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Detection of mycoplasma infection in sheep and goats by using PCR technique, with the associated pathological lesions

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SUMMARY

Sheep and goats are important agriculture animals in Egypt. Mycoplasma is the most frequent causes of morbidity, mortality and economic losses associated with sheep and goats. Three mycoplasma species were obtained in this study from sheep and goats at Cairo abattoirs .These mycoplasmas were found either as mixed or single infections and identified by biochemical and growth inhibition (GI) tests .Mycoplasma isolates were classified into three biochemical groups :group (1) contained 12 isolates (33.33%) from sheep and 6 isolates (23.1%) from goats *(M. agalactiae*). Group (2) contained 12 isolates (33.33%) from sheep and 10 isolates (38.5%) from goats (*M. ovipneumoniae*). Group(3) contained 12 isolates (33.33%) from sheep and 10 isolates (38.5%) from goats (*M.arginini*). PCR technique was found to be much faster than conventional microbiological techniques for isolation and identification and results could be obtained within five hours. In our study, six field isolates of *M. agalactiae* were tested by PCR, the results had confirmed that the field isolates were *M. agalactiae* by the presence of specific single band at 360 bp in each of field isolates and *M. agalactiae* reference strain .Also PCR amplification of *M. ovipneumoniae* strains gave a characteristic band at 1070 bp. Six field isolates were tested for detection of *M. arginini* from sheep and goats and all of them gave a characteristic common band at 280 bp. The histopathological examination revealed that the severity of both sheep and goats infection with *M. ovipneumoniae* is greater than those infected with *M. agalactiae* and / or *M. arginini* .Lesions were mainly found in the lungs in the form of fibrinous pneumonia with pleuritis . Hepatocellular necrosis, lymphoid depletion in the spleen and bronchial lymph nodes as well as degeneratic changes and necrosis of the tracheal and intestinal epithelium.

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INTRODUCTION

Sheep and goats are one of the most important agricultural animals in most countries all over the world. Therefore a great attention is directed toward caprine and ovine industry to meat with people requirements.

Mycoplasma is one of the causative agent of pleuropneumonia in sheep and goats that is considered by local veterinary authorities as the most serious epidemic disease causing high morbidity, mortality and consequently great economic losses.

The most important mycoplasma infections affecting sheep and goats caused contagious caprine pleurrpneumonia (CCPP) and contagious agalactia syndrome (CAS), other mycoplasma species were associated with conjunctivitis and mastitis (Egwa et al., 2000)

Aly and Dardeer (2003) studied an out break of respiratory affections in a herd of sheep. Different Mycoplasma spp. could be isolated included *M. arginini* (60%), *M. agalactia* (22%) and *M. conjunctiva* (35%).

Post mortem findings of mycoplasmas were found in the form of accumulation of reddish serofibrinous exudates and hepatized lungs. Microscopically, there was edema, thickening and inflammatory cell infiltrations in the pleura and interlobular septa, grey hepatization of the lungs with hyperplasia of bronchial epithelium which undergoes necrotic changes. There were multifocal haemorrhages and neutrophil infiltration in the liver and spleen as well as hepatic multifocal necrosis (Hernandez et al., 2006).

PCR was used for detection of *M. ovipneumoniae* in sheep and goats by using oligonucleotide primers which were designed on the basis of 16 sr DNA gene of *M. ovipneumoniae* standard strain. This PCR amplified only the target DNA fragment from *M. ovipneumoniae* which was at 1070 bp (Zhang et al., 2004) and also for identification of *M. agalactia* and evaluate the specifity of this PCR system (Bashiruddin et al., 2005).

The aim of this work is to determine the incidence of Mycoplasma spp. in sheep and goats, to assess the value of using the recent techniques in identification of the Mycoplasma spp. as well as studying the pathological lesions associated with the isolated Mycoplasma spp.

MATERIALS AND METHODS

A total of 50 slaughtered animals (30 slaughtered sheep and 20 slaughtered goats) were examined for Mycoplasma species from Cairo abattoirs (El-Bassatin and El-Monieb abattoirs). Representative tissue specimens from internal organs (lung, trachea, bronchial lymph nodes, spleen, liver and intestines) of these slaughtered animals were selected and

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divided into two parts. The first part were collected separately in sterile plastic bags and transported in ice box for mycoplasmal isolation and the second part was put in 10% neutral buffer formalin for pathological examination.

Mycoplasma isolation and identification:

A-Culture:

1. Primary isolation of mycoplasma and acholeplasma were done using hart infusion broth and agar as described by Sabry and Ahmed (1975).
2. Differentiation between mycoplasma and acholeplasma were done using Digitonin test according to Freundt (1983).

B-Biochemical identification:

1. Glucose fermentation test (Erno and Stipkovits, 1973): The suspected cultures were inoculated into glucose medium and incubated at 37 °C. The change of the color from red to yellow orange was considered positive, result.
2. Arginin deamination test (Erno and Stipkovits, 1973): The ability of the purified cultures to hydrolyse arginin was tested. The change of the color from red to pink was considered positive, result.
3. Film and spot formation test (Fabricant and Freundt, 1967): The medium was cultured with the tested isolates and incubated at 37 °C. The observation of pearly area clearing around areas of growth was considered positive, result.

C- Polymerase chain reaction (PCR):

a) Preparation of samples for DNA extraction (Yleana et al., 1995): 5ml of 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 100oC 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 supernatant containing chromosomal DNA was collected and stored at -20oC 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

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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 oC for 45 seconds, primer annealing at 60oC for 1 min., and extension at 72oC for 2 min.the amplifications were performed for 30 or 35 cycles with a final extention step at 72oC 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).

Pathological examination:

Representative tissue specimens from lungs, trachea, bronchial lymph nodes, spleen, livers and intestines were fixed in 10% neutral buffer formalin solution for at least 24 hours.

They were routinely processed by standard paraffin embedding technique sectioned at 4 µ and stained with:

1-Haematoxylin and Eosin (Bancroft et al., 1994).

2-Masson's trichrom stain for detection of fibrous connective tissue according to Clayden (1971).

RESULTS

Incidence of positive samples: Results obtained in Table (1) revealed that out of 180 samples from tissue organs of sheep, 42 were positive to mycoplasmal isolation with an incidence of (23.33%), while 32out of 120 samples from tissue organs of goats were positive with an incidence of (26.7 %).

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Table (1): Rate of isolation of mycoplasma species from tissue samples of sheep and goats.

Tissue samples	Sheep			Goats		
	No. of exam	No. of +ve	%	No. of exam.	No. of +ve	%
Lungs	30	9	30	20	7	35
Trachea	30	8	26.7	20	7	35
Bronchial lymph nodes	30	8	26.7	20	7	35
Liver	30	6	20	20	4	20
Spleen	30	6	20	20	4	20
Intestines	30	5	16.7	20	3	15
Total	180	42	23.33	120	32	26.7

Table (2): Number of mycoplasma and acholeplasma isolated from Tissue cultures of sheep samples

No. of positive samples For mycoplasma	Cultured isolated			
	Positive No. of Acholeplasma	% of +ve	Positive No . of Mycoplasma	% of +ve
(9) lungs	2	25	7	77.78
(8) Trachea	2	25	6	75
(8) Bronchial lymph nodes	2	25	6	75
(6) Liver	-	-	6	100
(6) Spleen	-	-	6	100
(5) Intestine	-	-	5	100
Total (42)	6	14.29	36	85.7

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Table (3): Number of mycoplasma and acholeplasma isolated from Tissue cultures of goats samples.

