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CLINICAL STUDY OF PNEUMONIC MYCOPLASMOSIS AND PASRTEURELLOSIS (CONCURRENT INFECTION) IN A COMMERCIAL SHEEP-FLOCK

(With 5 Tables and 8 Figures)

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دراســة إكلينيكية على مرض الالتهاب الرئوي المسبب بالميكوبلازما والبستيريلا (عدوى مشتركة) في قطيع أغنام تجارى

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تم إجراء الفحص الإكلينيكي لقطيع أغنام (٦١ رأس) بمحافظة أسـيوط يعـاني مـن أعـراض مرضيـة تنفسـية. الأعراض المرضية والآفات التشريحية والتحليـلات الجرثوميـة للأغنـام المريضة (٢٤,٥٩ %) والنافقة (٦,٥٦ %) تم وصفها بإسهاب والتي أشارت إلى إصابة الأغنـام بالتهاب شعبي رئوي نتيجة لعدوى الميكوبلازما -إما بمفردها أو مختلطة مع البكتريا (باسـتيريلا هيموليتيكا والأكتينوميسـيز بيوجيـن) . كـانت نتيجـة محـاولات عـزل فـيروس (Respiratory syncytial virus) من الرئات المصابة ظاهريا. سالبة.تـم مناقشـة التعـاون المشترك بين جرثومتي الميكوبلازما والبستيريلا في إحداث مرض الالتهاب الرئوي في الأغنـام. أظهرت نتائج التحليل البيوكيميائى والتحليل الكهربائي الراسي لعترات الميكوبلازما المعزولة إلى وجود ثلاثة أشكال مختلفة من العترات لنوع (صنف) واحد من الميكوبلازما (احتمال ميكوبلازمـا أجالاكتيا) بالإضافة إلى عترات ميكوبلازما أرجينيني. عدم كفاءة التهوية وتكدس الحيوانات قـد جُرما كعوامل مساعدة لانتشار عدوى الميكوبلازما في القطيع المختبر. أوضح اختبار الحساسـية بالمضادات الحيوية أن مركبا الانروفلوكساسين والتيامفينيكول ذاتا تأثير قوى على أغلب العترات المختبرة شاملة الميكوبلازما. كانت المحاولات العلاجية بدواء الانروفلوكساسين مـع الشـراب المنفث وإنزيم مضاد ألا لتهاب ناجحة في تقليص الأعراض المرضية للقطيع المصاب . هذا وقـد ازداد معدل الشفاء الإكلينيكي للأغنام زيادة معنوية بإعطاء دواء اليفاميزول (كـدواء مسـاعد- منشط مناعي) بجانب برنامج العلاج بالانروفلوكساسين.

SUMMARY

A sheep flock (n = 61 head) showed signs of respiratory affections was clinically examined. The clinical signs, gross lesions and the

microbiological examinations of the diseased (24.59 %) and dead (6.56 %) sheep were described in detail and referred to that the present problem was bronchopneumonia due to mycoplasma infection either alone or coupled with bacteria (*Pasteurella haemolytica* and *Actinomyces pyogens*). Isolation trials of respiratory syncytial virus from the grossly pneumonic lungs appeared to be negative. Synergistic situation between mycoplasma and pasteurella infections in induction of pneumonia in sheep was discussed. Biochemical and sodium dodecyl sulphate polyacrylamide gel electrophoresis analyses of the tested mycoplasmas isolates revealed that there were three different strains of one species of mycoplasma (may be *Mycoplasma agalactia*) plus strains of *Mycoplasma arginini* (serologically identified). Inadequate ventilation and overcrowded were incriminated as predisposing factors for spread of mycoplasma infection among the investigated flock. Antibiogram of the isolated microorganisms showed that enrofloxacin and thiamphenicol had strong inhibitory effects on the major tested strains including mycoplasma. Therapeutic trials with enrofloxacin plus expectorant and anti-inflammatory enzyme were successful in reduction of the clinical abnormalities of the investigated flock. The clinical recovery rate of the treated sheep was significantly ($P < 0.05$) accelerated by administration of levamisole (as ancillary, immuno-stimulant, drug) beside the enrofloxacin therapeutic course.

Key words: *Ovine mycoplasmosis and pasteurellosis, clinical, gross lesion, microbiology, SDS-PAGE and therapy*

INTRODUCTION

Pneumonia appears to be a complex disease that many infectious agents synchronized with stress and/or environmental factors were responsible for such disease causing a considerable level of economic losses of the infected animals. Fatalities and cost of medications were the major economic losses (Alley, 1975; Al-Darraji et al., 1982 and Sharma and Woldehiwet, 1991). Various types of the pathogenic bacteria belonged to class Schizomycetes, particularly family pasteurellae including *Pasteurella (P) haemolytica*, were culturally isolated from the nasal secretions and the lungs of the diseased sheep with pneumonia (Quinn et al., 1994). However, respiratory viruses were frequently incriminated as a primary cause of pneumonia in the domesticated animals including sheep (Elazhary et al., 1984 and Sharma and Woldehiwet, 1991). The most

important respiratory viruses in sheep and other domestic animals were reviewed and tabulated by Quinn et al. (1994). They epitomized that respiratory syncytial virus (RS-virus, pneumoviriniae, RNA) and ovine parainfluenza virus-3 (PI-3 virus, paramyxovirinae, RNA) considered the most commonly important viruses responsible for respiratory problems including pneumonia in sheep population. RS-virus appeared to be more prevalent than PI-3 virus (Elazhary et al., 1984 and Trigo et al., 1984).

