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ISOLATION AND IDENTIFICATION OF MYCOPLASMA SPECIES FROM SHEEP AND GOATS REARED UNDER DESERT CONDITIONS

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SUMMARY

In the present work one hundred and twelve mycoplasma isolates (10.89%) were recovered from 1028 samples (nasal swabs, tracheal swabs, lung tissues, pleural fluid, ear swabs, conjunctival swabs and milk). They were collected from 372 sheep and 127 goats reared under desert conditions. The incidence of mycoplasma in sheep was 6.87%, while it was 20% in goats. A total of 112 mycoplasma isolates were differentiated by digitonin sensitivity test into 79 isolates (70.54%) of Mycoplasma species and 33 isolates (29.46%) of *Acholeplasma* species. Using growth inhibition test (GIT) the 79 *Mycoplasma* isolates were serotyped. Only 73 isolates were fully identified as follow: *Mycoplasma arginini* (41 isoaltes), *Mycolplasma ovipneumonaei* (19 isolates), *Mycoplasma mycocides* subsp. *capri* (5 isoaltes), *Mycoplasma agalactiae* (6 isoaltes), *Mycoplasma putrefaciens* (2 isolates). Using the minimum inhibitory concentration test (MIC), azythromycin, enrofloxacin and oxytetracycline proved to be the most effective antimicrobial agents against the isolated *Mycoplasma* species.

INTRODUCTION

Mycoplasmas are distinguished phenotypically from other bacteria by their minute size (125-150 millimicron) and total lack of a cell wall, which explains many of the unique properties of the mycoplasma, such as sensitivity to osmotic shock and detergents, resistance to penicillin, and formation of peculiar fried-eggs shape colonies. Electron microscopic examination of mycoplasmas reveals that the cells are built essentially of three organelles: the cell membrane, ribosome and the characteristic prokaryotic genome (Sabry 2004).

Mycoplasma can cause diseases in all major species including animals and human. In small ruminants, they can cause respiratory diseases, mastitis, arthritis, genital diseases and eye lesions. The most important of these diseases are Contagious Caprine Pleuropneumonia (CCPP) and Contagious Agalactia (CA), which are designated by The Office of International Epizooties (OIE) as list B diseases because of their economic impact on livestock (Nicholas, 2002).

Clinical mycoplasmosis often lacks pathognomonic characteristics and symptoms can be shared by or can mimic other clinical infections (DaMassa, 1996).

Sheep and goats share their mycoplasmal flora. This can undoubtedly be explained by the close phylogenetic relationship of these two animal species, although host adaptation needs to be studied at the cellular and molecular levels (Rosendal, 1994).

A limited number of studies, however, have investigated the persistence or survival of Mycoplasma species under various environmental conditions (Pan and Ogata, 1969; Shimizu et al., 1990 and Nagatomo et al., 1997).

Few data are available regarding the effective control or eradication of mycoplasmal infections. Such a problem seems to be related to our lack of knowledge regarding the biological and biochemical properties or survivability of mycoplasma under various conditions. It seems that preliminary studies on the biological characteristics and/or persistence of mycoplasma organisms under various environmental conditions would certainly contribute to controlling or preventing the occurrence of mycoplasmal infections in the field (Nagatomo et al., 2001).

Consequently, this work was planned to clear out the isolation rate of mycoplasma infections among sheep and goats reared under desert conditions and to determine of the effective antimicrobial agents against field isolates using the Minimum Inhibitory Concentration assay.

MATERIAL AND METHODS

Samples: A total of 1028 samples were collected under aseptic condition from diseased and apparently healthy (living, slaughtered and dead) sheep and goats for mycoplasma isolation. These samples included 352 nasal swabs, 198 ear swabs, 106 tracheal swabs, 140 conjunctival swabs, 136 lung tissue, 4 pleural fluid samples from cases of pleurisy and 92 milk samples. The collected samples were obtained from sheep and goats of different ages and sexes at Maryot station-Desert Research Center, Al Amria, Alexandria Governorate and Sidi-Barani area, Matrouh Governorate, beside El Basateen abattoir in Cairo.

Primary isolation and identification of Mycoplasma: Specimens were cultured on Modified Hayflick's medium (Rosendal, 1994) and B.H.S.-L medium (Carmicheal et al. 1972) as described by Sabry and Ahmed (1975).

Genus determination: It was performed using digitonin sensitivity test as described by Freundt et al. (1973), where genus *Mycoplasma* was digitonin sensitive and genus *Acholeplasma* was digitonin resistant.

Biochemical characterization: It was carried out to differentiate the purified *Mycoplasma* isolates according to their biochemical pattern to limit the choice of specific antisera used for serotyping. Biochemical tests used were:

A- Glucose fermentation test (Sabry, 1968).

B- Arginine deamination test (Sabry, 1968).

C- Film and Spots formation (Cottew, 1983).

Serotyping of Mycoplasma isolates: It was carried out using growth Inhibition test (GIT). The test was carried out according to Clyde (1964) with reference antisera, which were kindly supplied by Prof. Dr. Hywel J. Ball (Veterinary Sciences Division, Department of Agriculture and Rural Development for Northern Ireland, Stoney Road, Stormont, Belfast BT4 3SD. e-mail: Hywel.Ball@afbini.gov.uk). The" Hywel.Ball@afbini.gov.uk). The typing antisera cover the following species:

1. *Mycoplasma agalactiae.*
2. *Mycoplasma arginini.*
3. *Mycoplasma mycoides subsp mycoides (LC).*
4. *Mycoplasma capricolum subsp capripneumoniae (strain F38).*
5. *Mycoplasma mycoides subsp. capri*
6. *Mycoplasma ovipneumoniae.*
7. *Mycoplasma conjunctivae.*
8. *Mycoplasma putrefaciens.*

