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ABSTRACT

Respiratory diseases are considered a major problem for calves especially with the development of intensive production .

Atotal of 200 buffalo calves of 1-12 month age from different localities in Alexandria governorate farms were clinically examined for respiratory affections where calves showed signs of respiratory manifestation and examined for the presence of the microbial causative agents of pneumonia.

Bacteriological examination revealed the isolation of the following pathogens : Pasteurella multocida , Pasteurella haemolytica , Haemophillus somnus , E-coli , Actinomyces pyogens and Staph. aureus from respiratory diseased calves. Moreover, All Pasteurella isolates recovered were pathogenic to mice and re-isolation of the organisms were occurred after the experimental infection of mice .Pathogenic Gram negative isolates were highly sensitive to Oxytetracyclin and Trimethoprim while Gram positive isolates were sensitive to Erythromycin and Penicilline. The prevalence of infection in different ages and different seasons were also investigated .

INTERODUCTION

Bovine respiratory affections are considered to be one of the most common and serious problems affecting both beef and dairy calves .

Respiratory affections were the main causes of mortality in feedlot calves . The morbidity can range from 0-91 % and mortality from 0-7 % , Van Donkersgoed et al.(1994) .

Reviewing the available literature , it was found that many organisms have been implicated as causes of pneumonia in calves from that Pasteurella multocida constitute one of the most common and prevalent pathogens affecting the respiratory system of calves in addition Pasteurella haemolytica , Haemophilus somnus , ACtino-myces pyogenes , Mycoplasma bovis , E-coli , Pseudomonas aeruginosa, Staphylococcus aureus and viruses , Fayed (1973), fisher (1978) , El-shahedy (1985), Abou El-Soud (1990) El-Sayed et al.(1992) , El-SAyed and El-Shaboury (1999) .

Pasteurella multocida is a common commensial organism found in the upper respiratory tract of cattle and buffaloes and playing an important role in induction of Haemorrhagic septicaemia and bronchopneumonia , Donkersgoed

et al.(1993). Moreover , Haziroglu et al.(1997) isolated Pasteurella multocida , Pasteurella haemolytica , and Haemophilus somnus and they decided that there was a close relation among these organisms and acute inflammation of the respiratory tracts in calves .

The present study was carried out to investigate and identify the prevalence of bacteria species causes pneumonia in calves in some farms in Alexandria and study the pathogenicity of some isolated bacterial isolates to white mice and in vetro study the sensitivity of the isolated bacterial isolates to certain antimicrobial agents .So as to use the obtained results to formulate the most efficient control measures .

MATERIAL AND METHODS

Animals :

A total numbers of 200 buffalo calves aged from 1-12 months and from different localities farms in Alexandria governorate and at different seasons were clinically examined to investigate diseased animals suffering from signs of respiratory manifestation such as (fever, nasal discharge , lacrimation and coughing).About 30 calves were dead or emergency slaughtered were subjected for postmortem examination .

Samples :

Nasopharyngeal swabs were collected by means of sterile cotton swabs from diseased calves , In addition , tracheal swabs and lung tissues were collected from parts showing congestion from cases of emergency slaughtered or recently dead calves for bacteriological examination .

Bacteriological examination :

The collected samples were inoculated into nutrient agar , blood agar and MacConkey's agar plates and incubated aerobically at 37°C for 24 – 48 hours , Chocolate agar plates also were used and in seeded plates were incubated at 37°C for 24-48 hours under 10% Co2 atmosphere . smears from colonies were stained with Gram's stain and examined microscopically , colony showing typical similar appearance and morphological characteristics was picked up and subcultured for purification and further identification . The pure colonies were identified by various diagnostic keys according to Koneman et al.(1983), Krieg and Holt (1984), and Mackie and MacCarteny(1996) .

