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MOLECULAR IDENTIFICATION OF RESPIRATORY BOVINE *MYCOPLASMA* ISOLATED FROM ARABIAN CAMELS IN EGYPT

By

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SUMMARY

A total of 369 samples were collected from adult Arabian camels for bacteriological examination of *Mycoplasma* associated with Respiratory signs. A 271 nasal swabs (from 126 diseased and 145 apparently healthy camels) and 98 lung tissues (53 abnormal and 45 apparently normal lungs) were examined bacteriologically and Mycoplasmas were recovered from 67 (18.2 %) samples, which were differentiated using Digitonin sensitivity test into *Mycoplasma* (35) and *Acholeplasma* (32) in percentages of 52.2 and 47.8 % of Mycoplasmas, and of 9.5 and 8.7 % of examined samples, respectively. *Mycoplasma* were differentiated according to biochemical reactions into biochemical groups III, IV and V in percentages of 68.6, 17.1 and 14.3% of isolated *Mycoplasma* and of 6.5, 1.6 and 1.4% of examined samples, respectively. All *Mycoplasma* isolates were identified molecularly on the genus level using PCR for detection of 16S rRNA gene, and on the species level as *M. bovis* and *M. bovirhinis* using PCR for detection of specific 16S rRNA gene for each species. *Mycoplasma* species were recovered from nasal swabs and lung tissues constituted 82.9 and 17.1% from isolated *Mycoplasma* (35), and recovered from nasal swabs, lung tissues and total samples in percentages of 10.7, 6.1 and 9.5 %, respectively. *Mycoplasma* was isolated from diseased and apparently healthy camels in percentages of 7.3 and 11.6 %, respectively. *Mycoplasma bovis* recovered from nasal swabs and lung tissues constituted 91.7 and 8.3% from totally isolated *M. bovis* (24), and recovered from nasal swabs, lung tissues and total samples in percentages of 8.1, 2 and 6.5%, respectively. *Mycoplasma bovis* was isolated from diseased and apparently healthy camels in percentages of 4.5 and 8.4%, respectively. *Mycoplasma bovirhinis* recovered from nasal swabs and lung tissues constituted 33.3 and 66.7% from totally isolated *M. bovirhinis* (6), and recovered from nasal swabs, lung tissues and total samples in percentages of 0.7, 4.1 and 1.6 %, respectively. *Mycoplasma bovirhinis* isolated from diseased and apparently healthy camels in percentages of 1.7 and 1.6%, respectively.

Keywords:

Arabian Camels, Respiratory, Nasal swabs, Lung, Mycoplasma and PCR.

INTRODUCTION

Camel is a multi-purpose animal with a huge productive potential and the most suitable domestic animal for use in climatic extremes **(Wernery, 2007**). The world's camel population of about 28 million heads, 80% of them lives in Africa, with 60% in the Horn of Africa and Arabian camels (Dromedaries) constitute 94% of the world's camel population **(FAOSTAT, 2014 and Yam and Khomeiri, 2015)**.

Mycoplasmas, belongs to class *Mollicutes*, in which *Mycoplasma* and *Acholeplasma* genera are included **(Stipkovits and Kempf, 1996)**. *Acholeplasma* has no known clear role in the disease occurrence **(O' Leary, 1989).** *Mycoplasma*, under certain conditions, either solely or with bacteria and/or viruses, may cause health disorders such as pneumonia **(Freundt, 1985)**. *Mycoplasma bovis* and *M. bovirhinis* were isolated from camels suffering from pneumonia **(Dardeer and Refat, 2004 and Hala, 2004).** *Mycoplasma bovis* is the most pathogenic and important bovine *Mycoplasma* causing severe economic losses worldwide **(Kumar *et al.,* 2011 and Maunsell *et al.,* 2011).**

Bacteriological isolation and identification of *Mycoplasma* species is difficult and time consuming, therefore molecular identification of *Mycoplasma* using PCR targeting specific genes considered a specific and sensitive method for identification **(Rong *et al.,* 2009)**.

MATERIAL AND METHODS

Animals and samples:

A total number of 369 adult Arabian camels from Northwestern coastal zone and El-Basatain abattoir were examined during the period from August 2014 to June 2016 for respiratory infection caused by *Mycoplasma*. Live camels were examined clinically for presence of respiratory signs as cough and nasal discharges, whereas slaughtered camels were examined for the pathological condition of their lungs.

Examined animals were classified according to health condition into 179 diseased (126 live and 53 slaughtered) and 190 apparently healthy (145 live and 45 slaughtered) camels.

A 271 nasal swabs (from 126 diseased and 145 apparently healthy camels) and 98 lung tissues (53 abnormal and 45 apparently normal lungs) with a total number of 369 samples were collected for bacteriological examination. Lungs showed consolidation, grey and red hepatization considered abnormal.