Antibiogram for the isolated mycoplasms using MIC method:

-Antimicrobials: The tested antimicrobial drugs included erythromycin, azythromycin, oxytetracycline, enrofloxacin, streptomycin and lincomycin. Sterile stock solutions containing 1000 ug/ml were prepared from each of these drugs in distilled water with or without alcohol. They were stored at 4°C and used freshly (Hannan, 2000). One field isolate representing each of the recovered Mycoplasma species was tested. These in clude

- *Mycoplasma agalactiae*
- *Mycoplasma arginini*
- *Mycoplasma mycoides* subspecies *capri*
- *Mycoplasma ovipneumoniae*
- *Mycoplasma putrefaciens*

-Titration of mycoplasmas:

For each selected mycoplasma isolate, a titer 10^3-10^5 colour changing units (ccu)/ml was prepared by the method described by Hannan (2000).

-Sodium Pyruvate: was added at concentration of 0.5% (w/v) to glucose broth for determination of Minimum Inhibitory Concentration of *M. agalactiae* as described by Hannan (2000).

-Determination of minimum inhibitory concentration (MIC):

The test was performed as described by Hannan (2000). The antimicrobials were tested in two fold serial dilutions at concentrations ranging from 10.0 to 0.039 ug/ml.

-End point reading:

The MIC was recorded as the lowest concentration of the antimicrobial that completely prevented a colour change. This typically occurred after 1 to 2 days. For comparison, a final reading was taken after 7-14 days incubation. The result was expressed in ug/ml of active compound. The breakpoints of this test are as follow; a value of 1ug/ml is a guide to mycoplasma susceptibility, with 2-4 ug/ml being intermediately susceptible and above 8 ug/ml being resistant as described by Ter Laak et al. (1993).

RESULTS

Out of 1028 bacteriologicaly examined samples from sheep and goats, 112 mycoplasma isolates (10.89%) were recovered.

Rate of mycoplasma isolation from sheep:

As shown in Table (1) and Fig. (1) bacteriological examination of 713 samples collected from apparently healthy (310) and diseased (403) sheep revealed the isolation of 49 mycoplasma isolates with a recovery rate of 6.87%. The highest rate of isolation was found among samples from slaughtered and dead animals with the isolation of 21 mycoplasma isolates out of 206 samples (10.19%) as compared with rate of isolation from living animals samples (28 out of 507; 5.52%). In samples taken from diseased sheep the rate of isolation reached to 38 mycoplasma isolates out of 403 samples (9.43%), which was comparatively higher (11 out of 310; 3.55%) than those from apparently healthy animals (Fig. 2).

The mycoplasma isolates recovered from of apparently healthy sheep samples included 3 isolates from tracheal swabs, 3 isolates from lung tissues and 5 isolates from milk samples. Comparing the type of the examined sample from diseased sheep to the rate of isolation revealed higher rate of isolation among pleural fluid samples (3 isolates out of 4 samples (75%), followed by lung tissue 11 isolates from 91 samples (12.09 %) nasal swabs 23 isolates from 201 samples (11.44 %) and tracheal swabs 1 isolate from 61 samples (1.64%). No isolation was obtained from ear, conjunctival swab and milk samples (Table 2).

Rate of mycoplasma isolation from goats:

Bacteriological examination of 315 samples collected from apparently healthy (266) and diseased (49) goats revealed the isolation of 63 mycoplasma isolates with a recovery rate of 20% (Fig. 3).

As shown in Table 3, the highest rate of isolation was found among the slaughtered goats (24 out of 40; 60.00%). The isolation rate was comparatively low in samples from living goats (14.18 %). The rate of mycoplasma isolation from diseased goats reached to 59.18 %, which was comparatively higher than those from apparently healthy ones (12.78%).

In diseased goats, the recovery rate was high from pneumonic lung samples and tracheal swabs (83.33%), followed by milk samples and nasal swab (66.66% and 27.27 %, respectively). No isolation was obtained from ear and conjunctival swabs. Among the 34 isolates recovered from the apparently healthy goats, 26 isolates were recovered from nasal swabs (30.59%), 2 isolates from each of tracheal swabs and lung tissues (25 % each) and 4 isolates from ear swabs (4.1%) No isolates could be obtained from conjunctival swab or milk samples (Table 4).

Identification of mycoplasma isolates:

The mycoplasma isolates were first tested to differentiate between *Mycoplasma* and *Acholeplasma* genera using digitonin sensitivity test, (Table 5). The isolates of genus Mycoplasma were further subjected to biochemical tests to be divided into 5 biochemical groups, (Table 6). Finally one isolate representing each biochemical group was tested against suspected reference mycoplasma antisera by the growth inhibition test (GIT).

- **Digitonin sensitivity test:** The results of digitonin sensitivity test of the recovered 112 mycoplasma isolates showed that 79 isolates (70.54%) were sensitive to digitonin (i.e. belonged to the Family *Mycoplasmataceae*, while only 33 isolates (29.46%) were resistant to digitonin (i.e. the Family Acholeplasmataceae).
- **Biochemical grouping:** The recovered mycoplasma isolates were divided into five groups according to their reactions to arginine utilization, glucose fermentation and film and spot formation:
- **Group I (Arginine positive, glucose negative, film and spot negative):**

  Forty one isolates were recognized in this group, 17 of them were of ovine origin and 24 of caprine origin.
- **Group II (Arginine positive, glucose positive, film and spot negative):**

  No, isolates could be recognized in this group.
- **Group III (Arginine negative, glucose negative, film and spot positive):**

  Only 6 isolates were identified from caprine or igin.
- **Group IV (Arginine negative, glucose positive, film and spot negative):**

  Thirty isolates were recognized in this grou 14 of them were of ovine origin and 16 of c prine origin.
- **Group V (Arginine negative, glucose positi film and spot positive):**

  Only 2 isolates were identified from capr origin.

