

Some studies on mycoplasmas infection in small ruminants using modern diagnostic methods

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SUMMARY

Sheep and goats are one of the important agricultural animals in Egypt. Respiratory affections and mastitis due to mycoplasma in both sheep and goats are regarded as a frequent cause of economic losses. Nasal, conjunctival swabs and milk samples from apparently healthy and diseased sheep and goats were collected from 75 sheep (195 samples) and 50 goat (140 samples) flocks in different areas in El-Fayom governorate. These samples were subjected to Mycoplasma isolation. The results revealed that, the total mycoplasma isolates from sheep were 135 and from goats were 54 which identified as follow : (38 isolates) *M. agalactiae* from sheep samples and (18 isolates) from goats. Also *M. arginini* was isolated from sheep (43 isolates) and goats (17 isolates) and *M. ovipneumoniae* was isolated from sheep (48 isolates) and goats (16 isolates). Also there

were 6 acholeplasma strains from sheep and 4 strains from goat samples. PCR confirm the isolation of *M. agalactiae* by the presence of 360 bp specific band, PCR amplification of *M. ovipneumoniae* strains gave a characteristic band at 1070 bp and PCR amplification of *M. arginine* strains gave a characteristic band at 280bp.

INTRODUCTION

Sheep and goats are one of the important agricultural animals in most countries all over the world. In Egypt, their number was estimated to be 3.5 and 3.3 million head respectively; therefore, a great attention is directed toward caprine and ovine industry to meet with people requirements (Anon, 1996). The main cause of respiratory affections in sheep and goats is the adverse physical and physiological stress combined with viral and bacterial infections (Brogden et

al., 1998). *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma mycoides* subsp. *mycoides* infections cause pneumonia with other clinical signs as conjunctivitis, mastitis, polyarthritis and abortion (Barton and Cottew, 1968 and Losos, 1986). *Mycoplasma* are associated with a number of various diseases of sheep and goats, most notable of which are contagious agalactia (CA) and caprine pleuropneumonias (CPP), which lead to significant economic losses as a result of decreased milk production, morbidity and occasionally, mortality (Nicholas, 2002). Other mycoplasma as *Mycoplasma arginini* was also involved as a causative agent of mild pulmonary changes in sheep and goats (Barile et al., 1968; Foggie and Angus, 1972 and Hassan, 1973). Egwu et al., 2000 reported that other *Mycoplasma* species are associated with pneumonia, conjunctivitis and mastitis. These are *Mycoplasma conjunctivae*, where infection results in the development of a serious, but self-limiting, keratoconjunctivitis (Baker et al., 2001), and *Mycoplasma ovipneumoniae*, the cause of a non-progressive (atypical) pneumonia (Gilmour et al., 1979). *Mycoplasma ovipneumoniae* (MO) is one of the most commonly isolated microorganisms from sheep with respiratory disease worldwide. (Davies, 1985). Recently this microorganism and *M. arginini* (MA) have been routinely recovered from young lambs with a respiratory disease that has been termed the "coughing syndrome." The

condition is associated with a severe paroxysmal cough, leading to rectal prolepsis. The disease is chronic and persists for several weeks in most affected lambs. Also the extended persistence of the MO organism in the respiratory tract of these lambs and the chronic nature of the disease may be due to failure of the immune system to generate protective immunity, (Niang, et al.:1998). Contagious agalactia (CA) is a serious disease of goats and sheep causing mastitis, arthritis and keratoconjunctivitis. Several mycoplasmas cause this disease: *Mycoplasma agalactiae*, *Mycoplasma capricolum* subsp. *Capricolum* (Mcc), *Mycoplasma mycoides* subsp. *mycoides large colony* (Mmm LC) and *M. putrefaciens*. CA occurs in Europe, Western Asia, USA and North Africa, (Bergonier et al., 1997). Greco et al., (2001) examined 56 samples (44 milk samples, 2 nasal swabs, 6 ocular swabs, 3 vaginal swabs and one sample of fibrinous exudates from carpal joint) from sheep and goats with clinical signs of contagious agalactia for the simultaneous detection of several species of small ruminant mycoplasmas. They used a multiplex PCR assay, using oligonucleotide primers specific for *M. agalactiae*, the multiplex PCR was able to amplify a 375 bp fragment *M. agalactiae* chromosomal DNA. Advances in polymerase chain reaction (PCR) technology have greatly improved the detection of mycoplasmas (Nicholas, 2002).

