

Animal Health Institute

Assiut Laboratory

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MICROBIAL RESPIRATORY DISORDERS IN BUFFALOE CALVES AND THE BEHAVIOUR OF SOME SERUM BIOCHEMICAL CONSTITUENTS

(With 2 Tables and one Fig)

By

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(Received at 26/9/1992)

الصور الميكروبيولوجية لمشكله الاضطرابات التنفسية الحادة فى عجول الجاموس مع الاهتمام الخاص ببعض التغيرات البيوكيميائية فى مصل الدم

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اجريت هذه الدراسة على ٤٠ عجل جاموسى يعانون من اضطرابات تنفسية حادة وعلى ١٠ عجول سليمة تماما من الناحية الاكلينيكية الظاهرية. تم عزل ميكروب الميكوبلازما من جميع عينات الرئة والسائل البلورى المصابة وكذلك ٧٥ % من عينات المسحة الانفية و٤٠ % من الغدد الليمفاوية الرئوية. وكذلك تم عزل الميكروب القولونى وخمائر الكانديديا من بعض العينات المصابة. ووجد ان ميكروب الميكوبلازما المعزول له درجة حساسية عالية للمضادات الحيوية الاتية: التتراسيكلين والايثرثروميسين والكلورامفينيكول والكنتاميسين وكذلك حامض النالدكسك. وبالفحص البيوكيميائى لمصل الدم للعجول المصابة وجد أن هناك نقصا معنويا جدا فى تركيز عنصرى الحديد والكالسيوم مصحوبة بزيادة معنوية جدا فى تركيز عنصر النحاس ولا يوجد اى تغيير معنوى فى تركيز عنصر الفسفور الغير عضوى.

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SUMMARY

This study was carried out on 40 diseased buffalo calves suffered from severe respiratory disturbances and 10 apparently clinically healthy calves that served as control group. Mycoplasma species were identified from all diseased samples of lung and pleural fluids, 75% of nasal swabs and 40% of pulmonary lymph nodes. *E.coli* and *Candidia albicans* were isolated from some nasal swabs, lung and pleural fluid of diseased samples. Antibiogram for the isolates of Mycoplasma revealed that the best sensitive antibiotics were tetracycline, erythromycin, chloramphenicol, kanamycin and nalidixic acid. Serum biochemical analysis showed highly significant ($p < 0.01$) decrease in iron and calcium levels in diseased sera with a highly significant ($P < 0.01$) increase in serum copper concentration without any significant ($P < 0.05$) alterations in serum inorganic phosphorus level.

INTRODUCTION

Respiratory disorders especially pneumonia represent one of the most important diseases of the farm animals (Hassan, 1987). Bacteria, Viruses and Fungi are the main causes of such diseases. The poor hygienic environmental conditions play an important Role as a predisposing factor of the infection (Bryson *et al.*, 1968).

Nowadays, Mycoplasma species are considered the most important bacteria that cause pneumonia in cattle (Belli *et al.*, 1989; Binder *et al.*, 1990 and Guadagnini *et al.*, 1991) and outbreaks of contagious bovine pleuropneumonia had been prevalent in elsewhere of the world (Trichard *et al.*, 1989). The disease is characterized by severe pneumonia that leads to death. P.M. Lesion showed varying degrees of red and grey hepatization as well as pus like copious pleural exudate (Dawson *et al.*, 1966).

There were many other organisms such as *E.Coli* had been detected to take place in the process of pneumonia and other respiratory disorders in cattle (Bryson *et al.*, 1978). Yeasts (*Candidia* species) and moulds were also encountered in cases of respiratory tract infection (Szige and Nagy, 1990).

Respiratory infections affect either directly or indirectly some serum biochemical parameters. Sayed (1988)

recorded significant elevation in serum copper level accompanied with significant decrease in serum iron concentration in respiratory diseased calves with high body temperature at Assiut, Sohage and Qena Governorates. Similar results were also reported by Beisal, 1974; Ghergeriua and kadar (1979) and Orr et al. (1990). The present investigation was carried out to detect the microbiological causes of a problem of severe respiratory disease in buffaloe calves at El-Hawatka farm at Assiut Governorate and the effect of this condition on some serum biochemical parameters.