Serotyping of Mycoplasma isolates (Fig. 4 and 5).: The result of growth inhibition test (GIT) on the members of the biochemical groups showed that:

In group I: Forty one isolates (51.90%) were identified serologically as *M. arginini*. 17 isolates (54.84%) of them were of ovine origin and 24 isolates (50.00%) were of caprine origin.

In group III: Six isolates were identified as *M. agalactiae* only from caprine origin (12.5%).

In group IV: Thirty isolates (24.19%) were identified into 2 serotypes as follows:

- Nineteen isolates antigenically related to *M. ovipneumoniae*, 12 isolates (38.71%) out of them were of ovine origin and 7 isolates (14.58%) were of caprine origin.
- Five isolates (10.42%) reacted to *M. mycoides* subspecies capri antiserum, all of them were of caprine origin.
- Moreover 6 isolates failed to react with the available antisera of this group, 2 (6.45%) of them were from ovine origin and 4 (8.33%) from caprine origine. i.e. untyped strains.

In group V: Only two isolates from caprine origin (4.17%) were identified as *M. putrefaciens*

Determination of MIC for Mycoplasma species: It was carried out on five representative field isolates (*M. agalactiae, M. arginini, M. ovipeumoniae, M .mycoides* subspecies capri and *M. putrefaciens*) using six different antibacterials (Erythromycin, Azythromycin, Oxytetracycline, Enrofloxacin, Streptomycin and Lincomycin). The in vitro activities of antimicrobial agents against mycoplasma isolates are shown in Table 7.

M. agalactiae was sensitive to azythromycin and enrofloxacin (0.078 ug/ml), followed by oxytetracycline (0.156 ug/ml), then erythromycin (0.312 ug/ml) and lincomycin (0.625 ug/ml) but less susceptible to streptomycin (5 ug/ml).

M. arginini was sensitive to erythromycin, azythromycin , lincomycin (0.039 ug/ml), oxytetracycline and enrofloxacin (0.312 ug/ml) and less susceptible to streptomycin (2.5 ug / ml).

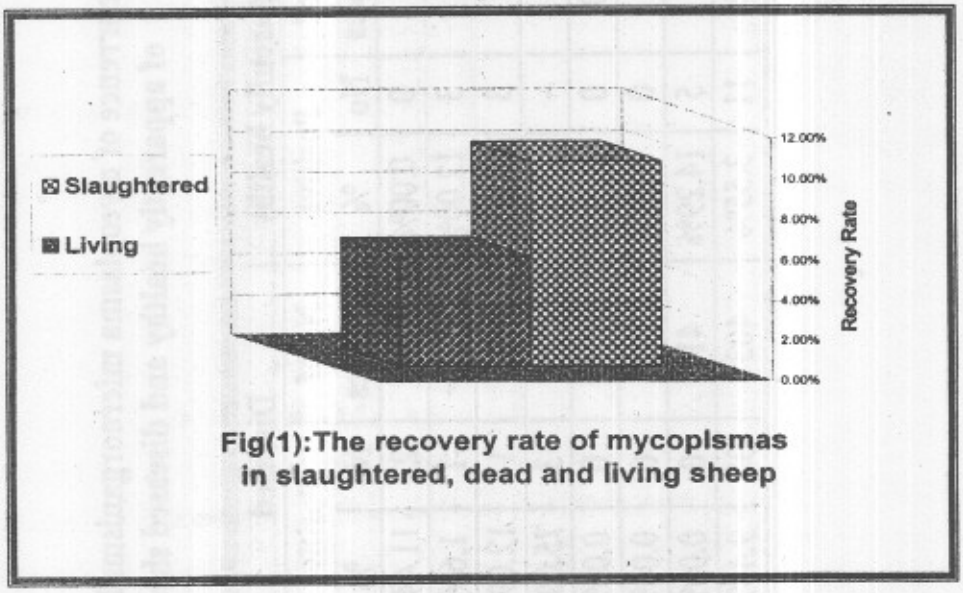
M. ovipneumoniae was sensitive to enrofloxacin (0.039ug/ml), lincomycin, oxytetracycline (0.078ug/ml) and azythromycin (0.156 ug/ml), followed by erythromycin (0.625 ug/ml) and less susceptible to streptomycin (1.25 ug/ml).

M. mycoides subspecies *capri* was sensitive to erythromycin and azythromycin (0.312 ug / ml), followed by enrofloxacin, lincomycin and oxytetracycline (0.625 ug/ml) and less susceptible to streptomycin (5 ug/ml).

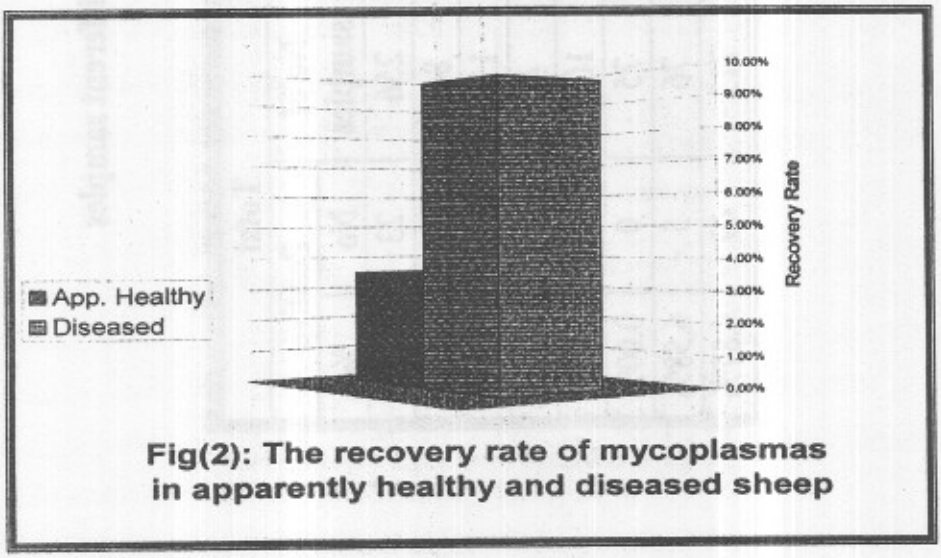
M. putrefaciens was sensitive to oxytetracycline (0.039 ug/ml) followed by azythromycin and enrofloxacin (0.156 ug/ml) and less susceptible to erythromycin (5 ug/ml) and streptomycin (10 ug/ml).