Therefore, the purpose of the present work is to assess the value of using the recent techniques in identification of different mycoplasma isolates from sheep and goats and also to determine its incidence, characteristic features, in addition to see the value of using biotechnology compared with the traditional procedures for diagnosis of mycoplasmosis in sheep and goats researches.

MATERIAL AND METHODS

1- Samples: One hundred ninety five samples were collected from 75 sheep flocks (195 samples) while (140 samples) were collected from 50 goats flocks in different areas in El-Fayom governorate. These samples were nasal, conjunctival swabs and milk samples from apparently healthy and diseased sheep and goats. (Table1).

Table (1): Type of samples collected from apparently healthy and diseased sheep and goats

Type of samples	Samples No. of apparently Healthy animals		Samples No. of diseased animals	
	sheep	goats	sheep	Goats
Nasal swabs	25	20	50	30
Conjunctival swabs	25	20	50	30
Milk	15	20	30	20
Total	65	60	130	80

2- Media used for cultivation and isolation of Mycoplasma:

2.1- Liquid and solid media for the isolation and propagation of mycoplasma were prepared as described by Sabry and Ahmed (1975).

2.2- Digitonin sensitivity test was done for the obtained isolates according to Erno and Stipkovits (1973).

2.3- 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).

3- Serological identification: was conducted by growth inhibition test as described by (Clyde (1964). Standard antisera - locally prepared- were kindly supplied by Dr. Nadra El-wgoud M.I.Infectious Department, Fac, Vet, Med, Giza Governorate, Egypt.

4- Polymerase chain reaction

(PCR):

A) Preparation of samples for DNA extraction (Yleana et al., 1995): 5ml of a 24 hour broth cultures of isolates were centrifuged for 10 minutes at 12000 r.p.m. The pellet was washed twice in 1 ml of phosphate buffered saline pH 7.2 (PBS) and suspended in 50 µl PBS. The cell suspension was heated directly at 100°C for 10 min. in a heat block to break the cell membranes, and then cooled on ice for 5 min. Finally, the cell suspension was centrifuged for 5 min. and the supernate containing chromosomal DNA was collected and stored at -20°C until used.

B) Oligonucleotide primers (Sigma):

Primer selection:

1- according to (Yleana et al., 1995): Two oligonucleotide primers were selected for the detection of *M. agalactiae*.

The sequence of primer (1) was: 5'- CCT TTT AGA TTG GGA TAG CGG ATG- 3'.

The sequence of primer (2) was: 5'- CCG TCA AGG TAG CGT CAT TTC CTA C-3'.

2-According to (Zhang et al., 2004) Two oligonucleotide primers were selected for the detection of *M. ovipneumoniae*.

The sequence of primer (1) was: 5'- AAC AGC GGC TAA TAC CAG ATA C-3'. and the sequence of primer (2) was: 5'- AGA CTT CAA TCC GAA CTG AGA C -3'.

3-According to (Van Kuppeveld et al., 1994) Two oligonucleotide primers were selected for the detection of *M. arginini* the sequences of primers (1) was: 5'- GGG AGC AAA CAG GAT TAG ATA CCC T -3'. and the sequence of primer (2) was: 5'-TGC ACC ATC TGT CAC TCT GTT AAC CTC -3'.

