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AEROBIC AND ANAEROBIC BACTERIAL CAUSES OF LUNG INFECTIONS IN BUFFALO CALVES.

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SUMMARY

A total of 88 lung samples from slaughtered bufflao calves were examined bacteriologically (aerobically and anaerobically). Fifty seven cases were proved as bacteriologically positive. *Corynebacterium pyogenes* was the most predominant isolates from 40 cases recorded single infection followed by *Streptococcus pyogenes* and *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Pasteurella multocida* were isolated in low incidence. *Peptococcus anaerobious* was isolated in percentage of 10% in single infection cases. Seventen cases revealed mixed infection with 2 bacteria (*Pasteurella multocida* + *Staphylococcus aureus)* were the most prevalance microorganisms, Bacteroides melaninogenicus + Pasteurella multocida were isolated from 2 cases in a percentage of 3.5 %. Isolated strains were identified biochemically and serologically.

INTRODUCTION

Bovine respiratory diseases cost the Egyptian live stock industry several million pounds per year. Therefore, effective preventive measures should be done. Since many respiratory disorders are closely associated with a particular system of management, it is logic to consider the differential diagnosis of respiratory disease in relation to the age of animal affected which is very important in the present work. The role of bacteria in the acute or chronic respiratory disease process is conveniently undertaken.

The respiratory disorders play an important role in calve diseases and losses of large numbers of

buffaloe-calves lost annually as a results of microbial affections (Corboz, 1982 and Erdag et al., 1993). Different types of pathogenic aerobic microorganisms were revealed from pneumonic lungs either in single or mixed infection (Vaissair et al., 1988).

The Gram negative non spore forming anaerobic rods are a part of the normal flora of human and animals occurring in the mouth and upper respiratory tract, they were found in clinical materials, sometimes are the only organisms present but most often in association with other organisms both aerobic and anaerobic (Gibbons and Engle, 1964).

Local infection of mouth almost contain anaerobic Streptococci as a part of the microflora. Aspiration from such lesions into the lungs may lead to pulmonary abscesses and empyemia and calf mortality (Bornstein et al., 1964).

So, the aim of this work was directed to detect the aerobic and anaerobic organisms that might be the causal agent of lung infection and to obtain a clear picture of different pathogens by the bacteriological examination of lung samples of slaughtered buffaloe calves.

MATERIAL AND METHODS

A total of 88 lung samples from recently slaughtered calves were collected separetly in sterile plastic bags, and examined bacteriologically under aerobic and anaerobic conditions.

Each sample was prepared, and was cultured aerobically on blood agar media, nutrient agar and MacConkey's bile salt agar media and incubated at 37°C for 24 hours. The suspected growing colonies were studied morphologically according to Cruickshank et al. (1975) and described for their appearance, haemolytic activtity and morphological characters, then examined microscopically using Gram's stain, one single colony showed the typical colonial appearance and morphologial characters was picked up and streaked into semi-solid agar media and incubated at 37°C for 24 hours for further identification.

All isolates were identified biochemically according to Baily and Scott (1986) and Konnemann et al. (1992) and some isolates were identified serologically according to Edwards and Ewing (1972).

The same sample was cultured anaerobically and inoculated into two tubes of freshly prepared cooked meat medium which had been boiled and cooled before to eliminate oxygen, one tube was heated in water bath at 80°C for 10 minutes (McClung and Lindberg, 1957) to eliminate the non spore forming aerobes, the other tube was

left without heating and streaked on neomycin sulphate 75 mg/ml agar for isolation of *Colistridium perfringes* and 10% sheep blood agar for isolation of non spore forming anaerobes. The heated tube streaked onto 10% sheep blood agar for growing of the other species of Clostridia.

All plates were incubated anaerobically at 37°C for 72 hours. The suspected colonies were described for their appearance, haemolytic activity and morphological characters. Gram's stained films were examined microscopically and identified according to Thomas and Hare (1954) and Sutter et al. (1980) and biochemically according to Williams et al. (1975).