A sterile cotton swabs were inserted into nostrils of examined camels, immediately inoculated into Modified Hayflick's broth medium **(Rosendal, 1994)**. Lung tissues collected from slaughtered camels were packed in sterile plastic bags separately. Samples were transported in an icebox to the laboratory for immediate bacteriological examination or stored in a refrigerator (4°C) or in deep freeze (-20°C) until be examined.

Bacteriological isolation and biochemical identification:

The collected lung tissues were seared in the laboratory using a red-hot spatula, cut open and swab was taken from the cut and inoculated into Modified Hayflick's broth medium.

Swabs from nostrils and lung tissues were cultivated on Modified Hayflick's solid medium **(Rosendal, 1994)** by direct method (samples were spread directly on agar plates) and indirect method (samples were inoculated into broth, which incubated for 3 and 6 days then plated, or re-inoculated in broth which plated after 3 and 6 days incubation).

All plates were incubated at 37°C in humidified candle jar for two weeks and examined microscopically using stereomicroscope (10X, Olympus CKX41) every 2-3 days until "fried egg" colonies appeared, followed by purification of the isolates according to **Sabry and Ahmed (1975)**. Isolated Mycoplasms colonies were maintained at-20°C either as 2-3 ml aliquots of actively growing broth culture or as agar blocks/strips in a sterile screw capped vials.

Purified Mycoplasms isolates were tested for Digitonin sensitivity **(Freundt, 1983)** to be differentiated into *Mycoplasma* (Digitonin sensitive, showing 6 - 10 mm zone of inhibition around Digitonin discs) and *Acholeplasma* (Digitonin resistant, showing no inhibition zone).

Identified isolates as *Mycoplasma* were tested for Arginine utilization, Glucose fermentation **(Sabry, 1968**) and Film and spot formation **(Cottew, 1983)**. Classified into 5 biochemical groups (From I to V).

Molecular identification of Mycoplasma species using PCR:

Molecular identification of all biochemically-grouped *Mycoplasma* on the genus level was done using PCR for detection of 16S rRNA gene belonging to all *Mycoplasma*, followed by identification on the species level for detection of specific 16S rRNA gene for each of *M. bovis* and *M. bovirhinis* and *ArcB* gene for *M. putrefaciens*.

Reference strains of *M. bovis* and *M. bovirhinis* (Reference GenBank: NCTC10131 and NCTC10118, respectively, kindly provided from Department of Mycoplasmology, Animal

Reproduction Research Institute, Giza, Egypt) were used as a positive control for molecular identification.

All biochemically identified *Mycoplasma* isolates and reference strains were subjected to DNA extraction through heating incubated broth culture in a heat block at 100 ºC for 10 min., cooling on ice for 10 min., centrifugation at 13,000 rpm for 3 min., the supernatant was aspirated and used as DNA template for PCR **(Fan *et al.*, 1995)**.

Each PCR tube included a mixture of 5 µl master mix (Jena Bioscience, Germany), 1 µl of each specific forward and backward primers (Metabion, Germany), 13 µl PCR grade water (Jena Bioscience, Germany) and 5 µl DNA template, with a total volume of 25 µl. PCR tubes gently mixed, placed in the thermal cycler (Biometra, Germany) for amplification of target genes. Each 10 µl of PCR product from each reaction and 5 µl of a 100 - 1000 Molecular weight DNA marker (Jena Bioscience, Germany) were mixed with 2 µl of gel loading buffer (Jena Bioscience, Germany), electrophoresed in 1.5% agarose gel stained with ethidium bromide for detection of target band. Gel visualized using ultraviolet trans-illuminator (BioRad, USA), photographed using Digital Camera (Sony Cyber-shot, Japan) and analyzed using Gel proanalyzer® v4 todetermine the product size.Target genes,specific oligonucleotide primers sequences, DNA amplification programs and product size presented in Table (1).

RESULTS

Bacteriological isolation of 369 nasal swabs and lung tissues collected from diseased and apparently healthy camels was done on Modified Hayflick's solid medium, colonies with a typical "fried-egg" appearance characteristic to Mycoplasmas (Photo 1) were recovered in 67 (18.2 %) samples.

The isolated 67 Mycoplasmas colonies differentiated using Digitonin sensitivity test (Photo 2) into 35 as *Mycoplasma* (Digitonin sensitive) and 32 as *Acholeplasma* (Digitonin resistant) in percentages of 52.2 and 47.8 % of isolated Mycoplasmas, respectively, and in percentages of 9.5 and 8.7% of examined samples, respectively.

The identified 35 *Mycoplasma* isolates were tested for Arginine utilization, Glucose fermentation and Film and spot formation, according to the resulting biochemical reactions *Mycoplasma* differentiated into three biochemical groups (Table 2) identified as groups III, IV and V in percentages of 68.6, 17.1 and 14.3% of the isolated *Mycoplasma*, respectively and in percentages of 6.5, 1.6 and 1.4% of examined samples, respectively. Biochemical groups I and II were unidentified.

All of the 35 *Mycoplasma* isolates, which biochemically-grouped into groups III, IV and V, were molecularly identified on the genus level using PCR for detection of 16S rRNA gene (Photo 3).