Table (1): The recovery rate of mycoplasma isolates from apparently healthy and diseased sheep.

Conditions of the examined animals	Apparently Healthy			Diseased			Total		
	No. of samples	Positive		No. of samples	Positive		No. of samples	Positive	
		No	%		No	%		No	%
Slaughtered	50	6	12.00%	156	15	9.61%	206	21	10.19%
Living	260	5	1.92%	247	23	9.31%	507	28	5.52%
Total	310	11	3.55%	403	38	9.43%	713	49	6.87%



Fig(1):The recovery rate of mycoplsmas in slaughtered, dead and living sheep



Fig(2): The recovery rate of mycoplasmas in apparently healthy and diseased sheep

Table (2): Occurrence of mycoplasma microorganisms in different samples of apparently healthy and diseased sheep.

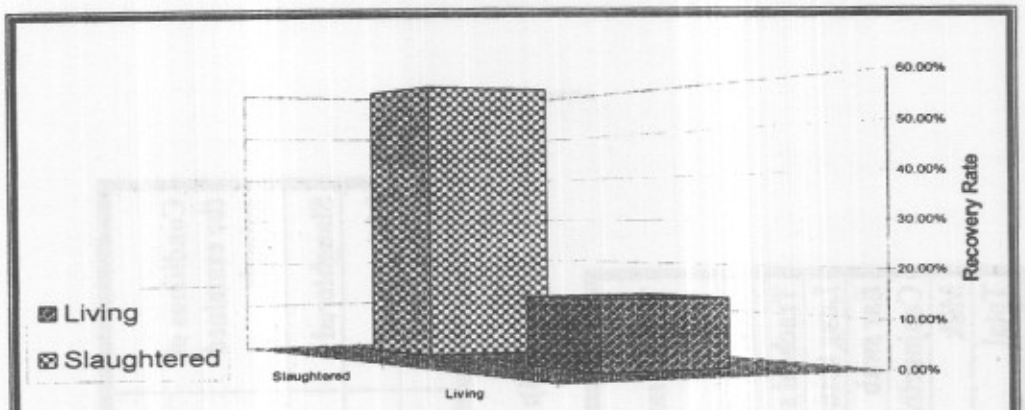
Type of samples	Apparently healthy			Diseased			Total		
	No of samples	Positive		No of samples	Positive		No of samples	Positive	
		No	%		No	%		No	%
Nasal swab	55	0	0.00%	201	23	11.44%	256	23	8.98%
Tracheal swab	25	3	12.00%	61	1	1.64%	86	4	4.65%
Lung tissue	25	3	12.00%	91	11	12.09%	116	14	12.07%
Pleural fluid	-	-	-	4	3	75.00%	4	3	75.00%
Ear swab	100	0	0.00%	0	0	0.00%	100	0	0.00%
Conjunctival swab	70	0	0.00%	5	0	0.00%	75	0	0.00%
Milk	35	5	14.29%	41	0	0.00%	76	5	6.58%
Total	310	11	3.55%	403	38	9.43%	713	49	6.87%

Table (3): The recovery rate of mycoplasma microorganisms from apparently healthy and diseased goats.

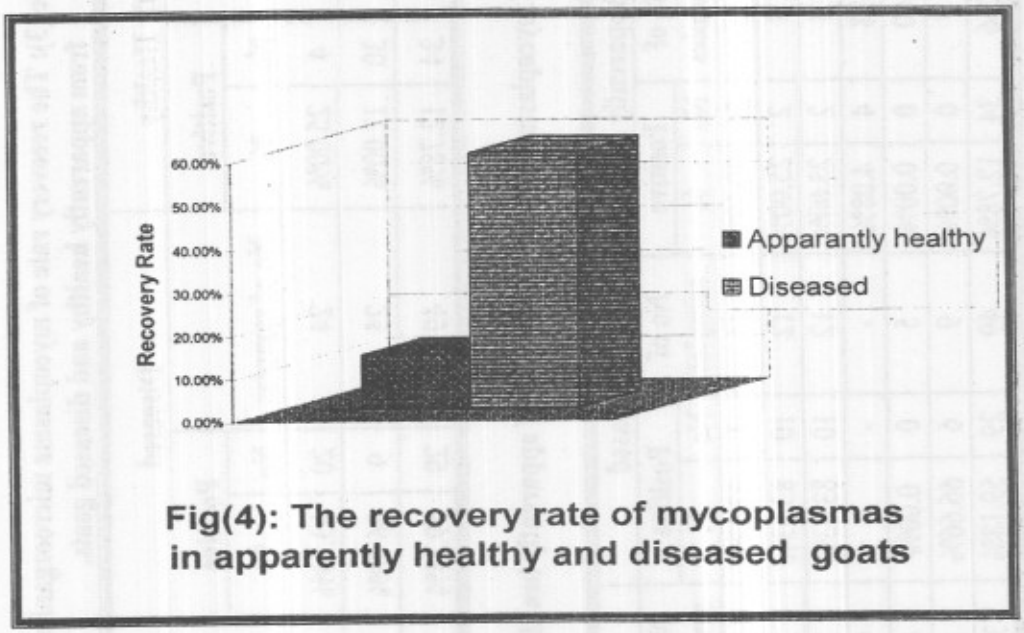
Conditions of the examined animals	Apparently Healthy			Diseased			Total		
		Positive			Positive			Positive	
	No of samples	No	%	No of samples	No	%	No of samples	No	%
Slaughtered	16	4	25.00%	24	20	83.33%	40	24	60.00%
Living	250	30	12.00%	25	9	36.00%	275	39	14.18%
Total	266	34	12.78%	49	29	59.18%	315	63	20.00%