C) Procedure for DNA amplification:

PCR amplification was performed in 50 µl reaction mixture consisting of 5 µl of 50 ng *M. agalactiae* genomic DNA, 10 µl of 10 x Taq buffer (10mM tris- HCl [pH 8.8], 50 mM KCl), 1 µl of 50 pM of each primer, 1.5 mM MgCl₂, 1 µl of 2U of Taq thermosTable DNA polymerase, 1 µl of 50 uM of each dNTP, and 31µl of DNase- RNase- free, deionized water. DNA amplification was carried out in PTC-100 programmable thermal controller (MJ, Research Inc.). The thermal profiles were as follows: Denaturation at 94 °C for 45 seconds, primer annealing at 60°C for 1 min., and extension at 72°C for 2 min. the amplifications were performed for 30 or 35 cycles with a final extension step at 72°C for 3 min. After the reaction, the amplified DNA was electrophoresed on 1.5% agarose gel for 90 min. at 100 volts, DNA Ladders: 100 bp (Pharmacia), Cat. No. 27-4001- 01, USA was added then stained with ethidium bromide. After electrophoresis, the gel was visualized by UV transillumination and photographed. Image analysis was made by ImageQuantTL-V2003.03 (Amersham Biosciences).

RESULTS

1-Incidence of positive samples:

Results obtained in Table (2) revealed that out of 65 samples from apparently healthy sheep 35 were positive to mycoplasma isolation with a rate of (53.8%), while 24 out of 60 samples from apparently healthy goats were positive with a rate of (40

%). As for diseased sheep 100 out of 130 samples were positive with a rate of (76.9 %) and 30 out of 80 samples from diseased goats were positive with a rate of (37.5 %). The Table also revealed that 59 out of 125 samples from apparently healthy animals were positive with a rate of (47.2 %), while 130 out of 210 samples from diseased animals were positive with an incidence of (61.9%).

Table (2): Rate of isolation of mycoplasma species from samples of diseased and apparently healthy animals.

Types of animals	Apparently healthy animals			Diseased animals		
	No. of examined samples	No. of positive samples	%	No. of examined samples	No. of positive samples	%
Sheep	65	35	53.8	130	100	76.9
Goats	60	24	40	80	30	37.5
Total	125	59	47.2	210	130	61.9

2- Primary isolation of mycoplasmas from sheep:

In Table (3) a total of 135 mycoplasma isolates were obtained from 195 samples with a rate of (69.2 %), 35 from apparently healthy animals with a rate of (53.8 %) and 100 from diseased animals with a rate of (76.9 %). In diseased sheep, the higher rate was from nasal swabs (40 isolates out of 50 samples) with an

incidence 80 %, while the lowest infection rate was from milk (21 isolates out of 30 samples) with an incidence 70%. As from apparently healthy sheep, the lowest infection was from nasal and conjunctival swabs (13 isolates out of 26 samples) with a rate of 52%, while the highest incidence rate was from milk (9 isolates out of 15) with a percentage of 60%. Table (3).

Table (3): Total recovery rate of mycoplasmas from apparently healthy and diseased sheep.

samples	Apparently Healthy			Diseased			Total		
	No. of examined samples	No. of positive samples	%	No. of examined samples	No. of positive samples	%	No. of examined samples	No. of positive samples	%
Nasal swabs	25	13	52	50	40	80	75	53	70.7
Conjunctival swabs	25	13	52	50	39	78	75	52	69.3
Milk	15	9	60	30	21	70	45	30	66.7
Total	65	35	53.8	130	100	76.9	195	135	69.2

Table (4): Total recovery rate of mycoplasmas from apparently healthy and diseased goats.

samples	Apparently Healthy			Diseased			Total		
	No. of examined samples	No. of positive samples	%	No. of examined samples	No. of positive samples	%	No. of examined samples	No. of positive samples	%
Nasal swabs	20	8	40	30	10	33.3	50	18	36
Conjunctival swabs	20	10	50	30	9	30	50	19	38
Milk	20	6	30	20	11	55	40	17	42.5
Total	60	24	40	80	30	37.5	140	54	38.6

3-Primary isolation of mycoplasmas from goats:

In table (4) a total of 54 mycoplasma isolates were obtained from 140 samples (38.6) %, 24 from apparently healthy animals (40 %) and 30 from diseased animals (37.5 %).

In diseased goats, the higher isolation rate was from milk samples 11 isolates out of 20 samples (55 %), while the lowest isolation rate was from conjunctival swabs 9 isolates out of 30 samples (30%).

As for apparently healthy goats, the higher isolation was from conjunctival swabs

samples 10 isolates out of 20 samples (50 %), while the lowest rate was from milk.