MATERIAL AND METHODS

A total of 50 buffaloe calves were used in this study, 40 calves showed severe respiratory disturbances of which 30 individuals died. Calf houses are old adapted bulidings, with a high stocking rate, high level of ammonia with extreme variation in temperature. Clinical signs were recorded and P.M examination was possible only for 25 calves. Nasal swabs, pleural fluids, specimens from the infected parts of lung, pulmonary lymph nodes, kidney and intestine were collected and transported using a sterile technique for microbiological examination. The collected samples were inoculated aseptically into nutrient broth at 37°C for 24 h. and then subcultured into the following: (Difico) nutrient agar, 5% sheep blood agar, MacConky agar, Sabouroud's agar as well as brain heart infusion agar with penicillin in 1,000 units per ml. For Mycoplasma isolation (Sadek et al., 1991).

The isolates were identified according to colonial morphology, pigment production, microscopically by Gram stain and serologically by growth inhibition test for Mycoplasma species (Clyde, 1964) and biochemically according to Bailey and Scott (1974). The isolated candida were identified according to their morphological and microscopical apperance (Monica, 1985). Mycoplasma antisera were obtained from Intervet, Boxneer Holland.

Antibiotic sensitivity tests for mycoplasma isolates were done using antibiotic discs (Biomerieux) of tetracycline (30ug) erythromycine (15ug), chloramphenicol (30ug), Kanamycin (30ug), naldixic acid (30ug), penicillin (10U), amoxicillin (20ug), neomycin (30ug), garamycin (30ug) and spectinomycin (20ug).

50 blood samples (10 from apparently clinically healthy and 40 from respiratory infected calves) were collected for serum biochemical study. the obtained clear sera were analysed biochemically for serum copper (ug/dl), iron (ug/dl) and inorganic phosphorus (mg/dl) levels using test kits supplied

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from Boehringer and after method of Cantrow (1962), Tabacco *et al.* (1981) and Zilversmit (1950) respectively. Serum calcium level (mg/dl) was determined using test kits supplied from Biomerieux (Bains./Frace) and after method of Glinder (1972). Statistical analysis of obtained serum biochemical data was preformed according to method of Kalton (1967) using t-test.

RESULTS

The most prominent clinical signs of infected calves were severe respiratory insufficiency including cough, nasal discharge, hyperpnoea. Varying degree of inappetence, followed by recumbancy and death were evident. Auscultation revealed wheezing and fluid sounds. In more advanced cases there were no respiratory sounds. In P.M. examination the carcasses were in poor condition. Thoracic cavity contained large amount of yellow turbid fluid. There were evidences of gross pneumonia where 40-50% of the lung substance showed macroscopic consilidation, thickness of pleura with fibrinous deposition. Nephritis and inflammation of intestine appeared in 10 cases.

Microbiological isolated, mean values and standard deviation of some serum biochemical parameters were demonstrated in Tables 1 & 2 and Fig., 1.

All isolates of *Mycoplasma* from infected materials were highly sensitive for tetracycline (++++), chloramphenicol (++++), Kanamycin (+++), eruthromycin (+++) and naldicic acid (+++) while these isolates possessed varying degree of resistance towared neomycin (+) and spectinomycin (+) but they are almost resistant to penicillin, ampicillin, trimethoprin and other antibiotics.

DISCUSSION

Respiratory diseases constitute a major cause of morbidity and mortilaty in feedlot cattle. Pneumonia and other respiratory tract infections were incriminated to be the principle causes of all calf death in 41% of the herds (Oxender *et al.*, 1970; Hassan, 1987 and Sayed, 1988).

The results recorded in Table (1) revealed that *Mycoplasma* was the main microbiological cause of the respiratory tract infections in diseased buffaloe calves. This result agrees with that previously reported by Belli *et al.* (1989) and Guadagnini *et al.* (1991). Contagious bovine pleuropneumonia (CBPP) or lung sickness caused by *Mycoplasma* species had been the prevalent respiratory disease which cause severe losses especially in intensively reared calves (Dawson *et al.*, 1966 and Trichard *et*

al., 1989). The authors observed that cough, nasal discharge, dyspnoea and hyperpnoea were the main clinical manifestations, while at the P.M. examination, the main lesions were lung consolidation, thickness of pleura with the presence of excessive amount of turbid fluids. These findings simulated both our observed clinical signs and P.M examination.

E.Coli and *Candida albicans* are also isolated as shown in Table (1). The probability of such finding is in accordance to Bryson *et al.* (1978) and Szige and Nagy (1990). They recorded that *E. Coli* and *Candida albicans* were pathogenic microorganisms frequently encountered in both upper and lower respiratory tract especially in calves reared at bad hygienic conditions.