Table (4): Occurrence of mycoplasma in different samples of apparently healthy and diseased goats.

Type of samples	Apparently healthy			Diseased			Total		
	No of samples	Positive		No of samples	Positive		No of samples	Positive	
		No	%		No	%		No	%
Nasal swab	85	26	30.59%	11	3	27.27%	96	29	30.21%
Tracheal swab	8	2	25.00%	12	10	83.33%	20	12	60.00%
Lung tissue	8	2	25.00%	12	10	83.33%	20	12	60.00%
Ear swab	98	4	4.08%	-	-	-	98	4	4.08%
Conjunctival swab	60	0	0.00%	5	0	0.00%	65	0	0.00%
Milk	7	0	0.00%	9	6	66.66%	16	6	37.50%
Total	266	34	12.78%	49	29	59.18%	315	63	20.00%



Fig(3): The recovery rate of mycoplsmas in slaughtered and living goats.



Fig(4): The recovery rate of mycoplasmas in apparently healthy and diseased goats

Table (5): Differentiation of mycoplasmas groups by digitonin sensitivity test.

Mollecutes	Sheep		Goats		Total	
	No.	%	No	%	No	%
Family Mycoplasmataceae	31	63.27%	48	76.19%	79	70.54%
Family Acholeplasmataceae	18	36.73%	15	23.81%	33	29.46%
Total	49	100.00%	63	100.0%	112	100.00%

Table (6): Biochemical Differentiation of genus *Mycoplasma* into 5 groups.

Biochemical groups	Sheep		Goats		Total	
	No	%	No	%	No	%
Group I (A+, G-,F/S-)	17	54.84%	24	50.00%	41	51.90%
Group II (A-, G-,F/S-)	-	-	-	-	-	-
Group III (A-, G-,F/S+)	-	-	6	12.50%	6	7.59%
Group IV (A-, G+,F/S-)	14	45.16%	16	33.33%	30	37.97%
Group V (A-,G+,F/S+)	-	-	2	4.17%	2	2.53%
Total	31	100.00%	48	100.0%	79	100.00%

