

## SOME STUDIES ON BACTERIAL RESPIRATORY DISEASES IN BUFFALOES

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### SUMMARY

The investigations are concerned with bacterial respiratory diseases in buffaloes and calves. Form the total examined samples (533) the prevalence of bacteria causing respiratory disease in buffaloes and calves was 50.09%. The incidences of bacterial isolates causing respiratory disease in apparently healthy buffaloes and calves were 25.45% and 22.94% , respectively and they were lower than in diseased buffaloes (83.33%) and calves (79.47%). Meanwhile, the incidence in dead calves was 66.67% . It is clear that the incidence of bacterial agents causing respiratory disease was somewhat higher in nasopharyngeal swabs than other collected samples, so the nasopharyngeal swabs may be used as a sufficient guide to indicate bacterial respiratory disease especially in life animals. There were a wide range of bacteria isolated from nasopharyngeal swabs, laryngeal swabs and lung tissue of apparently healthy and diseased buffaloes and calves and in dead calves including *A. pyogenes*, *C. bovis*, *Micrococcus* spp., *S. aureus*, *S. epidermidis*, *S. pneumoniae*, *E. coli*, *K. pneumoniae*, *M. haemolytica*, *P. multocida*, *P. vulgaris*, *P. aeruginosa*, and *Y. enterocolitica* in different incidences, and it was noticed that *S. aureus* was recovered in the highest incidence (10.32%). One point of interest in this work was the isolation of *Y. enterocolitica* from 3.19% from respiratory affections of buffaloes and calves although it is mainly an enteropathogen.

The incidence of positive samples yielding single bacterial infection (78.28%) was higher than the incidence of samples which yielded mixed isolates (21.72%). Moreover, there were variations among the incidence of single and mixed infection in different samples. The combination between many bacteria with each other to establish the respiratory disease in buffaloes and calves occurred mainly between *S. aureus* and *P. aeruginosa* ( 5 samples) followed by *S. aureus* and *S. epidermidis*, *S. aureus* and *Y. enterocolitica*, *S. pneumoniae* and *S. epidermidis*, *S. pneumoniae* and *Y. enterocolitica*, and *M. haemolytica* and *Y. enterocolitica* (each in 4 samples). Meanwhile, the lowest combination occurred between *S. aureus* and *K. pneumoniae* and *S. pneumoniae* and *P. aeruginosa* (each in 1 sample). In addition, other combination between many bacteria occurred. *P. vulgaris* and *Y. enterocolitica* could not induce a disease separately. Serological identification of *E. coli* revealed that the most predominant serogroups were O1, O119 and O126, followed by O44 then O26, O125, and O11. On the other hand, identification of *P. aeruginosa* revealed that serogroup K was the predominant isolates then H. In addition serogroups I, L and M were identified. There were differences in isolates susceptibilities and zones of inhibition to different chemotherapeutic agents. Amikacin, cefadroxil, chloramphenicol, doxycycline, enrofloxacin, norfloxacin, and trimethoprim + sulphamethoxazole, had great susceptibilities on most isolates. While, streptomycin was completely resistant. Pathogenicity in mice revealed that *A. pyogenes*, *C. bovis*, *S. pneumoniae*, *E. coli* O1, *K. pneumoniae*, *M. haemolytica*, *P. multocida*, *P. aeruginosa* serotypes H and K were highly virulent strains followed by *E. coli* O119 and O126, *Micrococcus spp.*, *S. aureus* and *Y. enterocolitica*. While, *S. epidermidis* was lower in its virulence. Treatment of some diseased animals using the sensitive chemotherapeutic agents in form of patent preparations as enrofloxacin ( Baytril ), gentamicin ( Garamycin ), doxycycline (Oxytetrac ) 5% and amoxicillin ( Vit-biotic ) were done.

## INTRODUCTION

Buffalo is one of the most important animals in Egypt since it is one of the

major sources of meat and milk production; therefore great attention was directed to buffalo industry to meet the increase in people demands. Respiratory disease is one of the major problems facing buffalo's calf industry in Egypt and the main factor causing economic losses as it causes high morbidity and mortality in calves (Healy et al., 1993). Respiratory affections are a complex syndrome involving stress factors, environmental factors and infectious agents (including viruses, bacteria and fungi). They generally develop when a calf's immune system is compromised or if the calf has a concurrent viral, mycoplasmal or bacterial infection (Roy, 1990). In addition, diverse environmental conditions can predispose to the disease (Hafez et al., 1991). Stress resulting from transportation and latent viral infection allows pathogenic bacteria primarily *Pasteurella* species to invade the lower respiratory tract resulting in pneumonic pasteurellosis (Wilson et al., 1985). Also, management practices may cause respiratory tract problems associated with high mortality rates (Losinger & Heinrichs, 1997). In buffalo's calves, respiratory manifestations (coughing, nasal discharge and dyspnea) most frequently occur (Pfutzner et al., 1980) and (Komoda et al., 1988). *P. multocida* was the main causative bacterial agent associated with respiratory affections (Mohamed, 2002). It is important when dealing with any respiratory disease to attempt to identify the pathogens involved in order that current therapeutic and future prophylactic regimes can be proposed.