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

Applying of digitonin test on positive samples from the primary isolation of mycoplasma revealed that all the isolates from both sheep and goats were digitonin sensitive so they belonged to genus *Mycoplasma* except 13 samples were *acholeplasma*, (9 *acholeplasma* were isolated from nasal swabs of apparently healthy sheep and 4 from apparently healthy goats) The recovery rate of mycoplasma from sheep and goats according to digitonin test is summarized in Table (5).

Table (5): Total recovery rate of mycoplasma from sheep and goats according to digitonin sensitivity.

Samples	Sheep			Goats		
	No. of positive samples	No. of mycoplasma	%	No. of positive samples	No. of mycoplasma	%
Nasal swabs	53	47	88.7	18	16	88.9
Conjunctival swabs	52	49	94.2	19	17	89.5
Milk	30	30	100	17	17	100
Total	135	126	93.3	54	50	92.6

S-Biochemical characterization of obtained isolates:

A total of 176 isolates (126 from sheep and 50 from goats) were subjected to the following biochemical tests; glucose fermentation, arginine deamination and film and spot formation. The results revealed the presence of three distinct biochemical groups of sheep mycoplasmas that are summarized in Table (6).

Group I: (Glucose negative, Arginine positive and film and spot formation negative):43 isolates were belonging to this

group (15 of them from nasal swabs, 20 from conjunctival swab, 8 from milk)

Group II: (Glucose positive, Arginine negative and film and spot formation negative): 48 isolates were belonging to this group (21 of them from nasal swabs, 21 from conjunctival swabs, 6 from milk)

Group III: (Glucose negative, Arginine negative and film and spot formation positive):38 isolates were belonging to this group (11 of them from nasal swabs, 11 from conjunctival swabs, 16 from milk)

Group	Glucose	Arginine	Film formation	Spot formation	Total
Group I	-	+	-	-	43
Group II	+	-	-	-	48
Group III	-	-	+	+	38
Total					129



Table (6): Results of the biochemical characterization groups of sheep mycoplasmas

Samples	Number of positive samples	Group I			Group II			Group III		
		Glucose -ve	Arginine +ve	Film and spot -ve	Glucose +ve	Arginine -ve	Film and spot -ve	Glucose -ve	Arginine -ve	Film and spot +ve
Nasal swabs	53 (47 were mycoplasma) (6 were acholeplasma)	15			21			11		
Conjunctival swabs	52	20			21			11		
Milk	30	8			6			16		
Total	135	43			48			38		

The results revealed also the presence of three distinct biochemical groups of goat mycoplasmas that are summarized in Table (7).

Group I: (Glucose negative, Arginine positive and film and spot formation negative): 17 isolates were belonging to this group (6 of them from nasal swabs, 7 isolates from conjunctival swabs, 4 isolates from milk)

Group II: (Glucose positive, Arginine negative and film and spot formation negative): 16 isolates were belonging to this group (6 of them from nasal swab, 5 from conjunctival swab, 5 isolates from milk)

Group III: (Glucose negative, Arginine negative and film and spot formation positive): 18 isolates were belonging to this group (4 of them from nasal swab, 5 isolates from conjunctival swabs, 9 from milk)

Group	Glucose	Arginine	Film and spot formation	Total
Group I	-ve	+ve	-ve	17
Group II	+ve	-ve	-ve	16
Group III	-ve	-ve	+ve	18
Total				51

Table (7): Biochemical characteristics of goat mycoplasmas

Table (7): Results of the biochemical characterization groups of goat mycoplasmas.

Samples	Number of positive samples	Group I			Group II			Group III		
		Glucose -ve	Arginine +ve	Film and spot -ve	Glucose +ve	Arginine -ve	Film and spot -ve	Glucose -ve	Arginine -ve	Film and spot +ve
Nasal swabs	18(16 mycoplasma +2acholplasma)	6			6			4		
Conjunctival swabs	19 (17 mycoplasma +2acholeplasma)	7			5			5		
Milk	17	4			5			9		
Total	54	17			16			18		

Results of PCR for detection of *M. arginini*: the results were shown in photo (1) for detection of *M. arginini* from sheep and goats where four field isolates were tested and all of them gave a characteristic common band at 280 bp.