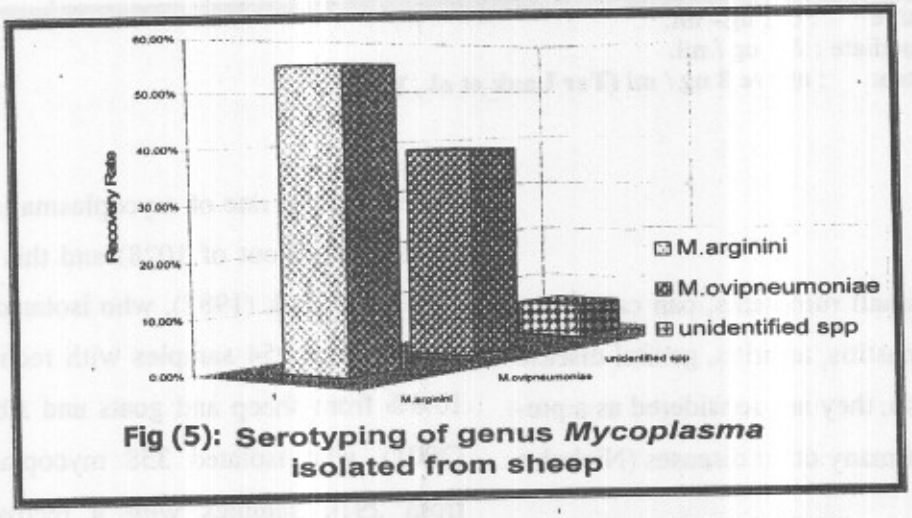


Fig (5): Serotyping of genus *Mycoplasma* isolated from sheep

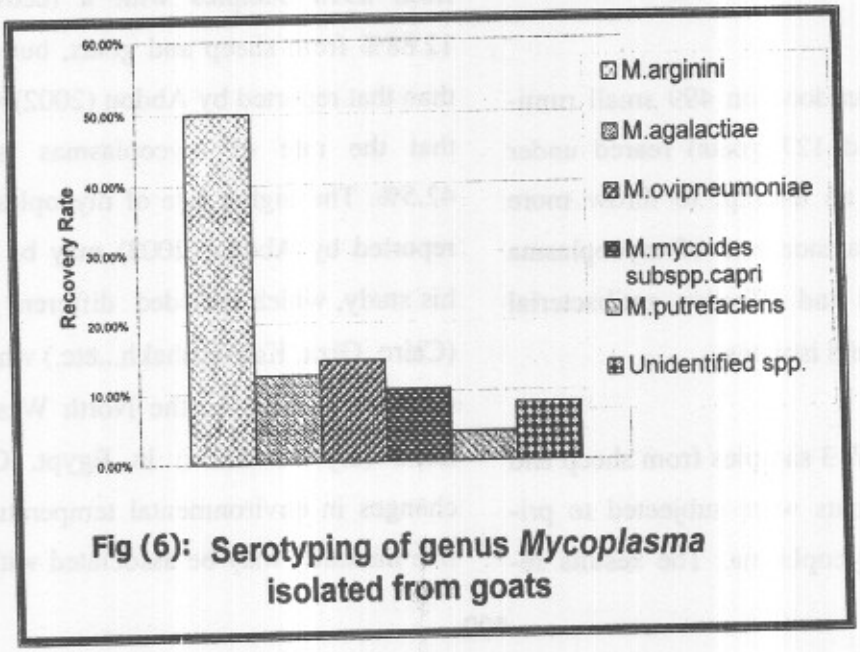


Fig (6): Serotyping of genus *Mycoplasma* isolated from goats

Table (7): MIC of six anti-microbial agents against five *Mycoplama* isolates.

Mycoplasma strains	Minimum Inhibitory Concentration (M.I.C) ug /ml					
	Erythromycin	Azythromycin	Oxytetracyclin	Enrofloxacin	Streptomycin	Lincomycin
M. agalactiae	0.312	0.078	0.156	0.078	5	0.625
M.arginini	0.039	0.039	0.312	0.312	2.5	0.039
M.ovipneumoniae	0.625	0.156	0.078	0.039	1.25	0.078
M.m..capri	0.312	0.312	0.625	0.625	5	0.625
M.putrefaciens	5	0.156	0.039	0.156	10	0.625

Sensitive : 0-1 ug / ml.
Intermediate : 2-4 ug / ml.
Resistant : above 8 ug / ml **(Ter Laak et al., 1993).**

DISCUSSION

Mycoplasmas in small ruminants, can cause respiratory disease, mastitis, arthritis, genital disease and eye lesions also, they are considered as a predisposing factor of many other diseases (Nicholas 2002).

The present work was done on 499 small ruminants (372 sheep and 127 goats) reared under desert conditions in an attempt to throw more lights upon the prevalence rate of mycoplasma among these animals and effective antibacterial drugs against some field isolates.

In the present study, 713 samples from sheep and 315 samples from goats were subjected to primary isolation of mycoplasma. The results revealed that the rate of mycoplasma isolation was 10.89 % (112 out of 1028) and this is in agreement with Resk (1987), who isolated 103 mycoplasma from 954 samples with recovery rate of 10.8% from sheep and goats and Abd El Lateef (2001), who isolated 358 mycoplasma isolaes from 2910 samples with a recovery rate of 12.88% from sheep and goats, but it was lower than that reported by Abdou (2002) who revealed that the rate of mycoplasmas isolation was 42.5%. The higher rate of mycoplasma isolation reported by Abdou (2002) may be attributed to his study, which included different governorates (Cairo, Giza, Kafr-Elshakh...etc.) while this study was limited only to the North Western Coastal Zone (dry condition) in Egypt. Consequently changes in environmental temperature and relative humidity may be associated with changes in

the incidence of mycoplasma survivability (Whittlestone, 1976 and Jones and Webster, 1984). Nagatomo et al. (2001) found that many strains of mycoplasma under dry conditions were unstable at 30°C, 37°C, room temperature and out door temperature.

Concerning the rate of mycoplasma isolation in sheep and goats, it was 6.87 % (49 positive isolates out of 713 samples) in sheep, as shown in Table (2), while it was 20 % (63 positive isolates out of 315 samples) in goats, as shown in Table (4). These results are nearly similar to that obtained by Haribabu et al. (1983) who isolated mycoplasma species from sheep with a rate of 5.08% and Al-Zeftawy (1979) and Abdou (1998), who recovered mycoplasma from goats with a rate of 25.66% and 27.75%, respectively. Consequently, the rate of mycoplasma isolation in goats was higher than that of sheep, which means that, mycoplasma are considered particularly prevalent in goats than in sheep, which may be attributed to cellular and molecular levels of mycoplasma host adaptation (Rosendal, 1994; DaMassa, 1996 and Cokrevski et al,. 2001).

Regarding the mycoplasma isolation rates in samples obtained from apparently healthy sheep and goats, they were 3.55% and 12.78%, respectively. These results are similar to that obtained by Resk (1987), Hussein (1998) and Abdou (2002). In this study, mycoplasma isolates were recovered from clinically normal sheep and goats. Therefore, mycoplasma themselves may be regarded as commensals, but during times of stress or hot weather (especially under desert conditions), subclinical infection may predispose sheep or goats to clinical infection such as acute fibrinous pneumonia, pulmonary abscessation, or pleurisy as mentioned by Ruffin (2001).

Concerning the sites of isolation of mycoplasma from apparently healthy sheep and goats, sheep mycoplasma were isolated from trachea and lung (12%), while no isolates were obtained from nasal swabs. These results may be explained as the colonization of mycoplasma in the nasal cavity of sheep is a transient phenomenon with intermittent excretion as mentioned by Ionas et al. (1985) and De La Fe et al. (2005). While in apparently healthy goats, the results of isolation of mycoplasma species from respiratory tract revealed that the highest rate of isolation was from nasal swabs (30.59%), which simulates results obtained by Al-Zeftawi (1979); Resk (1987); El-Ebeedy et al. (1989); Hussein (1998) and Abdou (2002). Also, in apparently healthy goats, 4 isolates were obtained from ear swabs and this is agreement with results recorded by Cottew and Yeats (1982); DaMassa (1996); Ribeiro et al. (1997) and De la Fe et al. (2005) who considered that occurrence of mycoplasmas in external ear of goat breeds is an important finding and plays important role in disease transmission.