Experimental infections of lambs with *P. haemolytica* (alone) and/or with RS-virus (alone) were unsuccessful to induce clinical respiratory abnormalities in the inoculated animals with non-critical minor exceptions, which rapidly abated within one week post infections (Al-Darraji et al., 1982). This experimental work may reveal that pasteurella (alone) and/or virus (alone) infection has no ability to induce clinical respiratory disease in sheep.

Severe clinical respiratory illnesses were experimentally induced in lambs by inoculation of RS-virus and then by *P. haemolytica* (Al-Darraji et al., 1982, Trigo et al., 1984 and Sharma and Woldehiwet, 1991). This may refers to the presence of a synergistic situation between viruses and bacteria in induction of the respiratory disease in sheep. Sharama and Woldehiwet (1991) experimentally investigated this synergism and they concluded that RS-virus not only increased the population density of *P. haemolytica* but also it increased the susceptibility of the mononuclear phagocytes in the peripheral blood and in the lungs of the RS-virus experimentally infected—lambs to *P. haemolytica*-cytotoxcin. Such conclusion may prove that RS-virus induce a favorable condition for the pathogenesis of *P. haemolytica* of the infected animals.

With exception of the different subspecies of *Mycoplasma mycoides*, ovine respiratory mycoplasmas, either alone or coupled with other pathogens were also incriminated as a principle etiologic factor responsible for pneumonia in sheep (Mohan et al., 1992; Tabatabayi et al., 1992; Srivastava et al., 1996 and Niang et al., 1998). However, attempts to induce clinical pneumonia in-groups of kids and lambs with the isolated respiratory mycoplasma (*Mycoplasma ovipneumoniae*) were unsuccessful (Mohan et al., 1992). Such result may be reveal that the ovine respiratory mycoplasmas (alone) incapable to induce clinical pneumonia in sheep. The different subspecies of *Mycoplasma mycoides* considered the most famous mycoplasma causing severe outbreak of pleuro-pneumonia with high mortality and fatality rates of the infected animals (Whitford et al., 1994). Otherwise, *Mycoplasma ovipneumoniae* (Mohan et al., 1992 and Niang et al., 1997) and *Mycoplasma arginini* (Tabatabayi et al., 1992) appeared to be

important respiratory mycoplasmas responsible for pneumonia in sheep. On the other hand, *Mycoplasma agalactia* was principally incriminated as etiological agent of ovine and caprine contagious agalactia in the Mediterranean and the Middle East countries (Whitford et al., 1994). However, severe ovine and caprine pneumonia has been occurred due to *Mycoplasma agalactia* infection under natural and experimental conditions (Pradhan et al. 1993 and Srivastava et al., 1996).

The fundamental goal of the present work was carried out to study the clinical picture of a fattened-sheep flock suffered from different degrees of respiratory problems, and to clear up the probable etiologic agent(s) responsible for such problems. Predisposing factor(s) that aided in spread of infection among the investigated flock was also monitored. Antibiogram of the isolated pathogens was done and therapeutic trials, thereafter, of the diseased animals with a choice drug were achieved.

MATERIAL and METHODS

Animal and history:

A indigenous sheep ("balady") flock consisted of 61 animals of different ages and sex located in Banoub village of Assiut Governorate. These sheep were purchased from different commercial markets as lambs or younger sheep and kept for fattening in a narrow semi-closed stable with apparently inadequate ventilation. The floor of this stable was bedded with straw and dried corncob material, which was usually wetness referring to the non- hygienic precautions. Sometime the flock was grazed in cultivated areas for soil fertilization, and in this journey, sheep might contact with other apparently healthy or probably diseased animals. On the first half of January 2000, three cases showed respiratory embarrassments in the form of persistent exaggerated coughing and haphazardly treatment with oxytetracycline tablets (Terramycin, Pfizer-Egypt,) and penicillin-streptomycin (streptopencid, Cid-Egypt) intra-muscularly injection was apparently successful to cool off the coughing. Three weeks later, February 2000, the number of sheep with respiratory problems gradually increased particularly in lambs and younger with ineffective therapy and some of them were succumbed. Frequent clinical visits to the respective flock were carried out

Collection of the samples and laboratory procedures:

Nasal and pharyngeal swabs:

Nasal and pharyngeal swabs from some diseased and apparently normal sheep were collected and subjected to bacteriological and mycoplasmal examinations. Brain heart infusion agar supplemented with 10 % citrated sheep blood, and serum dextrose agar media were used for bacteriological culturing. Isolation procedures and biochemical identification of the isolated bacteria were carried out (Quinn et al., 1994). On the other side, modified Hayflick broth and agar media were used for mycoplasma examinations. Bacterial irreversibility, genus determination and biochemical identification of the isolated mycoplasmas was carried out according to the protocol described by whitford et al. (1994). Moreover, Serological identification of some isolated mycoplasmas was done using growth inhibition test (whitford et al., 1994). Reference antisera were obtained kindly from Prof. Dr. H. Krichhoff "Institute für Mikrobiologie und Tierseuchen der Tierärtztiche, Hochschule. Hannover—Deushland"

Lungs tissues:

A - Bacterial and mycoplasmal examinations:

Lungs of the recently succumbed (n = 3) and the slaughtered cases (n =3) were taken and aseptically pieced. These pieces were microbiologically tested for the presence of bacteria and mycoplasma as described.

B - Viral isolation:

For RS-virus isolation, the affected lungs' areas of the slaughtered cases (n = 3) were aseptically swabbed and the swabs were immediately soaked in Eppendrof tubes containing 2 ml of Egale's minimal essential medium (EMEM) supplemented with 5 % of mycoplasma free—bovine fetal serum (BFS), 250 g of gentamicin sulphate (Garamycin, Memphis/Sch.Corp) and 20 g of amphotericin (Fungizone, Bristol Myer Squibb). These tubes were leaving for a half-hour and thereafter kept frozen at – 20 ^{O}C. As rapidly as possible these Eppendorf tubes transmitted in ice container to the Dept. of virology, Animal Health Research Institute, Dokki, Giza-Egypt. The time interval between samples' collection and viral analysis was less than twelve hours. Technique for RS-virus isolation was carried out according to the methods reported by Al-Darraji et al., (1982). Briefly, primary cell cultures of ovine fetal kidney were grown as monolayers in 25 cm^2 tissue culture flasks at 37°C. The cells were maintained in EMEM supplemented with 10 % BFS and gentamicin sulphate (100. When the cells were approximately 70 or 80 % confluent, the medium was removed and the cultures were inoculated with 250 clinical samples. Approximately, one hour later (after absorption) the cells were fed

with about 10 ml EMEM supplemented with BFS and bacterial and fungal inhibitors. The flasks were placed in humidified incubator with 5 % Co_2 at 37°C. The cells were examined daily for signs of a cytopathic effect (CPE).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE):

On the basis of biochemical analysis, the isolated strains of mycoplasma of the tested samples (n = 19, Table 3) were classified into two species. One of them was identified serologically as *Mycoplasma arginini* (26.32 %) whereas the second species could not be identified (73.68%, n=14 strains). These fourteen strains were subjected to SDS-PAGE to clear up the electrophoretic protein pattern of each isolated strain. SDS-PAGE antigens of the purified mycoplasma isolates were prepared according to the methods described by Thirkell et al. (1990). SDS-PAGE technique was applied according to the criteria of Laemmli (1970).

Blood-sera:

Thirty-seven blood samples of the apparently normal (n = 25) and clinically ill (n = 12) sheep were taken. The blood sera were thereafter collected and serologically tested for the presence of mycoplasma's antibody using growth inhibition technique (GIT) according to (Whitford et al., 1994). On the basis of SDS-PAGE, the non-serologically identified mycoplasma strains (n = 14) were sub-divided into three groups according to the electrophoretic protein pattern of each strain (Fig. F). These strains were incorporated in a mixed strain culture formed by pooling equal aliquots of the 3 individual strains. This mixed culture was diluted 1: 10 before used (running drop technique on Hayflick agar media) and commercially available filter paper discs (6mm.) saturated by each tested serum were prepared.

Antibiogram:

Antibiogram of the selected isolated strains including mycoplasma species to the different members of antibiotics (BioMerieux, France) that commonly used in the veterinary field were tested by disc diffusion methods. Muleller-Hinton medium (BioMerieux, France) used to bacterial isolates, Hayflick media to the tested mycoplasma strains. Results of the antibiotic sensitivity test were interpreted according to the guidelines of National Committee for Clinical Laboratory Standards (1984).

Therapeutic trials:

Therapeutic trails of the diseased sheep with the choice antibiotic (according to the results of antibiogram) and supportive treatments were summarized and tabulated in Table 5. The therapeutic course persisted to five successive days.

Prevalence of the disease:

From the epizoothiological point of view, the morbidity (%), mortality (%) and the case fatality (%) were estimated according to the methods described by (Thrusfield, 1995).

RESULTS

Clinical examinations:

A: Clinical findings:

The morbidity, mortality and case fatality rates (%) of the examined sheep-flock during the period of investigation were tabulated in Table 1. Severe respiratory signs were predominantly seen in lambs and younger (yearling) sheep than the adults were.