Results of PCR for *Mycoplasma agalactiae* isolates :

photo (2) shows the genomic similarity among *M. agalactiae* field isolates and reference strain. The banding pattern of the field isolates was highly similar to those of *M. agalactiae* reference strain. PCR results confirmed that the isolates were *M. agalactiae* by the presence of the specific band at 360 bp in each of the 4 field isolates and *M. agalactiae* reference strain.

Results of Polymerase chain reaction for *Mycoplasma ovipneumoniae* isolates recovered from sheep and goats:

The PCR assay was highly specific and sensitive for the detection of *Mycoplasma ovipneumoniae* isolated from sheep and goats.

photo (3) shows the electrophoretic pattern of the PCR products of *Mycoplasma ovipneumoniae* DNA isolated from sheep and goats which was detected by agarose gel electrophoresis and ethidium bromide staining and reveals that all *Mycoplasma ovipneumoniae* strains isolated from sheep and goats gave a characteristic band at 1070 bp



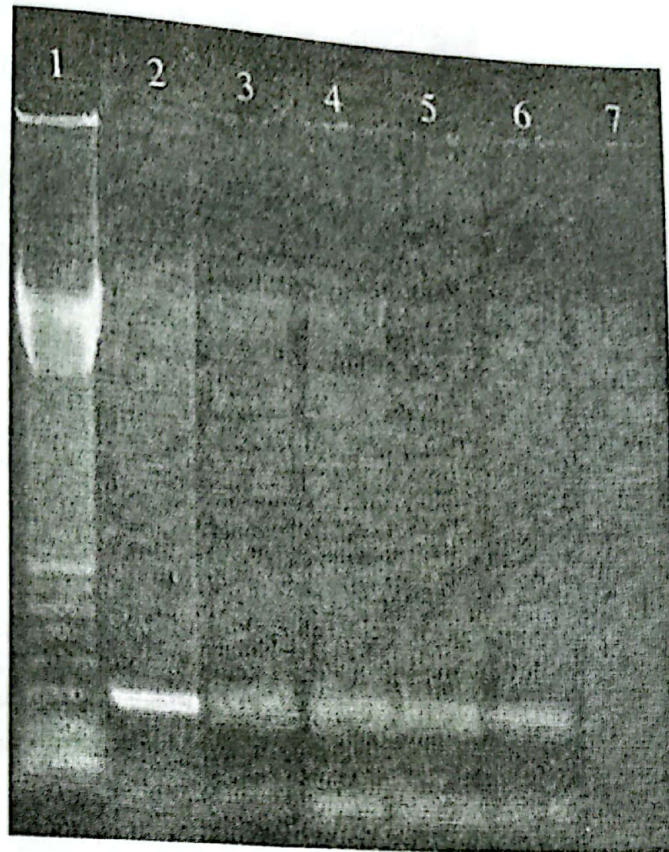


Photo: (1): Electrophoretic pattern of the PCR products of *Mycoplasma argentine*

DNA isolated from sheep and goats using the 16S rRNA gene.

Lane 1:- 100 bp DNA Ladder (Pharmacia)

Lane 2:-*M. argentine* reference strain

Lane 3:- *M. argentine* field isolate from sheep

Lane 4:- *M. argentine* field isolate from sheep

Lane 5:- *M. argentine* field isolate from goats

Lane 6:- *M. argentine* field isolate from goats

Lane 7:-control negative

Our results (Photo.1) revealed the presence of *M.* specific band at 280 bp.