Molecular identification on the species level was applied on *Mycoplasma* isolates belong to biochemical groups III (24), IV (6) and V (5) for detection of 16S rRNA gene specific for *M. bovis*, 16S rRNA gene specific for *M. bovirhinis* and *ArcB* gene specific for *M. putrefaciens*, respectively. All isolates belong to groups III and IV were molecularly identified as *M. bovis* (Photo 4) and *M. bovirhinis* (Photo 5) using PCR, respectively, whereas isolates belong to group V were unidentified as *M. putrefaciens* using PCR (Table 2).

The percentages of molecularly identified *Mycoplasma* species recovered from nasal swabs and lung tissues collected from diseased and apparently healthy camels were calculated in relation to the number of isolated *Mycoplasma* of the same species and to the number of the examined samples with consideration of health condition (Table 3).

Mycoplasma species recovered from nasal swabs and lung tissues constituted 82.9 and 17.1% from totally isolated *Mycoplasma* (35), and recovered from nasal swabs, lung tissues and total samples in percentages of 10.7, 6.1 and 9.5%, respectively. *Mycoplasma* isolates were recovered from nasal swabs collected from diseased and apparently healthy camels in percentages of 7.1 and 13.8%, whereas those isolated from lung tissues collected from diseased and apparently healthy camels in percentages of 7.6 and 4.4%, with total *Mycoplasma* isolation percentages of 7.3 and 11.6% from all samples collected from diseased and apparently healthy camels, respectively.

Mycoplasma bovis recovered from nasal swabs and lung tissues constituted 91.7 and 8.3% from all isolated *M. bovis* (24), and were recovered from nasal swabs, lung tissues and total samples in percentages of 8.1, 2 and 6.5%, respectively. *Mycoplasma bovis* isolates were recovered from nasal swabs collected from diseased and apparently healthy camels in percentages of 5.6 and 10.3%, whereas those isolated from lung tissues collected from diseased and apparently healthy camels in percentages of 1.9 and 2.2%, with total *M. bovis* isolation percentages of 4.5 and 8.4% from all samples collected from diseased and apparently healthy camels, respectively.

Mycoplasma bovirhinis recovered from nasal swabs and lung tissues constituted 33.3 and 66.7% from all isolated *M. bovirhinis* (6), and recovered from nasal swabs, lung tissues and total samples in percentages of 0.7, 4.1 and 1.6%, respectively. *Mycoplasma bovirhinis*

isolates were recovered from nasal swabs collected from diseased and apparently healthy camels in percentages of 0 and 1.4%, whereas those isolated from lung tissues collected from diseased and apparently healthy camels in percentages of 5.7 and 2.2%, with total *M. bovirhinis* isolation percentages of 1.7 and 1.6% from all samples collected from diseased and apparently healthy camels, respectively.

Mycoplasma species belong to biochemically identified group V and molecularly unidentified recovered from nasal swabs, lung tissues and total samples in percentages of 1.9, 0 and 1.4%, with total isolation percentages of 1.1 and 1.6% from all samples collected from diseased and apparently healthy camels, respectively.

DISCUSSION

Researches concerned with the ability of camels in resisting commonly known diseases had been well documented, whereas those concerned with *Mycoplasma* infection are still lacking **(Almaw and Molla, 2000 and Abbas and Omer, 2005)**.

Bacteriological isolation of 369 nasal swabs and lung tissues collected from diseased and apparently healthy camels revealed Mycoplasmas in 67 (18.2%) samples, which is nearly similar to those recorded by **El-Shabiny *et al.* (1998) and Abd El-Wahed (2001)** as 24 and 10.6%, respectively. **Dardeer and Refat (2004) and El-Ghazali *et al.* (2011)** recorded higher and lower percentages as 78.3 and 0.6%, respectively. Low isolation percentage of Mycoplasmas attributed to the presence of toxic or inhibitory substances in camel's tissue that hinder their cultivation **(Taylor-Robinson and Chen, 1983 and Tully and Whitcomb, 1992)**, in addition to the fastidious nature of many pathogenic *Mycoplasma* species**(Mederos-Iriarte *et al.*, 2014 and Tille, 2014)**.

The isolated 67 Mycoplasmas isolates were differentiated using Digitonin sensitivity test into *Mycoplasma* (35) and *Acholeplasma* (32) in nearly equal percentages of 52.2 and 47.8%, respectively, while **Dardeer and Refat (2004)** recorded higher isolation percentage (83%) of *Mycoplasma* and lower isolation percentage (17%) of *Acholeplasma*, whereas **El-Faki *et al.* (2002) and El-Gmaal (2007)** isolated only *Mycoplasma* without recovery of *Acholeplasma* from camels. Differences in isolation percentages may be attributed to the effect of environmental (including laboratory) temperature and relative humidity on the survivability of *Mycoplasma* and *Acholeplasma* **(Whittlestone, 1976; Jones and Webster, 1984 and Nagatomo, 2001)**.