The affected animals were anorexic, lethargic and they appeared to be not responding to the external stimuli. Fever (mean ± SD, 41.3 ± 0.43 °C), congested mucosae, with bilateral serous to mucopurulent nasal discharges (Fig. A and B) and moist-cough were the predominant clinical signs of the diseased animals. Such cough was easily stimulated by slightly touch on the upper trachea. Mildly compression on the upper tracheal ring of each affected case induced a strong coughing-fit with the involvement of intermittent contractions of the abdominal muscles, which persisted to approximately 1 - 1.5 min. post compression. Abdominal breathings were remarkable following tracheal compression of the affected cases. The respiratory cycle of the diseased sheep was rapid and was more superficial. The respiratory rate of the clinically infected sheep ranged from 37 to 69 times per minute. Two cases apparently showed prolonged expiration than inspiration. The femoral pulse was slightly increased in number (mean 83 ± 9/min.) than the apparently normal cases (mean 71 ± 4/min), with increased intensity of the heartbeat.

Tracheal auscultation particularly at the last part of the lower third cleared sounds similar to passing of the air current through semi-thick fluid media (purring) suggesting filling of the tracheal lumen with a fluid. Lung auscultation revealed moist gargling (bubbling) sound particularly on the anterior portions of upper and middle thirds of the lung area, whereas sound similar to the sea-waves was audible on the lower third of the lung area of auscultation referring to the increased bronchial and bronchiolar exudation. Auscultation on the second last intercostal spaces of both sides of lung areas, the lung sounds were clearly diminished and silent lung appeared to be evident.

***B: Gross lesions*:**

The tracheal mucosae of the examined sheep (n = 6) were severely congested and the tracheal lumens were partially (n = 1) or completely (n = 5) filled with frothy fluid (Fig. C), which was easily discharged by slight compression on the cranial lobes of the lung. The affected lungs of the recently succumbed (n = 3) and the slaughtered (n = 3) diseased sheep were diffusely congested with the presence of focal areas of consolidation and mutiple-abscesses (Fig. D). The abscesses were more predominant and they were circumscribed or roughly spherical in shape, 0.5 – 3.0 cm in diameter, grayish-white in color, demarcated by congested irregular borders similar to the saw-web (Fig. D). Some of the abscesses* coalesced with each other forming irregular shape (Fig. D) Some of the abscesses were protruded above the pulmonary surfaces and others were embedded in the lung parenchyma. Concerning the texture of the lung tissue (bilateral palpation), the affected pulmonary lobe was fairly similar to a semi-hard spongy bag, which contained firm various-sized marbles like bodies. The pulmonary consolidated areas were firm in texture with raised surfaces and some of them were confluen and othe s s owed hemorrhagic center. Patchy hemorrhages and emphysematous foci were also seen of some affected pulmonary lobes in the slaughtered cases. Regarding the cardiac gross lesions, three out of the examined sheep showed linear petechial hemorrhages along the coronary fat (Fig. E). The coronary vessels were distended (Fig. E).

***Viral (RS-virus) isolation*:**

The isolation trials of RS-virus by culturing technique revealed that no CPE were detected after 7 days in the first passage. The cultures were harvested and re-inoculated into new cells with negative results after one-week of inoculation.

Microbiological examinations:

Results of the bacterial and mycoplasmal examinations of the tested cases were summarized and tabulated in Table 2. Biochemical analyses of the isolated mycoplasma strains were illustrated in Table 3. The contents of the examined lung abscesses (n = 8) yielded *Actinomyces pyogenes* in a pure culture with exception of two samples were mixed infection (*Actinomyces pyogenes* and *Staphylococcus aureus*).

***Sodium dodecyl sulphate polyacrylamide gel electrophoresis*:**

Fig. G shows the electrophorretic pattern (SDS-PAGE) of the tested 14 mycoplasma strains on 10 % polyacrylamide gel with coomassie stain. It

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*: The abscesses were microscopically smeared and bacteriologically examined.

was found that there were dissimilarities protein bands at the different areas of the analytic mycoplasma isolates. These dissimilarities divided the tested isolates into three different groups (I, II & III, Fig. G). Each group contains highly similar strains with non-critical minute variations particularly in-group I. Group III appears to be identical

Serological test (GIT):

Serological testing (GIT, growth inhibition test) declared that the clinically diseased cases (100 %) gave strong positive reaction (mean ± SD of the inhibition zone, 13.9 ± 1.6 mm.). On the other hand, 16 % (n = 4) of the tested sera of the apparently normal sheep (n = 25) were serologically positive to mycoplasma infection.

Antibiogram and therapeutic trials:

The antibiogram of the isolated microorganisms including mycoplasmas revealed that all tested isolates were highly sensitive to enrofloxacin and thiamphenicol with minor exception (Table 4). On the other side, 71.43 % of the tested mycoplasma were insensitive to oxytetracycline. Therapeutic trials with enrofloxacin plus expectorant and anti-inflammatory enzyme (Group A, Table 5) was successfully reduced the clinical abnormalities of the treated cases and the clinical recovery period ranged from 5 to 9 days (mean ± SD, 7.33 ± 1.03) post the beginning of therapy. In the second group of the treated sheep (Group B, Table 5), the recovery period ranged from 3 to 7 days (mean ± SD, 5 ± 1.41) post the beginning of medications with levamizole beside enrofloxacin therapeutic course. The variation between the two means of the recovery periods was statistically significant ($p < 0.05$).