No. of samples	Cultured isolated			
	Positive No. of Acholeplasma	% of +ve	Positive No. of Mycoplasma	% of +ve
(7) lungs	2	28.57	5	71.29
(7) Trachea	2	28.57	5	71.29
(7)Bronchial lymph nodes	2	28.57	5	71.29
(4) Liver	-	-	4	100
(4) Spleen	-	-	4	100
(3) Intestine	-	-	3	100
Total (32)	6	18.75	26	81.25

2-Primary isolation of mycoplasmas and Digitonin test for differentiation between *Mycoplasma* and *Acholeplasma* genera from sheep and goats.

In table (2) and (3) a total of 36 mycoplasma isolates were obtained from 42 positive samples (85.7) % , from tissue organs of sheep and 26 mycoplasma isolates were obtained from 32 positive samples (81.25) from tissue organs of goats.

From sheep organs, the higher isolation rate was from lung (7) ,trachea, lymph node,liver and spleen samples(6) isolates from each , while the lowest isolation rate was from intestine 5 mycoplasma isolates. As for goats organs, the higher isolation rate of mycoplasma was from lung, trachea and lymph node (5) isolates from each. Four myocplasma isolates were obtained from liver and four from spleen samples. While the lowest rate of isolation was from intestine three isolates. Application 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 6 samples were acholeplasma ,(6 acholeplasma were isolated from lung, trachea, lymph node of sheep two

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for each).Also there were 6 acholeplasma isolates from lung, trachea ,lymph node of goats two for each.

3-Biochemical characterization of obtained isolates

A total of 62 isolates (36 from sheep and 26 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 and goats mycoplasmas that are summarized in Table (4).

Table (4): Biochemical characterization and serological identification of the obtained isolates

ORGANS	SHEEP					GOATS				
	Biochemical test					Biochemical test				
	No. of Ex.	G	A	F&S	Serological test	No .of Ex.	G	A	F&S	Serological test
Lung	7	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	2M.agalactia 2M.ovipneum. 3 M.arginine	5	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	1M.agalactia 2M.ovipneum. 2 M.arginine
Trachea	6	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	2M.agalactia 2M.ovipneum. 2 M.arginine	5	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	1M.agalactia 2M.ovipneum. 2 M.arginine
Lymph node	6	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	2M.agalactia 2M.ovipneum. M.arginine 2	5	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	1M.agalactia 2M.ovipneum. 2 M.arginine
Liver	6	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	2M.agalactia 2M.ovipneum. 2 M.arginine	4	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	1M.agalactia 2M.ovipneum. 1 M.arginine
Spleen	6	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	2M.agalactia 2M.ovipneum. 2 M.arginine	4	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	1M.agalactia 1M.ovipneum. 2 M.arginine
Intestine	5	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	2M.agalactia 2M.ovipneum. 1 M.arginine	3	-ve +ve -ve	-ve -ve +ve	+ve -ve -ve	1M.agalactia 1M.ovipneum. 1 M.arginine

A= arginine hydrolysis F&S= film and spot formation G = glucose fermentation

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4- Results of PCR for *mycoplasma agalactiae* isolates:

photo (1) 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.

5-Results of PCR for detection of *M. arginini*:_the results were shown in photo (2) 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.

6-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 reveales that all *Mycoplasma ovipneumoniae* strains isolated from sheep and goats gave a characteristic band at 1070 bp

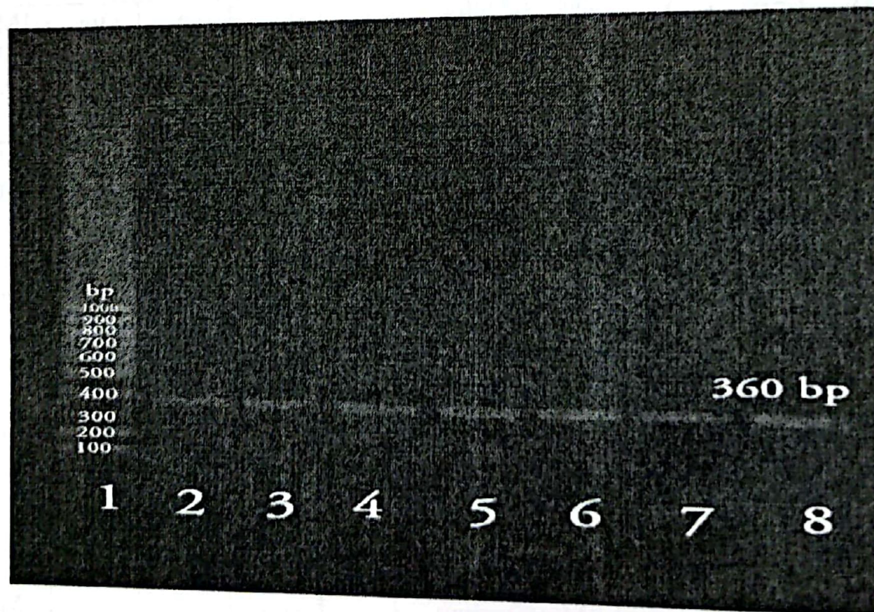

Photo (1): Electrophoretic pattern of the PCR products of *Mycoplasma agalactiae* DNA isolated from sheep and goats using the 16S rRNA gene.
Lane 1:- 100 bp DNA Ladder (Pharmacia).
Lane 2:-*M. agalactiae* reference strain.
Lane3-5:-*M. agalactiae* field isolates from sheep.
Lane 6 - 8:- *M. agalactia* field isolates from goats.
Our results (Photo.2) revealed the presence of *M. agalactia* specific band at 360 bp.