Isolated strain of *B. melaninogenicus* hydrolysed glucose, maltose, lactose, sucrose, cellobiose, starch and aesculin, they produced gelatinase but were non proteolytic, and did not form hydrogen sulphide or indole (Williams et al., 1975 and Schwabacher et al. 1947 and Quinn et al., 1994).

RESULTS

Out of 88 studied samples from examined lung buffalo calves, 57 specimen were recovered various kinds of microorganisms with a percentage of (64.7%). While the remaining 31 specimen (35.3%) were bacteriologically negative as shown in Table (1).

Table 1: Results of bacteriological finding of examined slaughtered buffaloe-calves.

Type of samples	Examined No. of Cases	Bacteriological finding			
		Positive		Negativ	
		No	%	No	%
Salughtered calves	88	57	64.7	31	35.3

Out of bacteriologically positive cases revealed the isolation of 40 pathogenic strains (single infection) with an incidence of 70.1%.

The distribution of the isolates and its incidence were represented in Table (2). The most prevalent organism was *Corynebacterium pyogenes* in a percentage of isolation 15.7% followed by *Streptococcus pyogenes, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Pasteurella multocida,*

Table 2: Incidence of microorganisms isolated from lung specimens of slaughtered buffaloe calves as sole infection.

Isolated strains	No. of positive cases	%
As sole infection	**40/57**	**70.2**
Corynebacterium pyogenes	9	15.7
streptococcus pyogenes	7	12.4
Escherichia coli	6	10.5
Pseudomonas aeruginosa	6	10.5
Klebsiella pneumoniae	3	5.3
Pasteurella multocida	2	5.6
Pasteurella haemolytica	1	1.7
Peptostreptococcus anaero-bious	6	10.5

Table 3: Prevalence of different microorganisms obtained from lung specimen of slaughtered buffaloe calves as mixed infaction.

Isolated strains	No. of positive cases	%
As Mixed infection	17/57	29.8
Pasteurella multocida + Staphylococcus aureus	6	10.5
Klebsiella rhinoscleromatis + Corynebacterium pyogenes	3	5.3
Klebsiella pneumoniae + Staphylococcus aureus	3	5.3
Streptococcus pyogenes + Proteus mirablis	2	3.5
Staphylococcus aureus + Corynebacterium pyogenes	1	1.7
Bacteroides melaninogenicus+ Pasteurella multocida	2	3.5

Pasteurella multocida, Pasteurella haemolytica with a percentage of isolation 12. 4%, 10.5%, 10.5%, 5.3%, 5.6%, 1.7% respectively. Six isolates of anaerobic organisms (*Peptostreptococcus anaerobius)* were revealed in a percentage of 10.5%.

Table (3) showed that, 17 examined specimen revealed the isolation of mixed infection with the recovery of 34 strains of different microorganisms concurrently mixed.

A total of 6 cases revealed mixed infection of *Pasteurella multocida* with *Staphylococcus aureus* 10.5%, 3 cases were due to concurrently infection of *Klebsiella rhinoscleromatis* with *Corynebacterium pyogenes*, 5.3%, 3 cases due to *Klebsiella pneumoniae* with *Staphylococcus aureus*, 5.3%, 2 cases revealed *Staphylococcus pyogenes* with *Proteus mirablis*, 3.5 %, 1 case revaeled *Staphyloccus aureus* with *Corynebacterium pyogenes*, 1.7%, and finaly 2 cases revealed the isolation of *Bacteroides melaninogenicus* with *Pasteurella multocida* 3.5%.

A total of 8 strains of *Pasteurella multocida* were isolated and identified biochemically and seologically as: 5 isolates were belonged to type II Robert, and 3 isolates were type IV Roberts.

Six isolates of *Escherichia coli* were idenified biochemically and serologically, as 4 isolates were belonged to O55: B5, and 2 isolates as O126: B16.

Identification of anaerobic isolates:

The anaerobic examination, revealed 6 isolated strains as Gram positive cocci and arranged in long chain which identified as *Peptostreptococcus anaerobious*. The other two isolated strains were appeared as a short Gram negative rods and were identified as *B. melaninogenicus*.

DISCUSSION

Buffaloes hold an important position in meat industries, it was surprising that this useful animal had received little attention from workers in the field of veterinary researches to increase its productivity.