The isolated *Mycoplasma* (n=35) were differentiated according to the resulting biochemical reactions into three biochemical groups III, IV and V in percentages of 68.6, 17.1 and 14.3%, respectively, whereas biochemical groups I and II were unidentified. Group III was recorded in percentages of 12.3, 12.8, 35.9 and 17.7%, whereas Group IV was recorded in percentages of 52, 38.5, 7.7 and 55.6% according to **Abd El-Wahed (2001), Dardeer and Refat (2004), Hala (2004) and El-Gmaal (2007),** respectively, they failed to identify groups II and V, and succeeded to identify group I in percentages of 35.7, 35.3, 35.9 and 26.7%, respectively.

All of the 35 *Mycoplasma* isolates were molecularly identified on the genus level using PCR for detection of 16S rRNA gene. On the species level, *M. bovis* (n=24, 68.6%) and *M. bovirhinis* (n=6, 17.1%) were identified molecularly using PCR for detection of specific 16S rRNA gene for each species. The same gene was targeted by **Dardeer and Refat (2004), Hala (2004) and El-Gmaal (2007)** for molecular identification of *M. bovis* and *M. bovirhinis* (isolated from respiratory system of camels) using PCR. None of the tested *Mycoplasma* isolates belong to biochemical group V using PCR targeting *ArcB* gene was identified as *M. putrefaciens* (Table 1and 2), there was no clear explanation for the failure of molecular identification especially in the absence of reference strain to *M. putrefaciens*.

Mycoplasma that recovered from nasal swabs and lung tissues constituted 82.9 and 17.1% from the totally isolated *Mycoplasma*, which nearly in agreement with **El-Gmaal (2007),** who recorded isolation percentages of 87.7 and 12.3%, respectively.

Mycoplasma recovered from nasal swabs in percentage of 10.7%, which is nearly in agreement with **Abd El-Wahed (2001)** and disagree with **El-Gmaal (2007)**, where they recorded isolation percentages as 7% and 23.8%, respectively. *Mycoplasma* recovered from lung tissues in percentage of 6.1%, which is nearly in agreement with **El-Faki *et al.* (2002)** and **El-Gmaal (2007)**, who obtained recovery percentages of 8.2% and 8.8%, respectively. From the totally examined samples *Mycoplasma* were recovered in percentage of 9.5%, as that (9%) obtained by **Abd El-Wahed (2001)**, and lower than that obtained by **El-Faki *et al.* (2002) and El-Gmaal (2007)** who recorded a percentages of 16 and 19.7%, respectively. The somewhat higher isolation percentage from nasal swabs than those recovered from lung tissues may be attributed to early infection and colonization of *Mycoplasma* at nostrils mucosa **(Uhaa *et al.*, 1990 a & b)**.

From diseased camels, *Mycoplasma* were recovered from nasal swabs and total samples in a relatively lower percentages (7.1 and 7.3%) than those from apparently healthy camels (13.8 and 11.6%), respectively. The relative lower isolation percentage from diseased animals may be attributed to the fragile nature of *Mycoplasma* in the environment due to lack of cell wall, consequently, a successful infection is restricted to close and repeated aerosol transmission **(Nicholas, 2004)** and also to the commensal nature of *Mycoplasma* which become infective as a result of decreased immunity following stress, resulting in subclinical infection which may be later on turned to clinical infection **(Ruffin, 2001)**, in addition to the overgrow of concurrent secondary bacterial infection and/or antibiotic administration during disease condition **(Nicholas and Ayling, 2003)**.

Mycoplasma recovered from pneumonic lungs in relatively higher percentage (7.6%) than that of apparently normal lungs (4.4%). **El-Gmaal (2007), Hasaneen *et al.* (2013) and Chitgar *et al.* (2014)**, recovered *Mycoplasma* from pneumonic lungs in percentages of 8.8, 8 and 8.2%, respectively. The relatively higher isolation percentage from pneumonic lungs may be attributed to that, the junction of alveolar duct and gas exchange site is the most susceptible site for *Mycoplasma* colonization than upper respiratory tract **(Caswell and Williamas, 2007)** with a concurrent decreased immunity resulting from stress **(Ruffin, 2001)**.

Mycoplasma bovis recovered from nasal swabs, constituted 91.7% from total *M. bovis* isolates, whereas **El-Gmaal (2007)** recorded a lower recovery rate (66.7%). *Mycoplasma bovis* live as commensal in upper respiratory tract **(Trevor, 1997)** and most of infected animals show inapparent infection and probably act as a chronic carriers **(Chavez *et al.,* 1995)** and the microorganism easily transmitted between sheep, goats, cattle and camel (**Pitcher and Nicholas, 2005)** and considered as a primary cause of pneumonia **(Mihai *et al.,* 2006)**. *Mycoplasma bovis* that recovered from lung tissues, constituted 8.3% from total *M. bovis* isolates, this disagrees with **Hala (2004) and El-Gmaal (2007)** who detected a higher detection rates as 42.1 and 33.3%, respectively. Low isolation rate may be attributed to the required needs of repeated / optimal sampling, optimal sample storage and decrease lapse of time between sampling and laboratory examination **(Nicholas and Ayling, 2003 and Kumar *et al.,* 2011)**.