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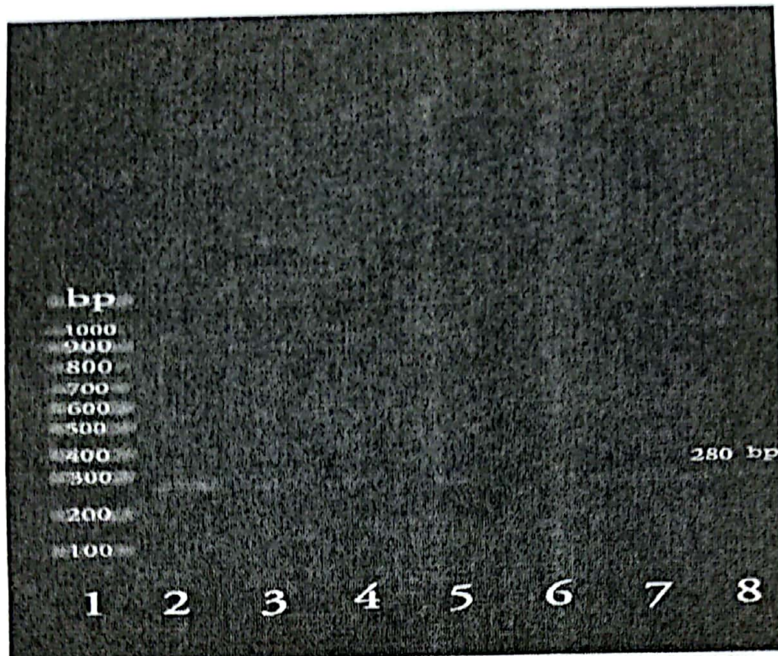

Photo(2): Electrophoretic pattern of the PCR products of *Mycoplasma argenine*
DNA isolated from sheep and goats using the 16S rRNA gene.
Lane 1:- 100 bp DNA Ladder (Pharmacia)
Lane 2:-*M. argenine* reference strain.
Lane 3 - 5:- *M. argenine* field isolates from sheep.
Lane 6 - 8:- *M. argenine* field isolates from goats.
Our results (Photo.1) revealed the presence of *M. arginine*
Specific band at 280 bp.

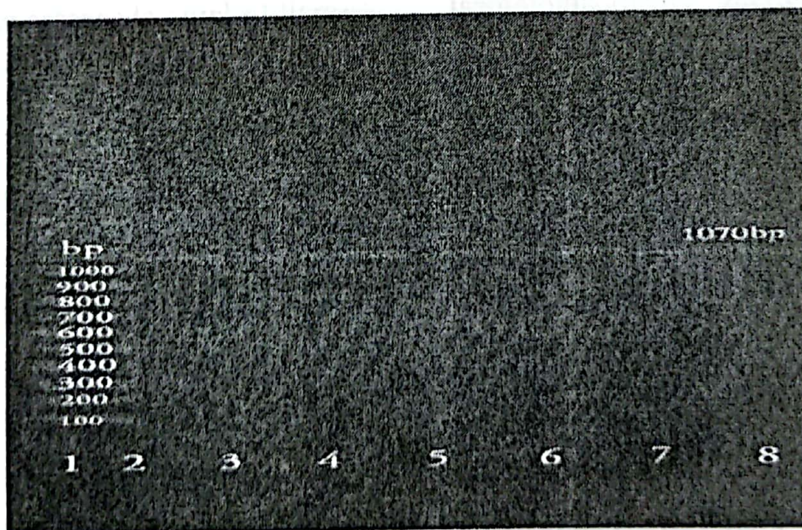

Photo (3): Electrophoretic pattern of the PCR products of *Mycoplasma ovipneumoniae* DNA isolates using the 16rDNA gene.
Lane 1:- 100 bp DNA Ladder (Pharmacia).
Lane 2:- control positive.
Lane 3- 5:- *M. ovipneumoniae* field isolates from sheep.
Lane 6 - 8:- *M. ovipneumoniae* field isolates from goats.
Our results (Photo.3) revealed the presence of *M. ovipneumoniae* specific band at 1070 bp.

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5-Gross pathology:

The lungs were bilaterally enlarged, edematous and congested. On palpation, extensive amount of air were recognized inside it The pleura was thickened and fibrotic. Petechial haemorrhages were seen on its parietal surface. In8 cases, the lungs exhibited numerous variable sized abscesses in the pulmonary tissue. On cut section, creamy whitish pus oozed out. The bronchial lymph nodes were enlarged, edematous and haemorrhagic. The trachea, spleen and intestines were congested. The livers were congested, enlarged and in most cases, there was numerous whitish foci 2.5 cm in diameter on its capsular surface. On cut section, creamy whitish pus oozed out.

6-Microscopical examination:-

The most characteristic histopathological feature indicated that the severity of sheep and goat infection with *M. ovipneumoniae* is greater than that with *M. argini* and *M. agalactiae*.

Lungs:

The pulmonary blood vessels were dilated, engorged with blood and sometimes surrounded by focal inflammatory cell aggregations (mainly mononuclear cells) (Fig. 1). Focal areas of emphsyma in the pulmonary parenchyma were also noticed and compensated with areas of collapse. Vasculitis with marked degenerative and/or necrotic lesions of the endothelial layers were recognized in some blood vessels. The alveolar lumina showed presence of cellular exudates encompassed of mononuclear cells mainly histocytes admixed with neutrophils with destruction of interalveolar septa were also evident in many instances (Fig.2&3). In most cases, the alveolar lumina were massively distended with faintly eosinophilic fibiller network of fibrin studded with mononuclear cells and polymorphnuclear leukocytes (fibrinous pneumonia). Meanwhile, other alveoli exhibited edematous fluid admixed with inflammatory cells (Fig.4). Thickening of internalveolar septa due to proliferation of pneumocyte type II cells with the presence of focal areas of atelectasis surrounded by compensatory emphsymatous parts (Fig.5) Furthermore, there were numerous small to large abscesses were recognized in the pulmonary tissue. Each abscess composed of a central deeply basophilic casated or calcified core surrounded by heavy aggregations of dead and intact neutrophils, lymphocytes, histiocytes and bounded by a thick fibrous connective tissue capsule which brings about pressure atrophy to the adjacent pulmonary tissue (Fig. 6A & B). Moreover, the bronchi and bronchioles displayed hyperplasia of their lining epithelium as well as mononuclear cell aggregations in the mucosal layer particularly around bronchial glands. Sometimes, cellular exudate admixed with exfoliated bronchial

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epithelium in their lumina were also evident (Fig. 7 & 8). The pleura appeared thickened by fibrous connective tissue proliferations which extended deep into the pulmonary tissue (Fig. 9A & B).

The bronchial lymph nodes revealed depletion of the lymphoid cells in both lymphoid follicles and cortical zones (Fig. 10).

Trachea:

The main tracheal lesions was necrosis of the epithelial lining the tracheal mucosa with mononuclear cell infiltrations in the sub mucosal layer (Fig. 11).

Spleen:

Marked depletion of lymphoid cells in the white pulp with necrosis of some lymphoid cells in the germinal centers were the most prominent lesions as well as wide dilatation of the splenic sinusoids were seen (Fig. 12 & 13).

Liver:

The hepatic tissue showed disorganization at the hepatic cords, vacuolar degeneration and individual cell necrosis of hepatocytes. The necrotic hepatocytes exhibited strongly eosinophilic cytoplasm with karyopyknotic nuclei (Fig.14). Increased fibrous connective tissue proliferation in the portal triads were evident in many instances and sometimes the proliferated fibrous connective tissue insinuate itself in between the degenerated and necrotic hepatocytes and brings about pressure atrophy on the adjacent cells. Leukocytic cell infiltrate in the form of histiocytes, lymphocytes and neutrophils were detected in the proliferated fibrous tissue (Fig. 15A & B). Proliferations of the biliary epithelim with formation of more than one bile ductules were also evident (Fig.16). Moreover, old calcified hepatic abscesses were noticed in the liver of some goats. The abscesses were embedded in the hepatic parenchyma and bounded by fibrous connective tissue capsule (Fig.17). The hepatic abscess caused pressure atrophy on the neighboring hepatic tissues.