The study of the antibiogram yields a good idea about the antibiotic of choice for the treatment. In this investigation, mycoplasma isolates is highly sensitive to tetracycline, erythromycin, chloramphenicol, Kanamycin and naldixic acid but it not inhibited by penicillin, ampicillin, trimethoprin and sulfametoxazol antibiotics. This result was previously reported by Kumar *et al.* (1989) and Leak *et al.* (1991).

In this study, serum biochemical analysis revealed a highly significant increase ($p < 0.01$) in serum copper level with highly significant decrease in both serum iron and calcium concentration with a minor alteration in serum inorganic phosphorous level Table (3).

These changes may took place as a result of effect of pathogens, mainly Mycoplasma which resulted in severe pleuritis, bronchitis, plumonary thrombosis and sometimes necrosis of lung tissue. Such behaviour was also discussed by Beisal *et al.* (1974), who interpreted the initial findings of Krebs (1928) in man with elevated serum concentration of copper in acute and chronic bacterial infections.

An explanation in this respect may be offered on the basis that these inflammatory and necrotic changes together with invading microorganisms may stimulate phagocytting cells (including neutrophils, macrophages and monocytes) to produce hormone like substance Known as Leukocytic endogenous mediator (LEM). This hormone like initiates a number of changes in host's trace element metabolism, thus causes a swift of serum iron and many amino acids into the liver. This is followed by an enhancement of liver RNA synthesis and production of a variety of glycoproteins especially ceruloplasmin and hence copper (Beisel *et al.* 1974).

It is interesting to mention that which had been described by Weinberg (1972) in his in vitro study which pointed that the increase of copper level had toxic effect on bacterial growth.

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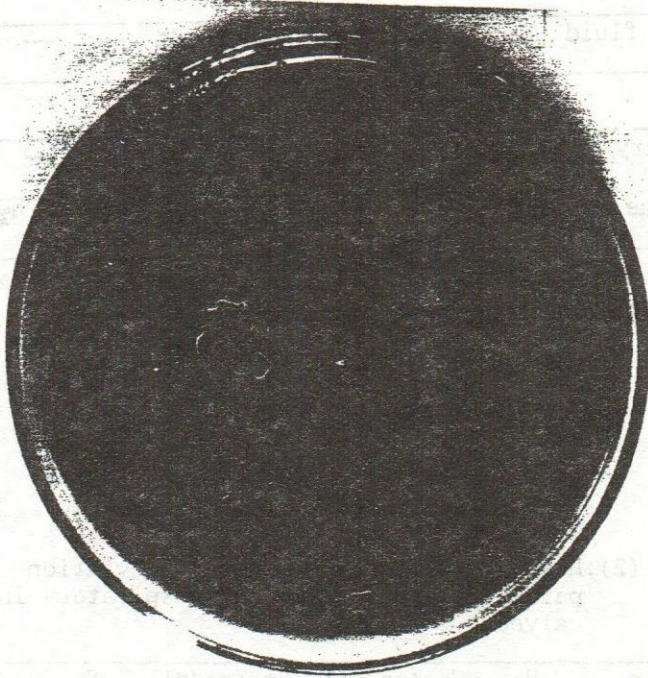


Fig. (1): Colonies of Mycoplasma isolates [note friry egg appearance].

Table (1): Types and incidences of microbiological isolates from respiratory infected buffalo calves.

Samples	No. of sample	Mycoplasma		E.coli		Salmonella		Candida	
		No.	Incid.	No.	Incid.	No.	Incid.	No.	Incid.
Nasal swabs	40	30	75.0%	5	12.5%	—	—	8	20.0%
Pleural fluid	25	25	100.0%	6	24.0%	—	—	5	20.0%
Lung	25	25	100.0%	10	14.5%	—	—	10	40.0%
pulmonary L.N	25	10	40.0%	5	20.0%	—	—	—	—
Intestine	10	—	—	6	60.0%	5	50.0%	—	—
Kidney	10	—	—	2	20.0%	—	—	—	—

Table (2): Mean values, \pm standard deviation of some serum parameters in normal and respiratory infected buffalo calves.

Animals	No.	Ca. (mg/dl)	Ph. (mg/dl)	Fe. (ug/dl)	Cu. (ug/dl)
Clinical healthy	10	11.2 \pm 0.3	6.1 \pm 1.1	190.1 \pm 3.1	170.4 \pm 1.8
Respiratory infected	40	7.6 \pm 0.9**	5.8 \pm 1.3	141.5 \pm 2.1**	201.9 \pm 6.2**

* L.S.D <0.05.

** L.S.D <0.01.