Mycoplasma bovis recovered from nasal swabs in percentage of 8.1%, which is higher than that (1.4%) obtained by **El-Gmaal (2007)**, and recovered from lung tissues in percentage of 2%, which is nearly similar (0.7%) to that obtained by **El-Gmaal (2007)**.

Mycoplasma bovis recovered from total samples in percentage of 6.5%, which disagree with **Abd El-Wahed (2001), Dardeer and Refat (2004), Hala (2004) and El-Gmaal (2007)** who recorded isolation percentages as 12.3, 12.8, 35.9 and 2.1%, respectively. Low isolation percentage regarded to low numbers of organisms in the sample as a result of intermittent shedding **(Maunsell *et al.*, 2011),** and the high sensitivity of the microorganism to environmental factors such as transportation, in addition to specific requirements needed during bacteriological cultivation **(Ayling *et al.*, 2004)**.

Mycoplasma bovis recovered from nasal swabs collected from diseased and apparently healthy camels in percentages of 5.6 and 10.3%, respectively. The relative higher isolation percentage from apparently healthy attributed to the inapparent or chronic nature of infection and / or antibiotic treatment **(Adegboye *et al.*, 1995)**.

Mycoplasma bovis recovered from lung tissues collected from diseased and apparently healthy camels in percentages of 1.9 and 2.2%, respectively. The relative lower isolation percentage from abnormal lungs may be attributed to the poor lung condition and/or gross bacterial contamination that inhibit isolation **(Adegboye *et al.*, 1995)**.

Mycoplasma bovis isolated in percentages of 4.5 and 8.4% from total samples collected from diseased and apparently healthy camels, respectively. Apparently healthy camels can harbor *M. bovis* in the respiratory tract without showing clinical signs and shed in nasal discharges for month or years as **(Pfützner, 1990**), consequently, act as source of infection for *M. bovis*, which considered as a primary cause of pneumonia in ruminants **(Dardeer and Refat, 2004; Hala, 2004 and El-Gmaal, 2007)**.

Mycoplasma bovirhinis recovered from nasal swabs and lung tissues in percentages of 33.3 and 66.7% from the totally isolated *M. bovirhinis*, respectively, whereas **El-Gmaal (2007)** recorded percentages of 86.5 and 13.5%, respectively. *M. bovirhinis* recovered from nasal swabs, lung tissues and total samples in percentages of 0.7, 4.1 and 1.6% respectively, whereas **El-Gmaal (2007)** recorded percentages of 11, 1.7 and 12.8%, respectively. Different isolation percentages of *M. bovirhinis* may be attributed to immune status of examined animal and the selected isolation method and time used for bacteriological examination **(Abo-Elnaga *et al.*, 2012)**.

M. bovirhinis recovered from nasal swabs collected from diseased and apparently healthy camels in percentages of 0 and 1.4%, respectively. Failure of isolation nasal swabs

collected from diseased camels may be due to its opportunistic nature, growth inhibition initiated by other microorganism and / or antibiotic treatment **(Nicholas *et al.*, 2008)**.

Mycoplasma bovirhinis recovered from lung tissues collected from diseased and apparently healthy camels in percentages of 5.7 and 2.2%, respectively, the somewhat higher percentage belong to diseased camels may indicate its pathogenic role in camel **(Sabry and Ahmed, 1986)**. The microorganism isolated in percentages of 1.7 and 1.6% from all samples collected from diseased and apparently healthy camels, respectively.

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Table (1): *Mycoplasma* species, target genes, oligonucleotide primer sequences, DNA amplification programs, product sizes and references.

Mycoplasma Species	Target gene	Oligonucleotide primer sequence (5`-3`)	Target DNA amplification program						product size (bp)	Reference
			Initial denaturation Temp (°C) / Time (min)	Second step			No. of cycles	Final extension Temp (°C) / Time (min)		
				Denaturation	Annealing	Extension				
				Temp (°C) / Time (sec)						
Mycoplasma	16S rRNA	F: GGG AGC AAA CAG GAT TAG ATA CCC T R: TGC ACC ATCT GTC ACT CTG TTA ACC TC	94/2	94/30	52/60	72/150	30	72/5	270	Abo-Elnaga *et al.*, 2012
M. bovis	16S rRNA	F: GCA ATA TCA TAG CGG CGA AT R: TCT CAA CCC CGC TAA ACA TC	94/2	94/30	52/60	72/150	30	72/5	227	Abo-Elnaga *et al.*, 2012
M. bovirhinis	16S rRNA	F: ATT ACT CGG GCA GTC TCC R: GCT GAT AGA GAG GTC TAT CG	94/9	94/30	60/60	72/60	35	72/7	316	Saad and Abdel Hameed, 2012
M. putrefaciens	*ArcB*	F: AAA TTG TTG AAA AAT TAG CGC GA R: CAT ATC ATC AAC TAG ATT AAT AGT AGC ACC	94/10	94/15	52/15	72/15	35	72/10	316	Peyraud *et al.*, 2003

Table (2): Biochemical and Molecular identification of isolated *Mycoplasma* species.