Intestine:

The mucosal and submucosal blood vessels were engorged with blood. The intestinal mucosa showed coagulative necrosis of the surface enterocytes manifested by homogenous strongly eosinophilic cytoplasm with karyolysis of the nuclei and exfoliation of the surface enterocytes into the intestinal lumina.The intestinal glands in most cases exhibited degenerative and necrotic changes and sometimes surrounded by fibrous connective tissue (Fig.18). Heavy infiltration of mononuclear and polymorphonuclear cells in the submucosa with formation of focal aggregations were also detected.

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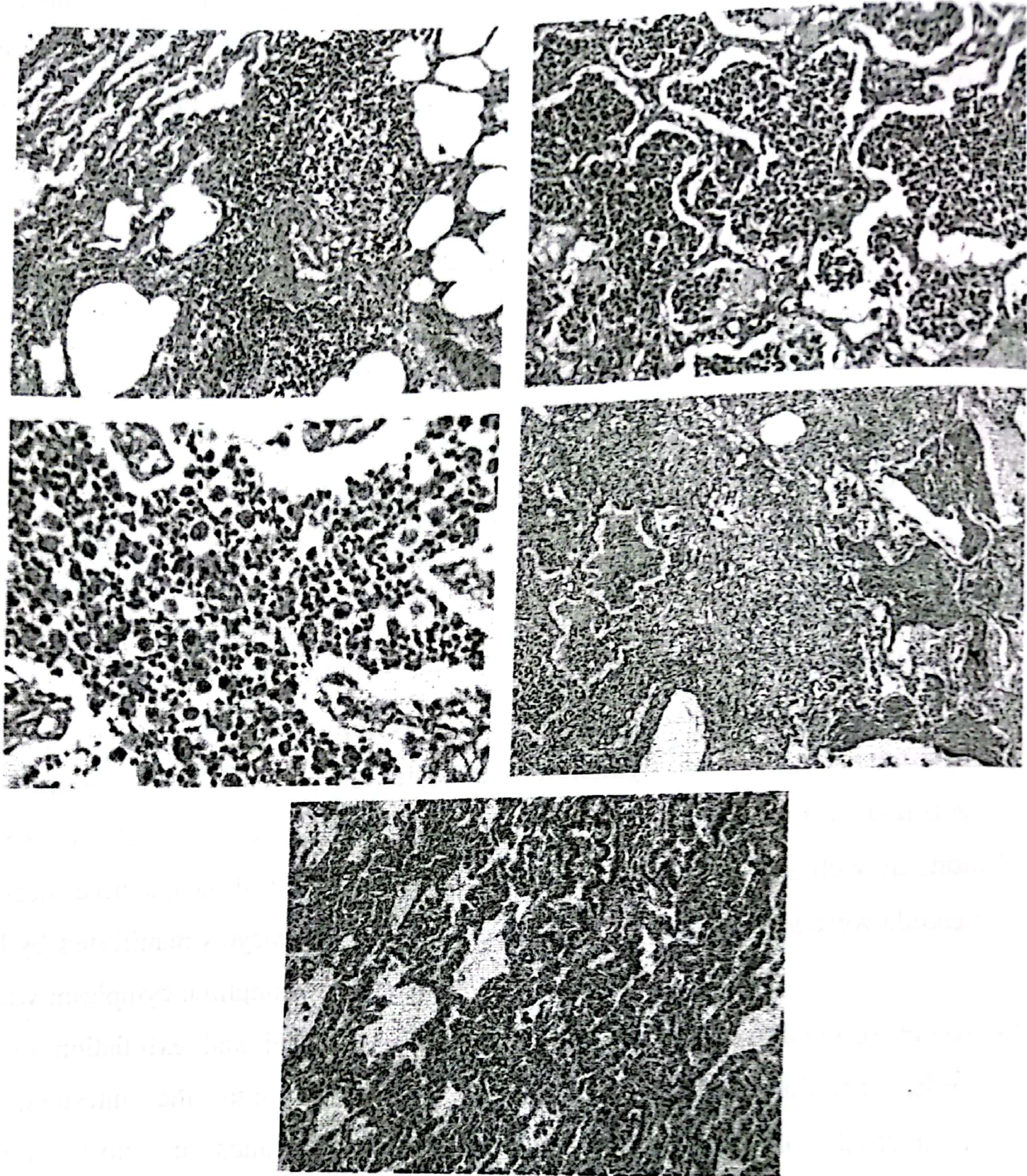


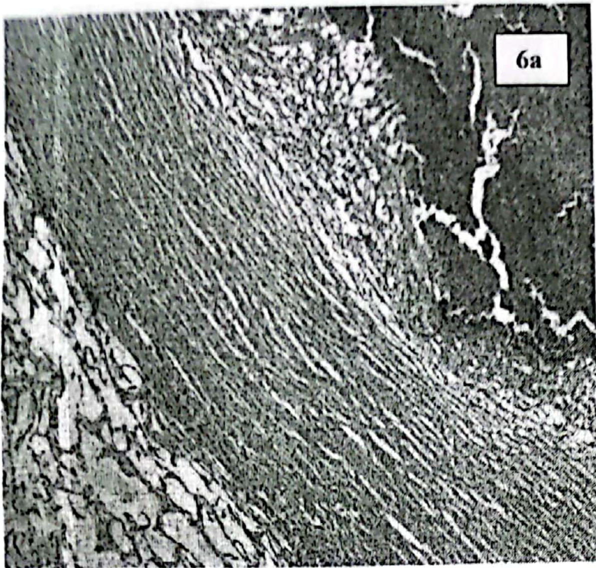
Fig. (1): Lung of sheep showing focal aggregations of mononuclear cells around pulmonary blood vessel. H&E × 200

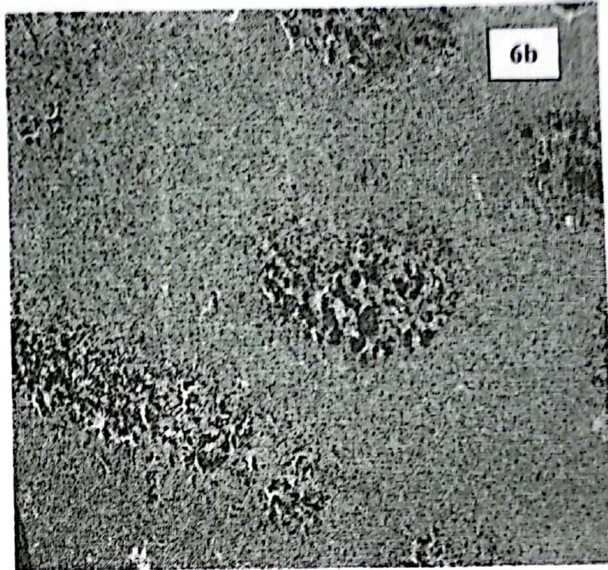
Fig. (2): Lung of sheep showing cellular exudates in lung alveoli composed of mononuclear cells mainly macrophages. H&E × 200