Concerning to the isolation of mycoplasma from

diseased sheep and goats, mycoplasma were recovered at rates of 9.43% and 59.18%, respectively, which are nearly similar to that obtained by Resk (1987). The rate of mycoplasma isolation from diseased sheep was high from pleural fluid (75%), followed by lung tissues (12.1 %) nasal swabs (11.4 %) and tracheal swabs (1.6%). In diseased goats, the recovery rate was high from pneumonic lung samples and tracheal swabs (83.33%), followed by milk samples and nasal swabs (66.66 % and 27.27 %, respectively). Nearly similar results were reported by Livingston and Gauer (1979); Rezk (1987); Malone et al. (1988); El-Ebeedy et al. (1989); Shouman et al. (1989); Al-Zeftawi and Shaker-Muna. (1997), Hussein (1998); Abd El Lateef (2001) and Abdou-Nadra, (2002). From these results it was clear that mycoplasma microorganisms tend to harbor the lower respiratory tract, which lead to higher recovery rates from pneumonic lung than that obtained from nasal swabs. These findings are supported by those obtained by Ammar et al. (1993) who recovered mycoplasma from 76.6% and 81.2% of pneumonic lungs of sheep and goats, respectively, compared with the isolation rate from nasopharynx of diseased sheep and goats (64.7% and 63.8%, respectively).

In the present work 9 Acholeplasma isolates were recovered from apparently healthy sheep (3 tracheal swabs, 2 lung and 4 milk samples), beside 9 Acholeplasma isolates recovered from diseased sheep (5 nasal swabs, 1 tracheal swabs, 2 lung and 1 pleural fluid). In apparently healthy goats 14 Acholeplasma were isolated from nasal swabs and one *Acholeplasma* isolate was recovered from mastitic milk of diseased goat. These results agree with Banerjee et al. (1979) and Pasic et al. (1990) who isolated Acholeplasma from nasal swabs and pneumonic lung of sheep and goat. Egwu et al. (2001) isolated *Acholeplasma* laidlawi from caprine milk samples from the apparently normal healthy udders as well as mastitic udders. They concluded that *A. laidlawi* could be regarded as commensals or agents of unknown pathogenicity. Also, Ayling et al. (2004) considered that *Acholeplasma* species were found to be more opportunistic than pathogenic. On the other hand Singh et al. (1990) mentioned that experiments on 8 lactating goats indicated that *Acholeplasma* species were pathogenic to goat udders, causing mastitis and leading to agalactia without any systemic reaction, and produced pathological changes similar to, but less severe than those reported in mycoplasmal mastitis in goats and ewes.

The serotyping of *Mycoplasma* isolates revealed that the highest rate of isolation was Mycoplasma arginini (54.84% and 50% from sheep and goats, respectively). Nearly similar results were reported by many investigators as Al-Zeftawi (1979); Radwan et al. (1985); Ribeiro et al. (1997); Hussein (1998); Abdou (2002) and Ayling et al. (2004) who identified *Mycoplasma*

arginini as the most predominant species isolated from different sites of apparently healthy and diseased sheep and goats. This result could be explained on the light of the observation of Nagatomo et al. (2001) who reported that *M. arginini* survive better than other mycoplasmas under dry condition.

By studying the rate of isolation of *M. arginini* in diseased sheep and goats, it is clear that the rate of mycoplasma isolations in diseased sheep was relatively higher than in diseased goats and this throw spotlight on the role of *M. arginini* in the respiratory disease of sheep and goats, which was studied by Bocklisch et al. (1987) and Goltz et al. (1986) who concluded that *M. arginini* was one of agents responsible for ovine pneumonia, on the other hand it did not induce lesions in experimental infection in goats.

Mycoplasma agalactiae was isolated from one apparently healthy goat (ear swabs). Similar results were recorded by Cottew and Yeats (1982) and DaMassa (1996) who considered the presence of pathogenic mycoplasma in the external ear canal of clinically normal goats as an important contributor to the spreading of mycoplasma to new host. They added that many clinically normal goats, may harbor several mycoplasma in diverse anatomical sites and showed that the rate of mycoplasma isolation of 80% or more can also be encountered in the ear canal.

The isolation of *Mycomlasma ovipneumoniae* form the respiratory tract of diseased goats was reported by many authors (Goltz et al. 1986; Mohan et al., 1992; Al-Zeftawi and Shaker-Muna, 1997 and Abdou, 1998).

The isolation of *Mycoplasma putrefaciens* from respiratory tract indicated its role in respiratory affections, which coincided with DaMassa et al. (1987) who mentioned that *Mycoplasma putrefaciens* was isolated from lung and pleural fluid. Also Rodriguez et al. (1994) who recovered *M.putrificience* in a pure culture from trachea.

About the isolation of *Mycoplasma putrefaciens* (1 isolate) from mastitic milk, this is similar to result obtained by Egwu et al. (2001) who isolated *Mycoplasma putrefaciens* (1 isolate) from mastitic caprine milk. The present results supported the capability of *Mycoplasma putrefaciens* to cause mastitis and pneumonia as mentioned by Bergonier et al. (1997).

With regard to determination the sensitivity of some representative field isolate to different antibacterial drugs using minimum inhibitory concentration method as conventional techniques cannot be used for the determination of mycoplasma- antimicrobial drug sensitivity since mycoplasma requires particular media to grow beside the test antibiotic will equilibrate across the plate before mycoplasmal growth, which make this method not applicable (Sabry, 2004 and

Francoz et al., 2005). MICs have been still generally considered to be the reference point for comparison and evaluation of other sensitivity tests and indeed the efficacy of all antimicrobials is described in terms of MICs and microbroth dilution is probably the most widely used method (Hannan, 2000). The present study showed that Azythromycin had high activity against five mycoplasma strains (0.078 ug/ml with *M. agalactiae*, 0.039 ug/ml with *M. arginini*, 0.156 with *M. ovipneumoniae* and *M. putrefaciens* and 0.312 ul/ml with *M. mycoides* suspecies capri) and this agreed with Renaudin and Bebear (1990); Hannan (1995); Leophonte (1995); Dunn and Barradell 1996 and Alvarez-Elcoro and Enzler (1999) who mentioned that Azythromycin is a new macrolides, which is more chemically stable and it has a broader antimicrobial spectrum.