Pathogenicity and virulence test :

Bacterial suspension was prepared from the original culture by plate washing technique , Stamp et al.(1954) and Bain et al. (1982) . 0.1ml $4x10^{6}$ of bacterial suspension from Pasteurella multocida , Pasteurella haemolytica and Haemophilus somnus were intraperitoneal (I/p) injected into mice . The mortality rate and post mortum changes were recorded , in addition smears from blood and spleen were prepared and stained with Leishman's stain for

demonstration of bipolarity , as well as re-isolation of the organisms on agar medium in pure form . Moreover, the survived injected animals were put under observation for at least one week .

Antibiotic sensitivity :

The antibiograms of the recovered pathogens were done using the disc diffusion method of Bauer et al.(1966) .The interpretation of zones of inhibition were estimated according to limits of given by Finegold and Martin (1982), and Bio-merieux (1984).

RESULTS

200 buffalo calves 1-12 months old belonging to different farms subjected to respiratory distress and had clinical respiratory manifestation out of the diseased calves 30 died or emergency slaughtered .

1- clinical examination :

The respiratory signs consisted mainly of dullness , anorexia , dyspnoea , coughing and sneezing with serous or mucopurulent nasal discharge .

2- Postmortem examination :

The postmortem findings of dead calves showed either mucoid , purulent or mucopurulent discharge in the upper respiratory tract lungs were congested and showed multiple abscess of variable sizes . Petchi and oedema with bloody tinged fluids were seen in the thorathic cavity . Variable degrees of congestions were seen along the gastrointestinal tract. The spleen was enlarged and congested .

3- Bacteriological examination :

Bacteriological examination of 200 calves of different ages and different sources and suffering from respiratory manifestation revealed the isolation of{ 185} bacterial isolates with a total isolation rate of (92.5 %) **Table (1)** .

It was found that the pathogenic and potentially pathogenic genera as Streptococcius, Staphylococcus , Corynebacterium , Pasteurella and Haemophilus somnus were higher in diseased calves than another microorganisms . Also , it was found that the Gram negative were predominating isolates{ 123} with an incidence of (66.48 %) in comparison to { 62} Gram positive cocci and rods (33.51%) as recorded in **Table (2)**.

The results present in **Table (3)** showed a higher prevalence of bacteria in the upper respiratory tract of respiratory diseased calves during winter season where {70} microorganisms could be isolated from {50} diseased calves in a rate of (37.83%) .

Results in **Table (4)** showed a high prevalence of pasteurella pathogens which consider the main cause for respiratory pneumonia , in calves aged between 2-4 months .

4-Pathogenicity test :

The pathogenicity of the isolated Pasteurella multocida and Manhamia haemolytica and Haemophilus somnus to white mice revealed that all isolates were highly pathogenic to mice after intraperitoneal (I/P) injection producing acute septicaemia and death within 2-5 days post inoculation . The injected bacteria were re-isolated again from all dead mice .

5- Drug sensitivity :

The drug sensitivity test revealed that the isolated bacteria were sensitive to Oxytetracyclin , Erythromycin ,Trimethoprim and Penicilline. Table (5).

DISCUSSION

Respiratory disease is often cause most significant losses in feedlot calves ,this due to the death of animals , costs of treatment and weight losses . From the clinical and postmortem examination of diseased and dead animals our findings appeared similar to those previously described by (Yousef et al. 1992 , Sivula et al. 1996,El-Sayed and El-shaboury 1999).

Bacteriological examination of the cultured swabs from the diseased calves revealed the isolation of bacterial pathogens were Pasteurella multocida , Pasteurella haemolytica , Haemophilus somnus , Actinomyces pyogenes , Staph. aureus and E-coli . These results nearly come in agreement with Aboul Saoud (1990), Abdel Ghany et al. (1991) ,Walker (1996) , El-Sayed and El-Shaboury (1999) , and Fayed et al.(2000) . The results present in **Table (3)** showed a high prevalence of the isolated bacteria causing pneumonia in the upper respiratory tract of respiratory diseased calves during winter season .

These results come in agreement with those obtained by Dutta et al.(1990) and Fayed et al. (2000) as they reported that high prevalence of causative bacteria occurred mainly during rain fall and cold season which adversely affect the mucocilliary apparatus function and pulmonary alveolar macrophages which are the innate immune system in the upper and lower respiratory tract .