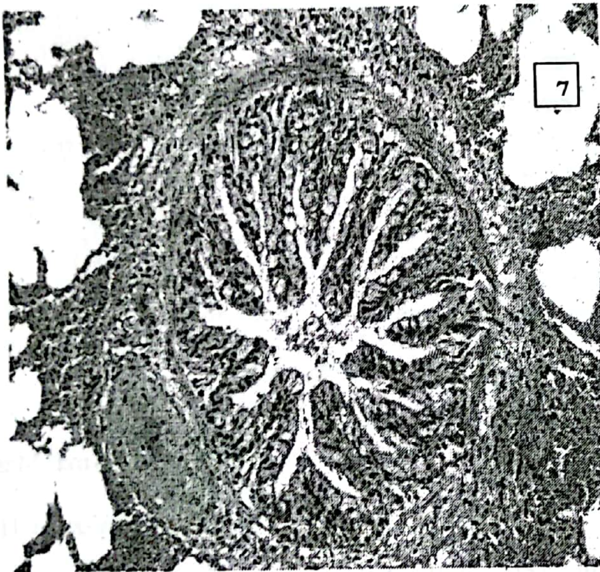
Fig. (3): High magnification of fig. (2), illustrating cellular exudates mainly polymorphonuclear cells, lymphocytes and macrophages. H&E × 400

Fig. (4): Lung of sheep showing fibrinous exudates in the alveolar lumina (right) , edemal fluid admixed with few inflammatory cells (left) and fibrous c. t. in the lung parenchyma. H&E × 200

Fig. (5): Lung of sheep showing areas of atelectasis.The alveolar septa appeared in an opposite position with slit - like openings. H&E × 200







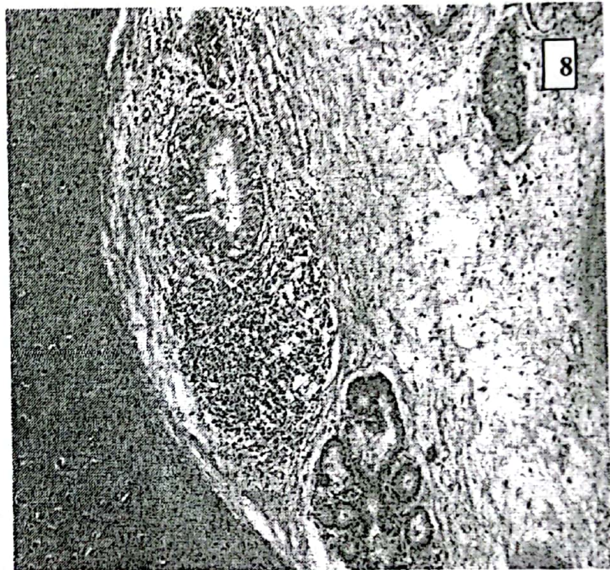


Fig. (6 A): Lung of sheep showing an old abscess composed of deeply basophilic calcified core surrounded by a layer of leukocytes mainly dead and intact neutrophils and bounded by a thick fibrous connective tissue capsule. H&E × 200

Fig. (6 B): Lung of sheep showing central core of the abscess revealing extensive necrosis of the pulmonary tissue with focal deposition of calcium salts. H&E × 100

Fig. (7): Lung of goat revealing hyperplasia of the epithelial lining of the pulmonary bronchi. H&E × 200

Fig. (8): Lung of goat showing focal mononuclear cell infiltrations around the bronchial glands. H&E × 200

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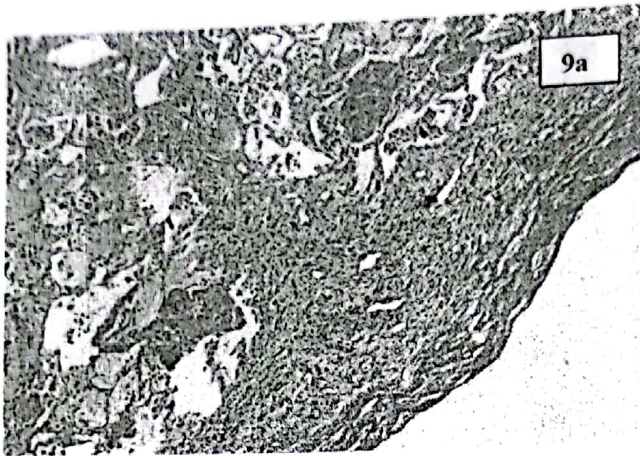

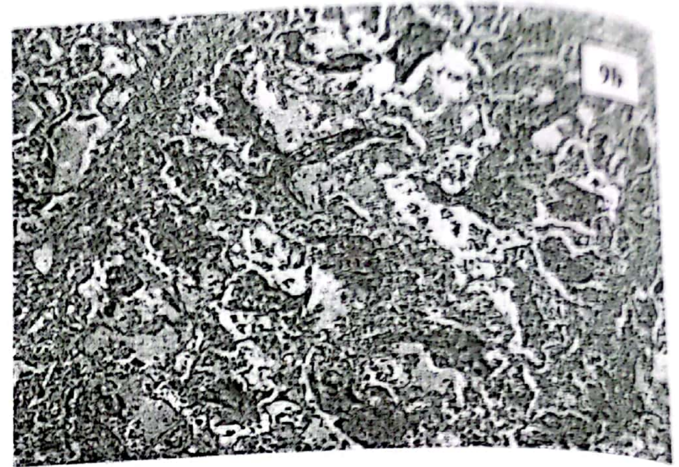

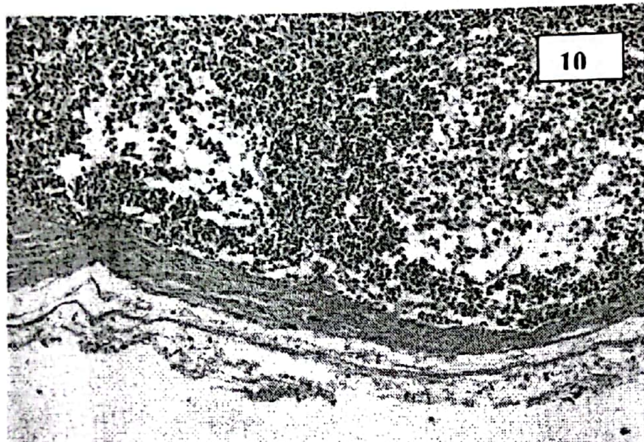

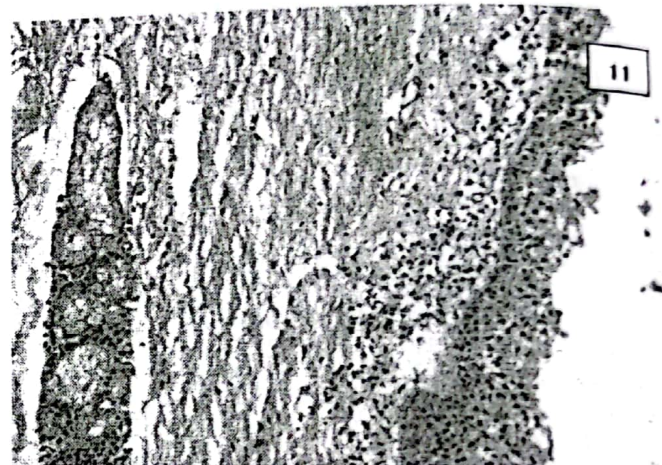

Fig. (9 A): Lung of sheep showing fibrous connective tissue proliferation in the pleura. H&E × 200