DISCUSSION

Pyrexia, bilateral muco-purulent nasal discharge with moist exhausting cough, and abnormal audible lung sounds in association with the abnormal necropsy findings including pulmonary abscesses suggesting that the present problem of the investigated sheep-flock was suppurative-bronchopneumonia. Calculations of the morbidity rate (Table 1) referred to the spread of infection among the investigated sheep-flock, and viral infection was primarily suspected. However, the isolation trials of RS-virus were unsuccessful. The negative results of viral analysis could not only attributed to the negativity isolation of the Rs-virus but also may ascribed to the time of transportation of the samples (less than 12 hours), where this virus was highly fragile agent (Al-Darraji et al., 1982 and Quinn et al., 1994). In general the role of viruses infections should not be completely

discounted because of not all-ovine respiratory viruses were tested. However, the highly isolation rate of mycoplasma (91.67 %) of the examined cases, either alone or coupled with bacteria, from the tested samples (Table 2 and Fig. 1) and the serologically positive results of the diseased sheep to the isolated mycoplasma may referred to the major role of this microbe in induction of the clinical bronchopneumonia. The spread of the respiratory mycoplasma infection among the investigated flock may ascribed to the over-crowded of sheep and inadequate ventilation.

Alley (1975) found that 73 % of the tested apparently healthy sheep (n = 184) were harbor *P. haemolytica* and other bacteria on their mucous membranes of the upper respiratory tracts (nasal flora) without clinical abnormalities. This may interpret the presence of *P. haemolytica* and other bacteria in the nostrils and pharynges of the clinically non-diseased sheep (Table 2). From the physiological point of view, Gilka et al. (1974) concluded that the deep pulmonary tissues (lung) should be bacterial-pathogens free. This induced under the effect of the repellant action of the ciliated epithelium of the trachea and any pathogen incidentally inhaled to the lung tissue would trapped and killed by the alveolar macrophage cells (lung clearance mechanism). Consequently, dysfunction of the lung clearance mechanism facilitates the upper respiratory tract—bacterial flora to invade the lung.

The microbiological examinations (Fig 1) revealed that 82 % of the mycoplasma positive cases (n = 11) yielded mycoplasma species coupled with *P. haemolytica* and all tested pneumonic lungs samples yielded mycoplasma species mixed with *P. haemolytica* and *Actinomyces pyogens* (Table 2). Such results may refer to synergistic situation between mycoplasma and bacteria in induction of pneumonia. Whitford et al. (1994), Niang et al. (1997) and Niang et al. (1998) concluded that ovine respiratory mycoplasmas firmly adhered to and colonized the mucous membrane of the respiratory system of the infected animal. Such adherence induced inactivation (ciliostasis) and destruction (ciliolysis) of the ciliated epithelium of trachea. The authors also concluded that those mycoplasmas inhibited the killing capacities of the alveolar macrophages of the infected sheep. Furthermore, under experimental conditions, Niang et al. (1997) found that the percentage of *Staphylococcus aureus* ingested by non-infected sheep alveolar macrophages was significantly higher than that of the infected macrophages with mycoplasma. Their results also indicated that mycoplasma suppressed the cytolytic effect of the sheep alveolar macrophages. Consequently it is probably to conclude that the isolated mycoplasma species may frustrated the defense barriers of the respiratory

system of the diseased sheep and induced a favorable microenvironment, which facilitated the pathway front the upper respiratory bacterial pathogens (*P. haemolytica, Actinomyces pyogens* and *Staphylococcus aureus*) to invade the deepest pulmonary tissues.

Unfortunately the most isolated mycoplasmas from the examined sheep were not identified serologically (Table 3) due to lack of specific antisera of ovine respiratory mycoplasmas in our hand. However, on the basis of biochemical analyses (Table 3), *Mycoplasma agalactia* was suspected. Concerning ovine mycoplasmas, Rosendal (1994) reported that the failure to ferment glucose and hydrolyse arginine is highly suggestive of *Mycoplasma agalactia*. The negative results of GIT of the apparently healthy sheep (84 % were negative), in contrast to the diseased cases (100 % were strong positive), suggesting the pathogenic role of the isolated mycoplasma. However, results of GIT as a monitoring serological test to the presence of mycoplasma' antibodies of the apparently normal animals was not sensitive (Mohan et al., 1992) because of it requires a high level of potent antibody, but it was highly specific (Whitford et al., 1994).

Although the results of biochemical characterizations of the isolated mycoplasmas referred to the presence of 14 strains of one species of mycoplasma (probably *M. agalactia*) other than *M. arginini* strains (Table 3), the electrophoretic pattern (SDS-PAGE) classified these 14 strains into three groups with some major differences between them (Fig.G). This conclusively may refer to the presence of multiple strains of one species of mycoplasma.