The present study also proved that *M. ovipeumonia, M. agalactiae M. putrefaciens* and *M. arginini* were sensitive to enrofloxacin (0.039 ug/ml, 0.078 ug/ml, 0.156 ug/ml and 0.312 ug/ml), respectively, which go hand to hand with results obtained by Otlu, (1997) and Eissa et al.(1999) who showed that *Mycoplasma arginini* and *Mycoplasma* ovipneumoniae were sensitive to enrofloxacin. Enrofloxacin was the most effective antimicrobial *in vitro* against *M. agalactiae* (Loria et al., 2003). On the other hand *M. mycoides subspecies capri* was less susceptible to enrofloxacin (0.625 ug/ml), this result was supported by Ter Laak et al. (1993) who mentioned that susceptibility or resistance to enrofloxacin depended on the species.

In the present study the sensitivity to oxytetracycline varied between the different species, as the most sensitive strains was *M. putrefaciens* (0.039 ug/ml) followed by *M. ovipeumonia* (0.078 ugml), *M. agalactiae* (0.156 ug/ml), *M. arginini* (0.312 ug/ml) and finally *M. mycoides* subspecies capri (0.625 ug/ml), which proved the efficacy of oxytetracycline in growth inhibition of *Mycoplasma* species in vitro especially *M. putrefaciens* and *M. ovipneumoniae* and this was coincided with that recorded by Harbi et al.(1981) and Otlu (1997) who concluded that oxytetracyclines were found to be the most effective drugs against *Mycoplasma* species.

The study of the sensitivity of lincomycin showed high efficacy against *M. arginini* (0.039 ug/ml) and *M. ovipeumonia* (0.078 ug/ml) followed by *M. agalactiae, M. mycoides suspecies capri* and *M. putrefaciens* (0.625 ug/ml). These results were supported by that reported by Zimmermann and Ross (1975); Inamoto et al. (1994) and Hannan (1995). Skoufos et al. (2005) concluded that administration of lincomycin is effective for the treatment and the prevention of mycoplasmal atypical pneumonia in lambs.

The present work showed that *M. arginini* was sensitive to erythromycin (0.039 ug/ml) followed by *M. agalactiae* (0.312 ug/ml). *M . ovi-*

peumonia was less susceptible than *M .arginini* (0.625 ug/ml), also, *M. putrefaciens* was less susceptible (5 ug/ml) and this result agreed with Otlu (1997) and Eissa et al. (1999) who recorded that *M. arginini* was sensitive to erythromycin (0.048 - 0.097 ug/ml), while, *M. ovipneumoniae* was less susceptible (0.097-0.39 ug/mL).

The sensitivity of mycoplasma isolates to streptomycin revealed that streptomycin had the lowest activity against the five mycoplasma species (*M. agalactiae, M. arginini, M. ovipeumoniae, M. mycoides* subspecies *capri* and *M. putrefaciens*), which were 5 ug/ml, 2.5 ug/ml, 1.25 ug/ml, 5 ug/ml and 10 ug/ml, respectively. The low susceptibility of these mycoplasma species to streptomycin was reported also by Harbi et al. (1981); Otlu (1997) and Eissa et al. (1999).

Elevation of MICs of *M. mycoides* subspecies capri which was observed in this study, was also observed by Stipkovits et al. (1984), which indicated development of resistance of this species to different antimicrobial agents.

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عزل وتصنيف عترات الميكوبلازما من الاغنام والماعز التي ترعي في البيئة الصحراوية

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فى هذه الدراسة تم عزل ١١٢ عترة ميكوبلازما من عدد ١٠٢٨ عينة تم فحصها (١٠,٨٩ %) . إشتملت هذه العينات علي مسحات أنفية ومسحات من القصبة الهوائية وأنسجة الرئة والسائل البلوري ومسحات من الأذن والعين وكذلك عينات من الألبان. تم جمع هذه العينات من عدد ٣٧٢ اغنام و١٢٧ ماعز تعيش في بيئة صحراوية. كانت نسبة إصابة الأغنام بالميكوبلازما هي ٦,٨٧% بينما كانت في الماعز ٢٠%. في هذا البحث تم تصنيف ١١٢ عترة معزولة من الميكوبلازما بإستخدام إختبار الحساسية للديجيتونين الي ٧٩ معزولة ميكوبلازما (٧٠,٥٤ %) و٣٢ معزولة لكوبلازما (٢٩,٤٦ %) . بإستخدام إختبار تثبيت النمو المناعي تم عمل تصنيف سيرولوجي لعدد٧٩ معزولة ميكوبلازما وتم تصنيفها إلي الأنواع التالية ميكوبلازما ارجينيني (٤ معزولة)- ميكوبلازما اوفونيمسوني(١٩ معزولة) ميكوبلازما ميكويديس كابري(٥ معزولات) ميكوبلازما اجالاكتي (٦معزولات) و ميكوبلازما بيترفيشيانس(٢معزولة). بإستخدام إختبار الحساسية لمضادات الحيوية تبين أن ازيسرومايسين والانرومايسين والإوكسيتراسيكلين لهم اكبر تأثير علي عترات الميكوبلازما المعزولة.