Fig. (9 B): Lung of sheep showing fibrous connective tissue proliferation in the pulmonary parenchyma. Masson's trichrom stain ×200

Fig. (10): Lymph node of sheep showing depletion of lymphoid cells in lymphoid follicles in the cortical zone. H&E × 200

Fig.(11): Trachea of goat revealing necrosis of epithelial cells of the trachea and subepithelial mononuclear inflammatory cells infiltrations. H&E × 200

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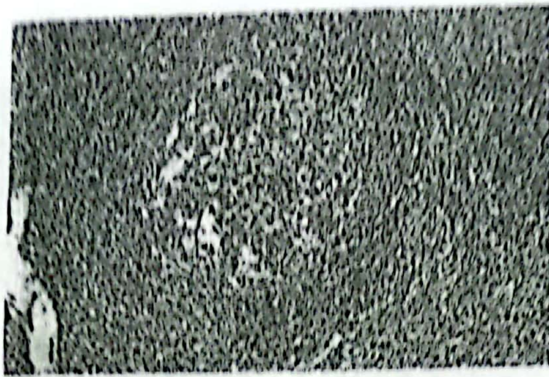

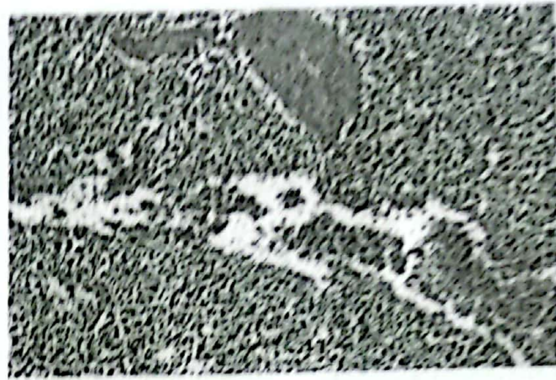

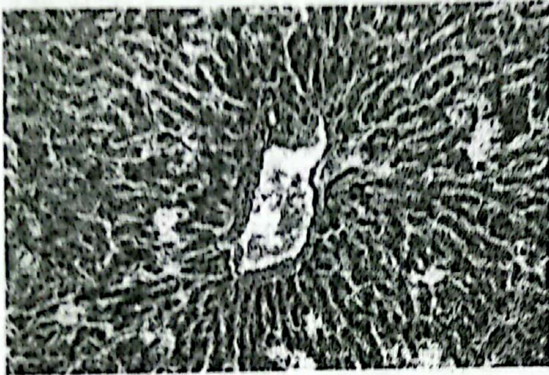

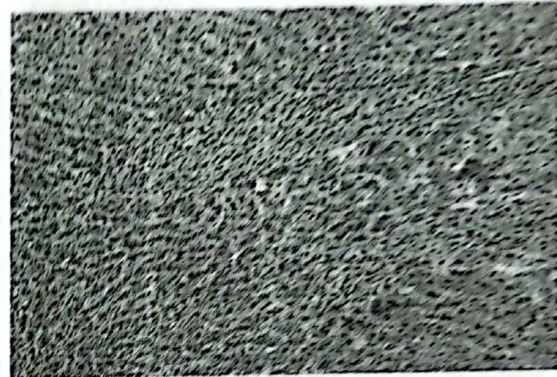

Fig. (12): Spleen of sheep revealing depletion of lymphoid cells in the white pulp. H&E × 200

Fig. (13): Spleen of sheep showing wide dilatation of the splenic sinusoids. H&E × 200

Fig. (14): Liver of goat illustrating distortion of the hepatic cords ,dilated hepatic sinusoids and necrobiotic changes of the hepatocytes around the portal area. H&E × 200

Fig. (15A): Liver of goat showing of fibrous c.t. proliferation in between the hepatocytes which infiltrated with mononuclear cells. H&E × 200

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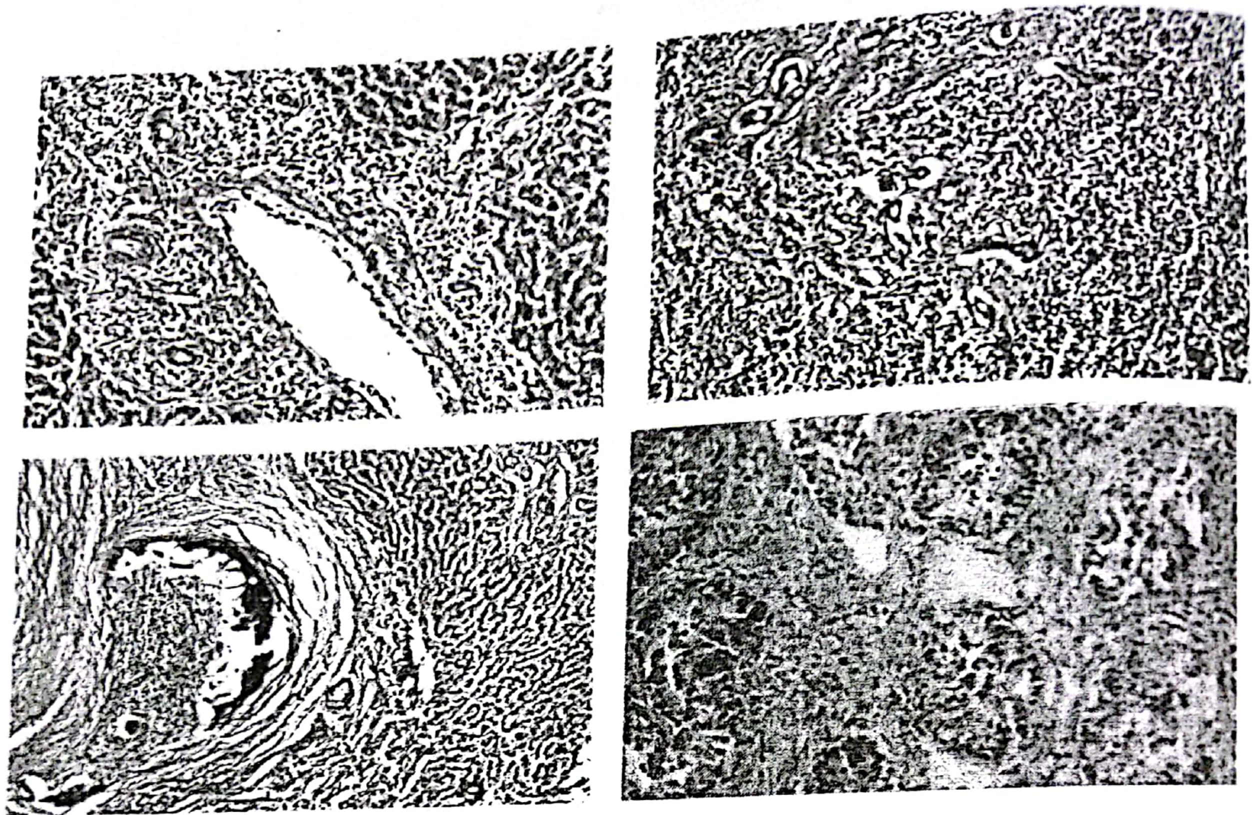