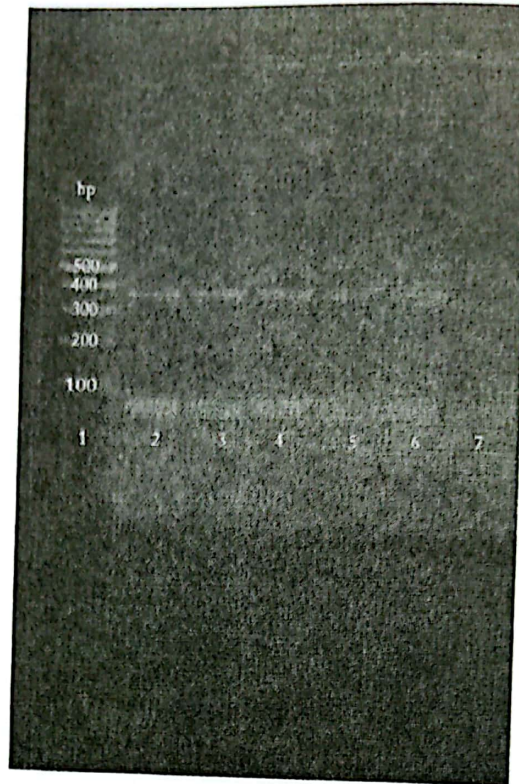


Photo: (2):-Electrophoretic pattern of the PCR products of *Mycoplasma agalactiae* DNA isolated from sheep and goats using the 16S rRNA gene.

Lane 1:- 100 bp DNA Ladder (Pharmacia)

Lane 2:- *M. agalactiae* reference strain

Lane 3:- *M. agalactiae* field isolate from sheep

Lane 4:- *M. agalactiae* field isolate from sheep

Lane 5:- *M. agalactia* field isolate from goats

Lane 6:- *M. agalactia* field isolate from goats

Lane 7:- control negative

Our results (Photo.2) revealed the presence of *M. agalactia* specific band at 360 bp.

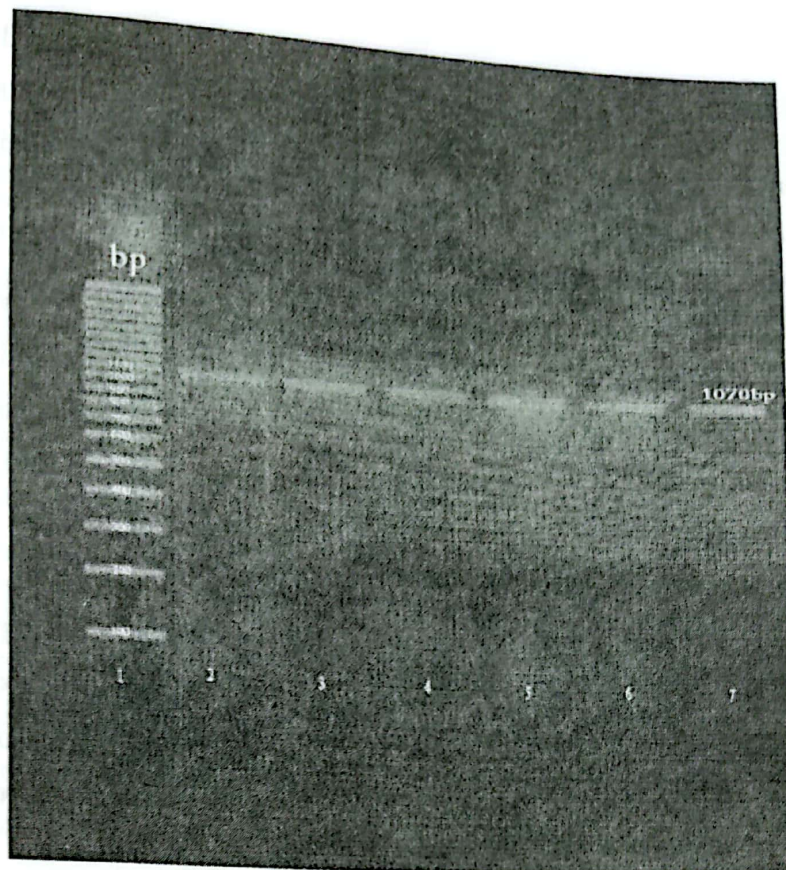


Photo (3): Electrophoretic pattern of the PCR products of *Mycoplasma ovipneumoniae* DNA isolate using the 16rDNA gene

Lane 1: 100 bp DNA Ladder (Pharmacia)

Lane 2: control positive

lane 3: *M. ovipneumoniae* isolate from nasal and ocular swabs of sheep

Lane 4: *M. ovipneumoniae* isolate from milk of sheep.