Results of antibiogram (Table 4) revealed that enrofloxacin and thiamphenicol had strong inhibitory effect on the tested bacterial and mycoplasmal isolates with some minor exceptions. Most of the tested mycoplasma isolates (71.443 %) were insensitive to oxytetracycline. Egwu and Aliyu (1998) concluded that tetracycline showed weak mycoplasmicidal activity. Such results may be interpreted the ineffective previous treatment of the pneumonic cases.

In regard to the therapeutic trials of the pneumonic sheep (Table 5), it was found that enrofloxacin coupled with expectorant and anti-inflammatory enzyme were apparently a choice treatment of pneumonia in sheep caused by mycoplasma either alone or coupled with other pathogens. This results may support the opinion of Egwu and Aliyu (1998) who reported that enrofloxacin considered to be the best mycoplasmicidal drug with minimum inhibitory concentrations (MIC, 0.01 – 0.06 μg). The obtained results also revealed that administration of levamisole (three doses, day after day) with daily enrofloxacin plus expectorant and anti-

inflammatory enzyme for five successive days was superior and significantly (P<0.05) accelerated the clinically recovery rates of the diseased sheep (Table 5). Sharma and Mahajan (1999) studied the effects of levamisole in goat-kids experimentally infected by mycoplasma (*M. mycoides subsp. mycoides*) and *pasteuerlla haemolytica*. Their results concluded that the experimental infections were significantly suppressed the immune response of those kids, and levamisole treatment was effective in restoration of the immune response referring to the immuno-stimulant role of levamizole. Such experiment and our therapeutic results may refer to the valuable role of levamisole (as ancillary drug) beside the choice antibiotic in treatment of ovine pneumonic mycoplasmosis and pasteurellosis.

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Table 1: Morbidity (%), mortality (%) and case fatality (%) rates of the investigated sheep-flock with respiratory problems.

Number of sheep	Number of cases showed respiratory signs	Morbidity (%)	Number of the dead cases	Number of the culled cases	Mortality (%)	Fatality rate (% affected)
61	15	24.59	4	3*	6.56	26.67

*: These cases were slaughtered due to very bad condition.

Table 2: Microbiological analysis of the examined samples:

Number of animals	Clinical status	Samples	Isolated microorganisms	
2	Apparently normal	NS	Micrococci coupled with Gram's positive and negative bacilli	
3	Apparently normal	NS & PS	Acholeplasma species *P. haemolytica*	Staphylococci Gram's negative bacteria
1	SND[@] Diseased	NS & PS	Mycoplasma species Acholeplasma species	*P. haemolytica*
2	Diseased	NS & PS	Mycoplasma species	
1+	Diseased	NS & PS	Acholeplasma species *Staphylococcus aureus*	*Actinomyces pyogenes* Streptococci
2	Diseased	NS & PS	Mycoplasma species Gram's negative bacteria	*P. haemolytica* *Staphylococcus aureus*
3#	Diseased	TS	Mycoplasma species	Acholeplasma species
		Lungs	Mycoplasma species	*P. haemolytica* *Actinomyces pyogenes*
2*	Diseased	TS	Mycoplasma species	Acholeplasma species
		Lungs	Mycoplasma species Acholeplasma species	*P. haemolytica* *Actinomyces pyogens* *Staphylococcus aureus*
1*	Diseased	TS	Mycoplasma species	Acholeplasma species
		Lung	Mycoplasma species *P. haemolytica*	Acholeplasma species *Actinomyces pyogenes*

NS: Nasal swabs. PS: Pharengeal swabs. TS: Tracheal swabs.
@: This case showed bilateral serous nasal discharge.
+: Serologically positive case to mycoplasma infection, however neither mycoplasma nor pasteuerlla could be isolated.
#: Recently succumbed cases.
*: Slaughtered cases due to very bad condition.

Table 3: Biochemical characterizations of the isolated mycoplasma* and acholeplasma strains from the microbiologically examined (apparently healthy, diseased and dead or slaughtered) cases.

Number of isolates	Biochemical characterizations (tests)					Suspected mycoplasma species
	DS@	GF	AD	Phos.	F&S	
14 (73.68 %)	sss	-	-	+#	+	*Mycoplasma agalactia*
5** (26.32 %)	sss	-	+	-	-	*Mycoplasma arginini*
13	r	Not carried out				

* : Nineteen mycoplasma strains could be isolated from the microbiologically examined diseased and dead or slaughtered cases (n = 12).
DS: Digitonin sensitivity. GF: Glucose fermentation.
AD: Arginine deamination. Pho.: Phosphatase. F&S: Film and spots.
@: All mycoplasma species are highly sensitive (sss) to digitonin in contrast to acholeplamas (resistant, r).
#: Three out of these strains showed weak phosphatase
**: These mycoplasma strains were serologically identified as *Mycoplasma arginini*.