Results in **Table(4)** showed a high prevalence of microorganisms isolated from diseased respiratory calves specially Pasteurella species in calves aged between 2-4 months.

These observations may be attributed to the lack of immunity in such animals, also may attributed to the degree of susceptibility of these calves as a result of concurrent. Infections by viruses and exposure to stress conditions especially cold , overcrowding and malnutrition , Carter and Alwis (1989) and Ajamal et al.(1992) .

The pathogenicity of isolates of Pasterurella species and

Haemophilus somnus to white mice revealed that all isolates were highly pathogenic to mice after intraperitoneal Inoculation , these finding come in agreement with the result obtained by Kennedy et al.(1960) , Ismaiel et al. (1993) , and El- Sayed and El- shaboury (1999) .

Regarding the drug sensitivity test , it was revealed that most pathogenic Gram negative isolates were sensitive to Oxytetracyclin and Trimethoprim while most Gram positive bacteria were sensitive to Penicilline , Erythromycin and Oxytetracycline .

These results were supported by Moareck et al. (1993), from these results its seems to be that long –acting Oxytetracyclin was very effective in treatment of pneumonic buffalo calves and this nearly similar to those obtained by Yousef et al . (1992) .

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Table (1) different species of bacteria isolated from buffalo calves .

Table (5): Drugs sensitivity test of the isolated bacteria recovered from infected

Bacterial species	Living 170 diseased calves		Emergency slaughtered or dead 30 calves		Totale 200	
	No .	%	No .	%	No .	%
P. multocida	29	17.05	11	36.66	40	20.00
M. . haemolytica	12	7.05	6	20.00	18	9.00
H.somnus	10	5.88	8	26.66	18	9.00
Staph..aurus	14	8.23	3	10.00	17	8.50
Staph.epidemidis	3	1.76	_		3	1.50
Coryn .bovis	6	3 .52	5	6.66	11	5.50
Arcinobacterium pyogenes	5	2.94	3	10.00	8	4.00
Strept . bovis	8	4.70	1	3.33	9	4.50
Strept . pyogenes	10	5.88	4	13.33	14	7.00
kleb . pneumonea	11	6.47	4	13.33	15	7.50
Proteus vulgaris.	2	1.17	2	6.66	4	2.00
Pseudomonas aeruginosa	4	2.35	3	10.00	7	3.50
Salmonella typhimurium	2	1.17	3	10.00	5	2.50
E . coli	8	4.70	8	26.66	16	8.00
Total	124	72.94	16	203.33	85	92 .5

Table (2) : bacterial groups isolated from respiratory affections of buffals calves.

Bacterial group	Diseased No	Emergency Slaughtered or dead No.	Total No.	Total %
Gram + ve				
Staphylo cocci	17	3	20	10.80
Corynebacterium	11	8	19	10.27
Streptococci	18	5	23	12.43
			62	33.51
Gram -ve				
Pasteurella	41	17	58	31.35
Haemophilus	10	8	18	9.72
E-coli	8	8	16	8.64
klebsiella	11	4	15	8.10
Pseudomonas	4	3	7	3.78
Proteus	2	2	4	2.16
Salmonella	2	3	5	2.70
			123	66.48

Table (3) : prevalence of microorganisms in respiratory diseased calves during different seasons .

Season	No . of samples collected from respiratory diseased calves	No . of + ve microorganisms
Winter	50	70 (37.83%)
Spring	50	50 (27. 02 %)
Autumn	50	35 (18.91 %)
Summer	50	30 (16.21 %)
Total	200	185

Table (4) : prevalence of microorganisms in respiratory diseased in relation to age specially Pasteurella species .

Age	No . of calves with respiratory symptoms	No . of the cases
0-2month	18	04 (22.22 %)
> 2- 4month	52	32 (61.53)
> 4-6 month	65	12 (18.46)
> 6-8 month	45	08 (17 .77)
> 8 – 10 month	20	02 (10 %)
	200	58 (29 %)