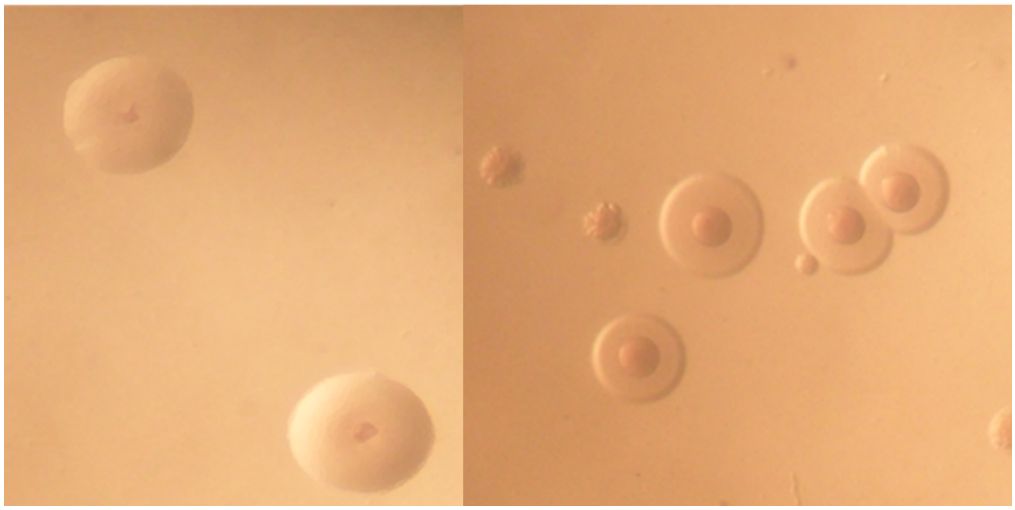
Biochemical tests			Biochemical Group	Identification of *Mycoplasma* species by						
				Biochemical identification				Molecular identification (PCR)		
Arginine utilization	Glucose fermentation	Film and spot formation		Species name (s)	No.	%1	%2	Target gene	Result	No.
+	-	-	I	*M. alkalescens* *M. arginini*	0	0	0	*Not tested*		
+	+	-	II	*M. capricolum* subsp *capricolum*	0	0	0	*Not tested*		
-	-	+	III	*M. bovis*	24	68.6	6.5	16S rRNA	Positive	24
				M. agalactiae				*Not tested*		
-	+	-	IV	*M. bovirhinis*	6	17.1	1.6	16S rRNA	Positive	6
				M. dispar *M. ovipneumoniae*				*Not tested*		
-	+	+	V	*M. putrefaciens*	5	14.3	1.4	*Tested and unidentified*		
				M. bovoculi				*Not tested*		
				Mycoplasma	35	100	9.5	16S rRNA	Positive	35

Percentages calculated in relation to the total number (35) of isolated *Mycoplasma* species (%1) and to the total number (369) of examined samples (%2).

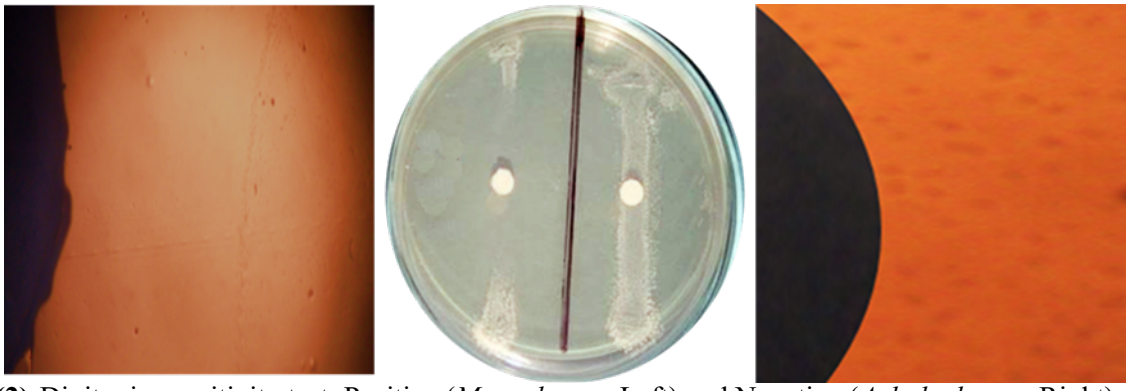
Table (3): *Mycoplasma* species isolated from nasal swabs and lung tissues collected from diseased and apparently healthy camels molecularly identified using PCR.

