844 - 4847.

Rong Q. Z, Kui N., Zuo-Y. Z. and Hong-L.L., 2009: Phylogenetic analysis of *Mycoplasma sius* isolates based on 16S rRNA gene sequence in China Vet Res Commun 33:855- 863.

Ruhnke, H.L. and Rosendal, S., 1989: Useful protocols for diagnosis of animal mycoplasmas. WAVLD Mycoplasma Workshop Vet. Microbiol. And Immunol. Dept. Ontario Vet. Collage, Guelph, Ontario, Canada NIC 2W1.

Sharif, A. and G. Muhammad, 2009. Mastitis control in dairy animals. Pakistan Vet. J., 29(3): 145-148.

Sheehan M, Cassidy JP, Brady J, Ball H, Doherty ML, and Markey BK., 2007: An aetiopathological study of chronic bronchopneumonia in lambs in Ireland. Vet J, 173: 630–637.

Ter Laak, E.A., Wentink G.H. and Zimmer G.M., 1992: Increased prevalence of *Mycoplasma bovis* in the Netherlands. Vet. Q., 14: 100-104.

Wade A., 2010: Isolation and characterization of *Mycoplasma mycoides* subsp *mycoides* small colony from the north and far-north regions of Cameroon. Unpublished MVSc dissertation, Univ. Maiduguri, Nigeria.

Waseem S., Rashid M., Mohammad S. K., Mansur-ud-Din A. M. and Rashid A., 2012: Characterization, Molecular Diagnosis and Prevalence of Caprine Mycoplasmosis in Different Areas of Pakistan Pakistan J. Zool., vol. 44(2), pp. 559-568.

Wit, M.Y.L.; Douglas, J.T.; McFadden, J. and Klatser, P.R., 1993: Polymerase chain reaction for the detection of *Mycobacterium leprae* in nasal swab specimen. J. Clin. Microbiol. 31: 502-506.

Yuan L, Huajun Z, Yang L, Yanwei J. and Zhiqiang S., 2011: The Complete Genome Sequence of *Mycoplasma bovis* Strain Hubei-1. PLoS ONE 6(6): e20999. doi: 10.1371/ journal.pone.0020999.

Zendulkova, D., Madanat, A., and Lany, P., 2007: Detection of *Mycoplasma agalactiae* by Polymerase Chain Reaction in Jordanian Sheep and goatherds. Acta. Vet. Brono., 76:71-77.

Zinka M., Christian DE L., and Maid R., 2013: Presence of Mycoplasmas in the respiratory system of small ruminants managed under an extensive production system.Turk. J.Vet.Anim. Sci.

الملخص العربى

القرابة الجينية للميكوبلازما بوفيس المعزولة من الماعز المصاب بالالتهاب الرئوى بناءا على تتابع جين 16S rRNA فى ترعة السلام بمحافظة شمال سيناء.

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قسم صحة الحيوان - مركز بحوث الصحراء

تعتبرميكوبلازما بوفيس من الميكروبات التنفسية الممرضة للمجترات والتى تؤثر سلبا علي الأقتصاد. لذا فقد قامت هذة الدراسة على اثبات تواجد هذا الميكروب فى الماعز المصابة بالالتهابات الرئوية المصحوبة بالاعراض التنفسية مع دراسة الفروق التركيبية الجينية بين العترات المعزولة لهذا الميكروب بمنطقة ترعة السلام بمحافظة شمال سيناء. فتم اخذ ٣٣٥ عينة من الماعز المصابة بأعراض تنفسية (٢٧٤ مسحة انفية و ٦١ مسحة نسيجية من الرئات المصابة) وذلك لمعرفة مدى تواجد الميكوبلازما وخاصة ميكوبلازما بوفيس باستخدام العزل البكتيرى و جهاز البلمرة المتسلسل معتمدا على البادئ المتخصص لجين 16S rRNA للميكوبلازما بوفيس. فوجد أن ميكوبلازما بوفيس قد وجدت بنسبة ٨% و٩,٨% و١١,٤% و ١٦,٤% فى المسحات الانفية والرئات المصابة بواسطة العزل البكتيرى و جهاز البلمرة المتسلسل معتمدا على البادئ المتخصص لجين 16S rRNA على التوالى (وهذا بالنسبة لعدد العينات الكلى) . اثبتت نتائج الدراسة أن ميكروب ميكوبلازما بوفيس من أهم مسببات الالتهابات الرئوية فى الماعز. كما أثبتت الدراسة أن هناك فروق فى التركيب الجيني بين العترات المعزولة للميكوبلازما بوفس مما يعني انه يوجد سلالات جديدة من هذا الميكروب. وقد دعم الهستوباثولوجى أعراض الميكوبلازما.

وتعتبر هذة الدراسة أول دراسة للتركيب الجيني لجين 16S rRNA للميكوبلازما بوفيس المعزولة من الماعز المصابة بالالتهاب الرئوى فى هذة المنطقة.