Lane 5: *M. ovipneumoniae* isolate from milk of goats.

Lane 6: *M. ovipneumoniae* isolate from nasal swabs of goats

Lane 7: *M. ovipneumoniae* isolate ocular swabs of goats

Our results (Photo.3) revealed the presence of *M. ovipneumoniae* specific band at 1070 bp.

DISCUSSION

Our results are in agreement with those stated by Al-Momani et al. (2008) recorded the isolation of three mycoplasma species from sheep and goats in Jordan including *M. agalactiae*, *Mycoplasma putrefaciens* and *Mycoplasma capricolum subsp. capricolum* (Mcc). They also added that the isolation rate was 35% from sheep and 65% from goats which means that goats are more susceptible to mycoplasma than sheep. Nicholas (2002) isolated *M. arginini* and *M. agalactiae* from small ruminants in the mediterranean region and added that contagious agalactiae (CA) caused by *M. agalactiae* is estimated annually to cost at least US \$ 30 million mainly as a result of milk production losses but mortality and poor growth in young may also be significant

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. Keeping sheep and goats in mixed flocks facilitate the transmission of mycoplasma infection from one species to another (Abo-Shehada et al., 2002). Ayling et al. (2004) isolated *M. ovipneumoniae* and *M. arginini* from sheep and goats, and Aly and

Dardeer (2003) recorded the isolation of *M. arginini* (60%), *M. agalactiae* (22%) and *M. conjunctivae* (35%) from an outbreak of respiratory affections in a herd of sheep in Alexandria Governorate. Our results about the recovery rate of *M. ovipneumoniae* were similar to that recorded by several authors as Su et al., (1983) who reported the common isolation of *M. ovipneumoniae* from nasal secretion of healthy and pneumonic lungs of goats, El-Ebeedy et al., (1989) who carried out a study on 135 samples from respiratory tract, milk, synovial fluid and conjunctiva of diseased sheep, 48 mycoplasma strains were isolated, 4 isolates from them were *M. ovipneumoniae*, Richard et al., (1989) who reported that *M. ovipneumoniae* represented 37% of nasal bacterial flora and 27.5% of lung bacterial isolates of goats, Mohan et al., (1992) and Hussein (1998) who concluded that sheep and goats act as a carrier of *M. ovipneumoniae* isolates and were obtained from nasal swabs randomly selected from apparently healthy and diseased flocks. Many authors were agreed with our results of isolation of *M. agalactiae* as El-Ebeedy et al., (1989) who carried out a study on samples from respiratory tract, milk, synovial fluid and conjunctiva of diseased sheep and recovered *M. agalactiae subsp. Agalactiae* strain, Rezk (1987) who reported the identification of *M. agalactiae* isolates from mastitic ewes' milk and mastitic doe milk. *M. agalactiae* was recovered also from the

respiratory tract of diseased lambs. Also, Ammar et al., (1993), Aly and Dardeer (2003), Mostafa (2003), and Marwa Ibrahim (2008), revealed the identification of *M. agalactiae* isolates, from nasal swabs and lung tissues of sheep and goats in an outbreak of respiratory affections and the most clinical symptoms were fever, respiratory distress and high mortality in sheep. 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. Keeping sheep and goats in mixed flocks facilitate the transmission of mycoplasma infection from one species to another (Abo-Shehada et al., 2002). Ayling et al. (2004) isolated *M. ovipneumoniae* and *M. arginini* from sheep and goats. Aly and Dardeer (2003) recorded the isolation of *M. arginini* (60%), *M. agalactiae* (22%) and *M. conjunctivae* (35%) from an outbreak of respiratory affections in a herd of sheep in Alexandria Governorate. Results of recovery rate of *M. arginini* agreed with results obtained by Al-Zeftawi (1979), Radwan et al., (1985), who concluded that *M. arginini* was the most prevalent species recovered from many sites of normal and diseased sheep and goats. Also Pasic et al., (1990), Ammar et al., (1993), El-Shabiny et al., (1996), Hussein

(1998), isolated *M. arginini* from nasal swabs of clinically diseased with pneumonia and other respiratory signs. Nevine et al., (2001), Adehan and Youssao (2002), Aly and Dardeer (2003), Mostafa (2003) and Rania (2006) who isolated *M. arginini* from samples of nasal swabs and from samples of pneumonic lungs which were collected from dead animals.