Table 4: Antibiogram of the important isolated micro-organisms .

Antibiotic disc	*Mycoplasma species* Tested strains							*P. haemolytica* Tested isolates			*A. Pyogens* Tested isolates		*S. aureus* Tested isolates	
	1*	2*	3*	4*	5*	6*	7*	1	2	3	1	2	1	2
Cepha. 30 µg	s	r	r	ss	s	r	s	sss	ss	sss	sss	sss	sss	sss
Enro. 10 µg	sss	sss	ss	sss	sss	sss	sss	sss	sss	sss	sss	sss	sss	sss
Eryth, 15 µg	s	ss	ss	r	r	r	ss	ss	sss	sss	ss	sss	ss	sss
Genta. 10 µg	r	ss	ss	s	r	s	sss	sss	ss	sss	sss	sss	sss	ss
Linco. 2 µg	ss	ss	ss	ss	sss	s	ss	sss	sss	ss	sss	sss	sss	sss
Oxy. 30 µg	sss	r	s	r	r	r	ss	ss	sss	sss	ss	sss	s	ss
Thia. 30 µg	ss	sss	sss	sss	sss	ss	sss	sss	sss	sss	sss	sss	sss	sss

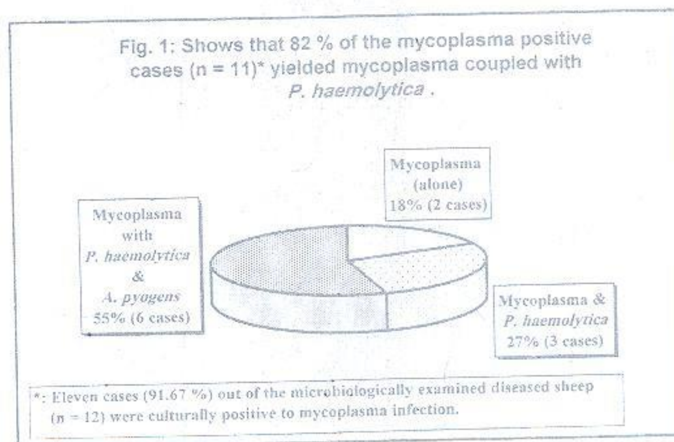
- *: Mycoplasma strains from 1 to 6 are probably *Mycoplasma agalactia*, whereas strain No. 7 is *Mycoplasma arginini*.
- Ceph. Cephalexin. Enro.: Enrofloxacin Eryth.: Erythromycin
  Genta.: Gentamicin Linco.: Lincomycin Oxy.: Oxytetracycline
  Thia.: Thiamphenicol.
- r: Resistant strain.
- s: Intermediate sensitivity, the inhibition zone ranged from 12 to 18 mm.
- ss: Sensitive, the inhibition zone ranged from 19 to less than 23 mm.
- sss: Highly sensitive, the inhibition zone was more than 23mm.

Table 5: Therapeutic trials of the diseased sheep (n = 12):

Group	Number of cases	Therapeutic lines	Clinical recovery period Mean ± SD
A	6	*Enrofloxacin (Enro-Flox)*: 5mg / kg BW/ IM / daily. *Expectorant (bronchistal)*: 15 ml. / case/ orally/ twice daily. *Anti-inflammatory enzyme (Chymotrypsin)*: 5 mg / case / IM/ daily.	7.33 ± 1.03 (range = 5 ~ 9 days)
B	6	*Enrofloxacin*: 5mg / kg BW / IM / daily. *Expectorant (bronchistal)*: 15 ml. / head/ orally / twice daily. *Anti-inflammatory enzyme (chymotrypsin)*: 5 mg / case / IM daily. *Levamisole Hcl (Levamisole)*: 7.5 mg /Kg BW, IM day after day.	5 ± 1.41* (range = 3 ~ 7 days)

Enrofloxacin 10 % w/v, VETWIC, El-Naser for Pharmaceutical chemichals Co., Egypt
Bronchistal: Expectorant and soothing syrup, Kahira Pharm. and Chem. Ind. Co., Egypt
Levamisole: 7.5 % w/v levamisole, Egyptian Co. for chemical and pharmacutical (ADWIA).
Chymotrpsine: Alphachemotrypsine, Leuquin Co. M: Intra-muscular BW: Body wieght
*: significant decrease ($P < 0.05$).

Fig. 1: Shows that 82 % of the mycoplasma positive cases (n = 11)* yielded mycoplasma coupled with *P. haemolytica* .



*: Eleven cases (91.67 %) out of the microbiologically examined diseased sheep (n = 12) were culturally positive to mycoplasma infection.