Samples and health condition			*Mycoplasma* species			
			M. bovis	*M. bovirhinis*	Unidentified *Mycoplasma* spp.	Total *Mycoplasma*
Nasal swabs	Diseased (126)	N	7	0	2	9
		%1	29.2		40	25.7
		%2	5.6		1.6	7.1
	Apparently healthy (145)	N	15	2	3	20
		%1	62.5	33.3	60	57.1
		%2	10.3	1.4	2.1	13.8
	Total (271)	N	22	2	5	29
		%1	91.7	33.3	100	82.9
		%2	8.1	0.7	1.9	10.7
Lung tissues	Diseased (53)	N	1	3	0	4
		%1	4.2	50		11.4
		%2	1.9	5.7		7.6
	Apparently healthy (45)	N	1	1	0	2
		%1	4.2	16.7		5.7
		%2	2.2	2.2		4.4
	Total (98)	N	2	4	0	6
		%1	8.3	66.7		17.1
		%2	2	4.1		6.1
All samples	Diseased (179)	N	8	3	2	13
		%1	33.3	50	40	37.1
		%2	4.5	1.7	1.1	7.3
	Apparently healthy (190)	N	16	3	3	22
		%1	66.7	50	60	62.9
		%2	8.4	1.6	1.6	11.6
	Total (369)	N	24	6	5	35
		%1	100	100	100	100
		%2	6.5	1.6	1.4	9.5

Percentages calculated in relation to the number of isolated *Mycoplasma* of the same species (%1) and to the number of examined samples (%2) with consideration of health condition.



Photo(1): Characteristic"fried-egg"appearance of mycoplasmas colonies understereomicroscope (10X).



Photo(2): Digitonin sensitivity test: Positive (*Mycoplasma*, Left) and Negative (*Acholeplasma*, Right).

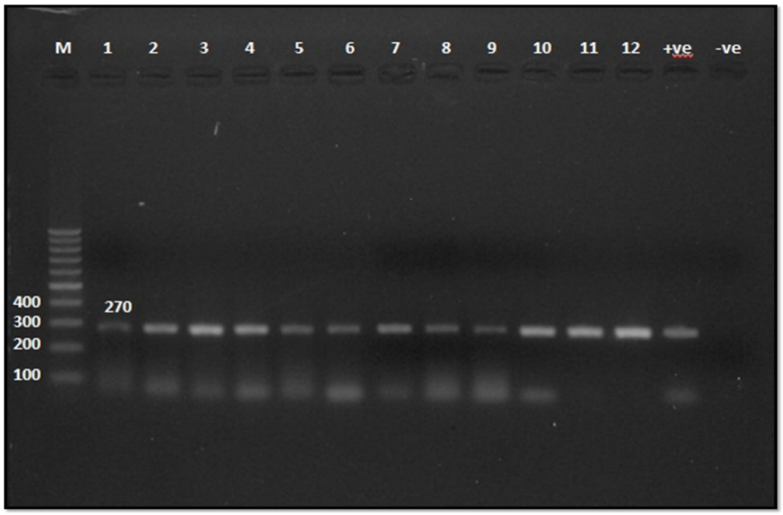


Photo (3): PCR product of 16S rRNA gene specific for *Mycoplasma*. Lane M: A 100-1000 bp DNA ladder. Lanes 1-12: Positive samples at 270 bp. Lane +ve: Positive control. Lane-ve: Negative control.

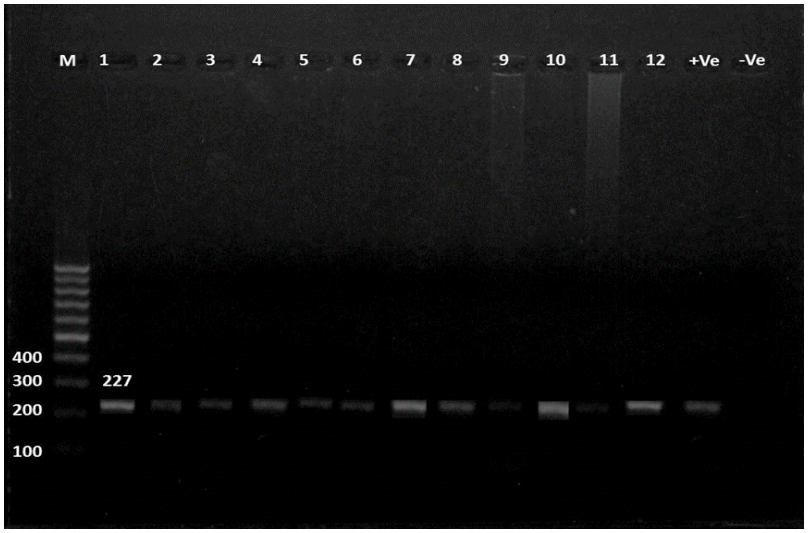


Photo (4): PCR product of 16S rRNA gene specific for *M. bovis*. Lane M: A 100-1000 bp DNA ladder. Lanes 1-12: Positive samples at 227 bp. Lane +ve: Positive control. Lane -ve: Negative control.