Bacteriological examination was made to identify aerobic and anaerobic microorganisms that might be the causal agent of pneumonia in bufflaoe calves. All isolates were extensively studied for their morphological and biochemical characteristics. Serological identification was carried out on certain microorganisms encountered with this work,. With all these points of view, a total of 88 lung samples of slaughtered buffaloe-calves were investigated.

Out of 88 lung specimens examined bacteriologically, 57 (64.7%) harboured bacteria and the remaining 31 (35.3%) were pathogenic free as shown in Table (1), nearly similar results have been described by El Battrawy et al. (1992) who investigated twenty six cases of bovine pneumonia in calve due to bacterial infection with an incidence of 91%, the same results were reported by Yamamoto et al. (1976) and Fischer (1978).

A total of 40 strains of different microorganisms were secured as single sole infection from diseased lungs of slaughtered buffaloe-calves as shown in Table (2), the most predominant pathogenic species were *Corynebacterium pyogenes* 15.7%, *Streptococcus pyogenes* 12.4%, Pseudomonas aeruginosa 10.5% Escherichia coli 10.5 % and *Klebsiella pneumoniae* 5.3%. This nearly coincides with the results stated by Yamamoto et al. (1976) and Riad (1989) who described an outbreak of calf pneumonia in a breeding establishment the onset of infection appeared between the 10th day and 12th week of life (Jenning and Clover, 1952) isolated *Corynebacterium pyogenes, Staphylococci, Escherichia coli, Pseudomonas aeruginosa,* and *Proteus* species from pneumonic lungs of calves in Liverpool and El Battrawy et al. (1992) reported the same findings.

In this study, *Pasteurella multocida* isolates were recovered in a percentage of 5.6% and *Pasteurella haemolytica* were isolated with an incidence 1.7%, these results agree with Fischer (1978); Schulz et al. (1990) and Sheikh et al. (1994) who reported nearly the same findings.

From Table (2), it was of interest to note that mixed microorganism recovered from 17 slaughtered buffaloe-calves. The most prevalent mixed infection were *Pasteurella multocida* with *Staphylococcus aureus* 10.5%, *Corynebacterium pyogenes* and *Klebsiella rhinoscleromatis* 5.3%, *Klebsiella pneumoniae* and *Staphylococcus aureus* 5.3%, *Streptococcus pyogenes* with *Pasteurella multocida* 3.5%, the comparison of

the present data with those reported from other laboratories (Yamamoto et al., 1976); Fischer, 1978; Corboz, 1982; Brylin, 1986; Riad, 1989; Saudhu et al., 1987 and Erdag et al., 1993) shows little considerable differences in the incidence.

The role and the incidence of anaerobic organisms in the respiratory affection in buffaloe calves in this study were studied, the anaerobic organisms represented by *Bacteroides melaninogenicus* and *Peptostreptococcus anaerobious* which isolated in an incidence 3.5% and 10.5% respectively. *Bacteroides melaninogenicus* was revealed in combination with *Pasteurella multocida*. *Peptostreptococcus anaerobious* was isolated as a single infection from slaughtered pneumonic calves. These findings go hand to hand with that proved by Siering (1986); Blunden and Mackintosh (1991) and Sweeney et al. (1991).

Number and incidence of mixed infection of bacterial isolates from pneumonic lungs proved that most of the concurrently mixed infection were obtained from calves above 3 month old, these results were nearly in agreement with that of Corboz (1982) and Garoiu et al. (1982).

The second observation was the presence of *Bacteroides melaninogenicus* in mixed infection with *Escherichia coli*, or *Pasteurella multocida* and its very difficult to grow and mentain in pure culture, this is probably a reflection of the requirement of *Bacteroides melaninogenicus* for vitamin K, since a variety of other organisms (both aerobic and anaerobic) synthesize napthoquinons (Gibbons and Engle, 1964).

The last observation related to *Peptostreptococcus* species which frequently found in abscesses in gastrointestinal regions in pur culture, this local infection of mouth by aspiration food to calf pulmonary abscesses and empyemia (Bornstein et al., 1964 and Quinn et al., 1994).

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