Polymerase chain reaction technique is much faster than conventional microbiological techniques for isolation as well as identification as the results can be obtained within 5 hours and it has the advantage of the ease of use, standardization and is more suitable for processing large number of specimens. In the present study, PCR amplification of *M. ovipneumoniae* strains isolated from sheep and goats gave a characteristic band at 1070 bp which agrees with that reported by Zhang et al., (2004) and Ayling et al., (2003). In the present study, PCR amplification of *M. agalactiae* strains isolated from sheep and goats gave a characteristic band at 360 bp which agrees with that reported by Dardeer et al., (2006), Hussein (2003) and Yleana et al., (1995).

This result also agrees with Nicholas (2002), Bashiruddin et al., (2005) and Woubit et al., (2007) who used PCR for identification of *Mycoplasma agalactiae*. This result disagrees with Tola et al., (1996 b) who developed a polymerase chain reaction test for the detection of *M. agalactiae* which isolated

from sheep but by using different oligonucleotide primers than that in this study and disagrees with them also as they amplified a 375 bp fragment of *M. agalactiae* chromosomal DNA. Our results (photo 2) revealed the presence of *M. agalactiae* specific band at 360 bp which agreed with the results reported by Yleana et al., (1995) and Hussein (2003). Greco *et al.*, (2001). Also our results agree with Ayling *et al.*, (2003), Zhang *et al.*, (2004) were developed a rapid and specific PCR test for the specific detection and identification of *M. ovipneumoniae* depending on 16 S ribosomal DNA gene. This PCR amplified only the target DNA fragment from *M. ovipneumoniae* which was at 1070 bp. Nicholas (2002), Hussein (2003), Bashiruddin *et al.*, (2005) and Dardeer *et al.*, (2006) were used PCR for identification of *Mycoplasma agalactiae* and evaluated the specific of PCR system.

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بعض الدراسات على عدوى الميكوبلازما في المجترات الصغيرة باستخدام طرق التشخيص الحديثة

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الأغنام والماعز من الحيوانات الزراعية المهمة في مصر. تعتبر الميكوبلازما من أهم الأسباب المتكررة لإصابة الجهاز التنفسي و التهاب الضرع مؤدية الي خسائر إقتصادية متكررة. العينات التي تم جمعها في هذه الدراسة من ٧٥ من الأغنام (١٩٥ عينة) و ٥٠ من الماعز (١٤٠ عينة) من قطعان في مناطق مختلفة في محافظة الفيوم. وكانت هذه العينات مسحات أنفية ومسحات من الملتحمة و عينات من الحليب من الأغنام والماعز المريضة والسليمة ظاهرياً كما خضعت العينات للفحص البكتريولوجي لعزل ميكروب الميكوبلازما. أوضحت نتائج العزل ما يلي الميكوبلازما المعزولة من مجموع الأغنام 135 معزولة و ٥٤ معزولة من الماعز حددت على النحو التالي ٣٨ ميكوبلازما أجلاكتيا من الأغنام و (١٨ معزولة) من الماعز. و(٤٣ معزولة) ميكوبلازما أرجيني معزولة من الأغنام و(١٧ معزولة) من الماعز. و (٤٨ معزولة) ميكوبلازما أوفينوميني من الأغنام (١٦ معزولة) من الماعز. تم عزل 6 عترات أكيلو بلازما من الأغنام و ٤ سلاطات من الماعز العينات. تم التأكد من المعزولات باستخدام إختبار انزيم البلمرة المتسلسل عند ٣٦٠ bp للأجلاكتيا وعند ١٠٧٠ bp للأوفينوميني بينما اعطت سلاطات أرجيني ٢٨٠ bp.