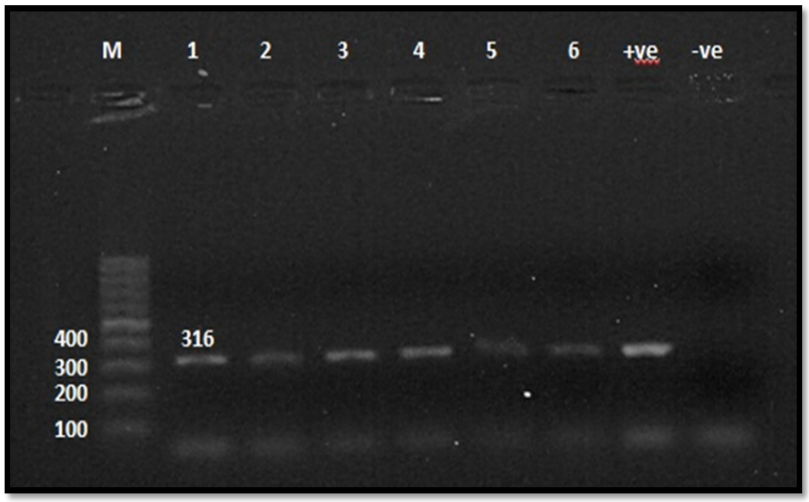


Photo (5): PCR product of 16S rRNA gene specific for *M. bovirhinis*. Lane M: A 100-1000 bp DNA ladder. Lanes 1-6: Positive samples at 316 bp. Lane +ve: Positive control. Lane -ve: Negative control.

التصنيف الجزيئي لميكوبلازما الجهاز التنفسي للماشية المعزولة من الإبل العربية في مصر

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الملخص العربي

تم تجميع 369 عينه من الإبل العربية البالغة للفحص البكتريولوجي للميكوبلازما المصاحبة للأعراض التنفسية. تم إجراء الفحص البكتريولوجى لعدد 271 مسحه أنفيه (من 126 جمل مريض و145 سليم ظاهريا) و98 أنسجه رئوية (53 رئة مصابة و45 سليمه ظاهريا). تم عزل الميكوبلازماس من 67 (18.2%) عينه وبإستخدام إختبار الحساسية للديجوتنين تم تحديد الميكوبلازما فى 35 عينه و الأكيلوبلازما فى 32 عينه بنسبه 52.2 % و47.8 % من الميكوبلازماس على الترتيب وبنسبه 9.5 % و8.7 % من العينات التى تم فحصها على الترتيب. تم تقسيم الميكوبلازما بإستخدام التفاعلات البيوكيميائيه إلى المجموعات الحيويه الثالثة والرابعة والخامسة بنسبه 68.6 % ,17.1% ,14.3 % من الميكوبلازما المعزولة على الترتيب وبنسبه 6.5 % و1.6% و1.4% من العينات التى تم فحصها على الترتيب. تم إجراء التعرف الجزيئي لجنس الميكوبلازما بإستخدام تفاعل البلمرة المتسلسل بالبحث عن جين 16S rRNA كما تم إجراء التعرف الجزيئي لنوع الميكوبلازما بوفس وبوفيرينس بالبحث عن جين 16S rRNA المتخصص لكل من نوعى الميكوبلازما. تم تحديد نسبه الميكوبلازما المعزولة من المسحات الأنفية و أنسجه الرئة بـ 82.9 % و17.1 % من إجمالى (35) الميكوبلازما المعزولة على الترتيب وبنسبة 10.7 %, 6.1 % ,9.5 % من المسحات الأنفية والرئة و إجمالى العينات على الترتيب. تم تحديد نسبه الميكوبلازما المعزولة من العينات التى تم جمعها من الإبل المريضة والسليمة ظاهريا بـ 7.3% و 11.6% على الترتيب. تم عزل الميكوبلازما بوفس من المسحات الأنفية و أنسجه الرئة بنسبة 91.7 % و8.3 % من إجمالى الميكوبلازما بوفس المعزولة (24) على الترتيب ومن المسحات الأنفية والرئة و إجمالى العينات بنسبه 8.1% و2 % و6.5 % على الترتيب ومن العينات التى تم جمعها من الإبل المريضة والسليمة ظاهريا بنسبه 4.5% و8.4% على الترتيب. تم عزل الميكوبلازما بوفيرينس من المسحات الأنفية و أنسجه الرئة بنسبة 33.3% و66.7 % من إجمالى الميكوبلازما بوفيرينس المعزولة (6) على الترتيب ومن المسحات الأنفية والرئة و إجمالى العينات بنسبه 0.7 % و4.1% و1.6 % على الترتيب ومن العينات التى تم جمعها من الإبل المريضة والسليمة ظاهريا بنسبه 1.7% و 1.6% على الترتيب.