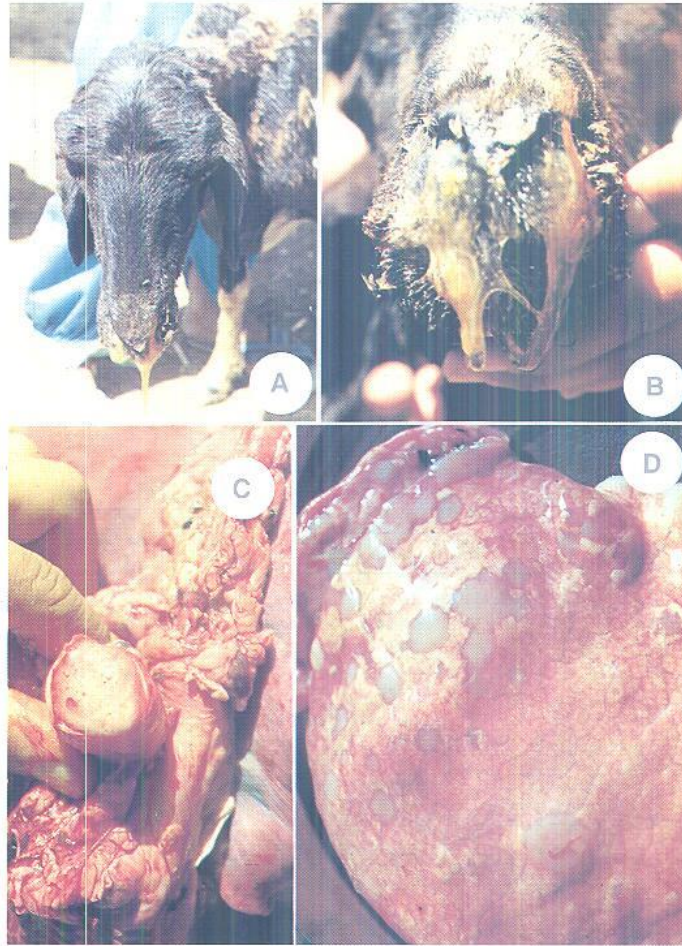


Fig. A: Bilateral copious nasal discharge of the infected sheep. *Fig.B:* Close up-photograph of the nostril of the infected case showing voluminous viscous nasal discharge post tracheal compression. *Fig.C:* Close up-photograph of the trachea of the infected case showing severely congested mucosa and the lumen fills with frothy fluid. *Fig.D:* Close up-photograph of the affected pulmonary lobe showing diffuse congestion, multiple various sizes of single/coalesced abscesses and consolidated areas. Each abscess demarcates by congested irregular border. Some of the consolidated areas are confluent and others show hemorrhagic center.

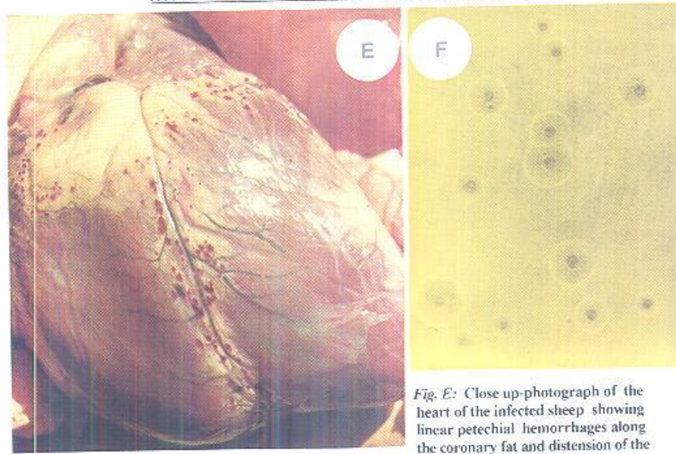


Fig. E: Close up-photograph of the heart of the infected sheep showing linear petechial hemorrhages along the coronary fat and distension of the coronary vessels. *Fig. F:* Various sizes of mycoplasma species on modified Hayflick agar medium, 72 hours incubation (fried-egg colony appearance, X 40).

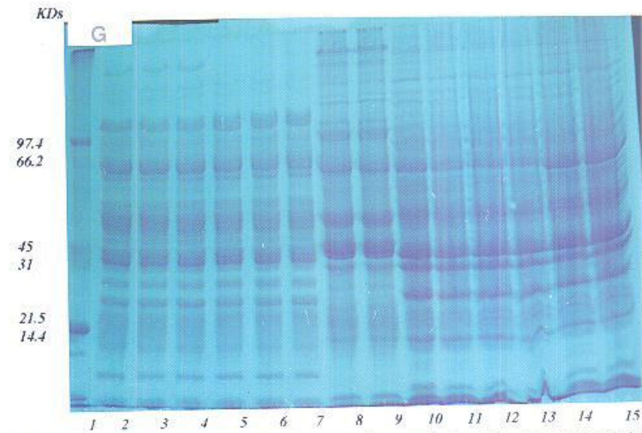


Fig. G: Electrophoretic patterns of the isolated mycoplasma species (probably *Mycoplasma agalactia*).
1: Low molecular weight standard (Bio-Rad) .
2 – 7: Six similar strains with minute differences (Group I).
8 – 9: Two strains appear to be identical (Group II).
10 – 15: Six strains appear to be identical (Group III).