Fig.(15B): Liver of goat showing fibrous c.t. proliferation in the portal area with infiltration of mononuclear cells . H&E × 200

Fig.(16): Liver of goat showing numerous newly formed bile ductules .H&E × 200

Fig.(17): Liver of goat illustrating an old abscess embedded in the hepatic parenchyma. H&E × 200

Fig.(18):Intestine of goat showing fibrous C.T. proliferation around necrosed intestinal glands. H&E × 200

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DISCUSSION

The role of mycoplasma in the diseases of sheep and goats in Egypt has been studied by Al-Zeftawi (1973, 1979) constituting the first systematic study. In the present study, 7mycoplasma isolates were obtained from 30 lung samples from sheep with an incidence of 23.33 %, while5 isolates was obtained from 20 lung samples from goats with an incidence of 25 %.These results nearly agree with Rania (2006)who isolated 6 mycoplasma isolates out of 12 lungs of diseased goats with an incidence of 50 %. These results agree with Shaheen et al., (2001) who recovered mycoplasma in pneumonic lungs of goats with an incidence of 35 %, also agree with Otlu (1997) who isolated 61 mycoplasma isolates out of 247 pneumonic lungs of sheep with an incidence of 25 %.

However, these results disagree with Mostafa (2003) who recovered mycoplasma from lungs of diseased sheep with an incidence of 75 % (3 isolates out of 4 samples) and recovered mycoplasma from lungs of diseased goats with an incidence of66.67 % (2 isolates out of 3 samples), also these results don't agree with Hussein (1998) who recovered mycoplasma from lungs of diseased sheep with an incidence of 72 % (18 isolates out of 25 samples) and recovered mycoplasma from lungs of diseased goats with an incidence of 100 % (2 isolates out of 2 samples).

The digitonin sensitivity test for differentiation between mycoplasma and acholeplasma genera was applied and revealed that all obtained isolates from both sheep and goats belonged to genus mycoplasma except 6sheep and 6 goat samples belonged to genus acholeplasma. These results agree with Rania (2006) who recovered 14 acheloplasma isolates (14 from sheep and 4 from goats) and also revealed that these isolates were from nasal swabs and were more frequently isolated from apparently healthy animals than diseased ones, Rahman and Singh (1990) who isolated acholeplasma but from pneumonic lungs of slaughtered goats, Rezk (1987) who isolated 2 acholeplasma isolates from sheep and these results don't agree with Hussein (1998) and Mostafa (2003) as they did not isolate any acheloplasma in their work.

Biochemical characterization tests using arginine deamination, glucose fermentation and film and spot formation tests were performed and all obtained isolates from sheep and goats were classified into . 3 biochemical groups:Group (1)contained 12 isolates (33.33%) from sheep and 6 isolates (23.1 %) from goats (*M.agalactia)* .

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Group (2) contained 12 isolates (33.33%) from sheep and 10 isolates (38.5 %) from goats (*M.ovipneumone*).

Group (3) contained 12 isolates (33.33%) from sheep and 10 isolates (38.5 %) from goats (*M.arginine*).

Similar results of biochemical characterization were recorded by Rania (2006) who classified all obtained isolates into three biochemical groups.

These results nearly agree with Hussein (1998) and Mostafa (2003) who classified mycoplasma isolates of sheep and goats into four groups.

However, these results disagree with Nadra (2002) who classified mycoplasma isolates of sheep into five biochemical groups and classified mycoplasma isolates of goats into four biochemical groups.

These results agree with Mostafa (2003) who recovered 19 mycoplasma isolates from goats belonged to group (1) with an incidence of 35.18 % and Nadra (2002) who recovered 38.99 % of mycoplasma isolates from goats belonged to this group.

These results agree with Rania (2006) who recovered 6 mycoplasma isolates from goats belonged to group(2) with an incidence of 13.95 % and recovered 22 mycoplasma isolates from sheep belonged to this group with an incidence of 23.40 % and Hussein (1998) who recovered 32 mycoplasma isolates from sheep belonged to this group with an incidence of 22.53 %.

However, these results disagree with Mostafa (2003) who showed that group(2) recorded lower incidence rates in sheep and goats (3.75 % and 7.40 %) respectively and Hussein (1998) who recovered 2 mycoplasma isolates from goats belonged to this group with an incidence of 3.37 %.

Serological identification was carried out using growth inhibition test for the isolates in each biochemical group against specific antisera which revealed the identification of three mycoplasma species from sheep and also the same three *Mycoplasma* species from goats.

Similar results were recorded by several authors asNevine et al.,(2001) who isolated *M. arginini, M. ovipneumoniae* from pneumonic lungs collected from goats with an incidence of 60.82% and also Pasic et al.,(1990) who identified *M. arginini* an *M. ovipneumoniae* d from pneumonic sheep lungs with an incidence of 17.94%. Bocklisch et al.,(1987) who isolated *M. arginini* from pneumonic sheep lungs with an incidence of 14.83%.

Similar results were recorded by Aly and Dardeer (2003) who isolated 22 % of *M. agalactiae* from lung tissues of sheep and this result nearly agrees with that obtained in this study (32 %) and Gupta and Verma (1984) who

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isolated *M. agalactiae* from sheep by a percentage of 34 %.

The 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).

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).

Regarding to the pathological examination, the macroscopical pathology showed congestion in all examined organs with mild enlargement of the liver and spleen and marbling appearance of the lungs in association with abscesses in both lungs and livers. Similar results were observed by Kumar et al., (1994); El-Manakhly (1995) and Quinn et al., (2002). Meanwhile Hernandez et al., (2006) found areas of consolidation in the lungs, pleuritis and no gross lesions in other organ except for haemorrhagic intestine.

The histopathological study revealed that sheep and goats infected with *M. ovipneumoniae* were more severely affected than those with *M. arginini* and/or *M. agalactiae* as mentioned by Mary & David (1994) who found subacute fibrinous pleuritis in goats infected by *M. ovipneumoniae* while goats infected with *M. arginini* is of doubtful pathogenicity. While Goltz et al., (1986) mentioned that *M. arginini* is less fastidious than *M. ovipneumoniae* as he did not found significant lesions in experimentally infected goats with *M. arginini*.

Histopathologically, the lung tissue showed cellular exudates in some alveolar lumina while most cases exhibited fibrinous exudates. Similar results were observed by Goltz et al., (1986); El-Manakhly (1995); Hernandez et al., (2006) and Rania (2006).Also Jubb et al., (1993) suggested that the production of profuse fibrinous exudates correlates with a fulminating pulmonary inflammation. Moreover, the detection of fibrinocellular exudates (fibrin admixed with histiocytes, neutrophils and plasma cells) in other alveolar lumina as well as in the pleural sac was attributed by Mac Gavin and Zachary (2007) to increased permeability of the blood-air barrier, increased procoagulant activity and diminished profibrinolytic activity of the lung, furthermore, fibrin is chemotactic to leukocytes

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particularly neutrophils. Bronchitis, bronchiolitis, thickened interalveolar septa as well as thickened alveolar walls (interstitial pneumonia) brings about immune suppression, cilliary dysfunction and unregulated production of TNFα (Mac Gavin et al., 2001 and Shahriar et al., 2002). Similar results were observed by Mary&David (1994); El- Manakhly (1995); Gutierrez et al., (1999) and Rodriguez et al., (2001).

The appearance of multiple old abscesses in the hepatic and pulmonary tissue were also detected in our study. Lomas and Hazell (1983) found pituitary abscess in a goat due to *M. arginini*. Adegboye et al., (1995) mentioned that mycoplasma as one of the causative agents of bovine bronchopneumonia with abscessation. Fibrinous pleuritis was also detected in our study as mentioned by Rania (2006) and Mac Gavin and Zachary (2007) .

The lesions of the bronchial lymph nodes were depletion of lymphoid cells in both lymphoid follicles and cortical zones .These results agreed with Goltz et al., (1986) and Wesonga et al., (2004).

The tracheal lesion was epithelial necrosis with inflammatory cell infiltrations. Jones et al., (1985) mentioned that the tracheal lesions were ciliostasis, loss of cilia and clusters of the organism attached to the epithelial surfaces. *M.ovipneumoniae* produces a polysaccharide capsule with variable thickness that facilitates adherence of the organism to the ciliated epithelium (Niang et al., 1998).

The lesion of the spleen was depletion of lymphoid cells of white pulp while the liver lesion consiststed of degeneration and necrosis of the hepatic cells, vasculitis, abscessiation and fibrosis with inflammatory cell infiltrations. Similar findings were observed by Nakagawa et al., (1976); Goltz et al., (1986); Adegboye et al., (1995) and Singh et al., (2004). Meanwhile, Crawford et al., (1980) found amyloid in the spleen and liver. Moreover, Hernandez et al., (2006) found multifocal and neutrophilic infiltrations in the liver and spleen as well as hepatic cell necrosis. They suggested that the pathogenic role of this microorganism was to invade the already damaged tissue.

On the other hand, Damdinsur (1989) mentioned that the major role played by mycoplasmosis was first as liver damage, spleen and lymph nodes and to be subsequently proliferated to lungs and other organs. Our results revealed presence of necrotic enteritis as previously mentioned by El-Manakhly (1995) and Nayak and Bhowmik (1991).

Conclusion

Our study discussed mycoplasma infection in sheep and goats beginning with

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studying it from the cellular level (isolation, differentiation, biochemical and serological identification) to the molecular level (genetic identification by DNA banding by PCR) and histopathological investigation.

The conclusion of the present study may be summarized as following:

1. Mycoplasma plays an important role in sheep and goats diseases.

2. The incidence of species of genus mycoplasma was predominant than those of genus acholeplasma in both sheep and goats.

3. *Mycoplasma arginini* in sheep was isolated from pneumonic lungs, trachea.

4. The high incidence of *Mycoplasma agalactiae* , *Mycoplasma ovipneumoniae* in sheep and goats reflects its role in sheep and goat diseases.

5. Applying a PCR with a set of oligonucleotide primers specific for gene sequence proved to be a rapid, reliable, sensitive and highly specific technique for detecting and confirming the diagnosis of mycoplasma and can contribute the need of fast identification and detection methods for use in monitoring and control measure programs in flocks.

6. The pathological studies are important as it reflects and explains the histopathological features associated with mycoplasma species in the affected tissues.

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تحديد الآصابة بالميكوبلازما في الأغنام والماعز باستخدام تقنية أنزيم البلمرة المتسلسل والتغيرات الباثولوجية المصاحبة لها .

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الأغنام والماعز من حيوا نات الزراعة المهمة فى مصر . تعتبر الميكوبلازما أكثر الامراض شيوعا ومرتبطة بالخسائر الاقتصادية فى الأغنام والماعز. كان الفحص في هذه الدراسة لثلاثة أنواع للمبكوبلازما (بصورة مختلطة أو وحيدة) تم عزلها من الأغنام والماعزمن مجازر القاهرة و قد تم تحديدها بالاختبارات البيوكميائية وباختبارأعاقة النمو (G I) . تم تقسيم الميكوبلازما المعزولة الى ثلاثة مجموعات بيوكميائية. مجموعة (١) تحتوى على ١٢ معزولة بنسبة (٣٣.٣٣ ٪) من الأغنام و ٦ معزولات بنسبة (٢٣.١ ٪) من الماعز(المبكوبلازما اجالاكتيا).المجموعة (٢) تحتوى على ١٢ معزولة بنسبة (٣٣.٣٣ ٪) من الأغنام و ١٠ معزولات بنسبة (٣٨.٥ ٪) من الماعز(ميكوبلازما اوفينيمونى). مجموعة (٣) تحتوى على ١٢ مغزولة بنسبة (٣٣.٣٣ ٪) من الأغنام و ١٠ معزولات بنسبة (٣٨.٥ ٪) من الماعز (ميكوبلازما أرجنينى). باستخدام تقنية أنزيم البلمرة المتسلسل وجد أنة أسرع بكثير من التقنيات الميكروبيولوجية التقليدية لعزل وتحديد الميكوبلازما ويمكن الحصول على النتائج في غضون خمس ساعات. ستة معزولات حقلية من الميكوبلازما اجالاكتيا تم اختبارها بانزيم البلمرة المتسلسل،و أكدت النتائج عند٣٦٠ bp .و سلالات الميكوبلازما اوفينيمونى المميزة عند ١٠٧٠ bp. ستة معزولات حقلية للكشف عن الميكوبلازما أرجنينى أعطى عند ٢٨٠ bp.

أظهر الفحص الهستوباثولوجى للأنسجة أن شدة العدوى بالميكوبلازوما أوفينيمونى فى الأغنام والماعز أكبر من تلك العدوى من الميكوبلازما أجالاكتيا و / أو الميكوبلازما أرجنينى. ووجدت الاصابات الأساسية في الرنتين على شكل التهاب رنوي فيبرينى مع التهاب للغشاء البلورى ووجود تنكرز لخلايا الكبد،وأضمحلال للخلايا اللمفاوية في الطحال و العقد اللمفاوية الشعبية وكدلك وجد أستحا لة وتنكرز للخلايا الطلائية المبطنة للقصبة الهوائية والأمعاء .

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