**The aim of this work is directed to the following items:**

\* Studying bacterial affections causing respiratory syndromes in apparent-

ly healthy and diseased buffaloes and calves and dead calves.

- \* Serological identification of some bacterial isolates.
- \* Determination the antibiogram of most bacterial isolates.
- \* Pathogenicity of some bacterial isolates in mice.
- \* Treatment of some diseased animals using the sensitive chemotherapeutic agents.

## **MATERIALS AND METHODS**

### **\* Sampling :**

533 samples were collected from abattoirs and buffalo farms including 237 nasopharyngeal swabs from adult buffaloes (50 apparently healthy and 20 diseased). While from buffalo's calves (90 apparently healthy, 70 diseased and 7 dead), and 148 samples were collected from each laryngeal swabs and lung tissue (30 apparently healthy and 11 diseased from adult buffaloes, while 40 apparently healthy, 60 diseased and 7 dead from buffalo's calves). The swabs were collected in sterile test tubes while lung samples were collected in sterile polyethylene bags, and they were sent to laboratory in an ice box with a minimum of delay.

- \* **Isolation & identification of bacteria associated with respiratory diseases:** according to **Koneman et al. (1996)** and **Quinn et al. (2002)**. Samples were cultured onto nutrient broth for 24h at 37°C and then a loopfull was taken and cultured onto the following solid media according to its specificity: Nutrient agar, 5% sheep blood agar, MacConkey agar, DAS Media, Baird Parker medium, S.S agar and Yersinia

selective agar with selective supplement. All inoculated plates were incubated at 37°C for 24h then the colonies were identified.

- \* **Serotyping:** of the isolated strains of *E. coli* and *P. aeruginosa* was done according to **Edward and Ewing (1972)** and **Homma (1982)**, respectively. They were obtained from Denka Seiken Company, Tokyo, Japan.
- \* **Antibiogram of the recovered bacterial isolates:** This was done by using disc diffusion standard technique according to **Finegold and Martin (1982)** and **Quinn et al., (2002)** using the following disc (Oxoid), including amikacin (30), amoxicillin (25), cefadroxil (30), chloramphenicol (30), ciprofloxacin (5), doxycycline(30), enrofloxacin (5), erythromycin (15), norfloxacin (10), streptomycin (10), and trimethoprim + sulphamethoxazole (1.25 + 23.75). The results were interpreted according to the manual supplied by Manufacture Company.
- \* **Pathogenicity of the recovered bacterial isolates in mice:** It was carried out according to **Hala ( 2003 )**. 85 white mice, about 28-30 days old were used to investigate the pathogenicity of 16 isolates. All mice were examined bacteriologically to ensure their freedom from pathogens and were divided into 17 groups, each contained 5 mice which received 0.1 ml / mouse of 24 hours pure culture of an isolate suspended in sterile saline containing  $1.5 \times 10^8$  C.F.U /ml. of tested isolate I/P and Kept separately. Last group was kept as a control and injected only with sterile saline. All mice were kept under observation for 7 days. The number of dead mice was recorded and dead mice were subjected to bacteriological investigation for reisolation of the inoculated strains.

\* **Treatment of some diseased animals:** Table (8) using the most sensitive chemotherapeutic agents in form of commercial products as Enrofloxacin, Garamycin, Oxytetrac 5% and Vit-biotic (amoxycillin).

## **RESULTS AND DISCUSSION**

Respiratory diseases are still serious causes of low productivity and mortalities in buffalo's calves. Therefore, it is essential to identify the bacterial agents responsible for these disorders to devise strategies to minimize their economic losses and know the ideal treatment.

The investigations concerned with respiratory diseases in buffaloes were little in the available literature. The data in this work revealed that the prevalence of bacteria causing respiratory disease in buffaloes and calves was 50.09% from the total examined samples (533). These results indicate that not all samples yielded positive results for bacterial isolation and respiratory disease may be related to other causes than bacterial infection. The incidence of bacterial isolates causing respiratory disease in apparently healthy buffaloes and calves were 25.45% and 22.94%, respectively and they were lower than in diseased buffaloes (83.33%) and calves (79.47%). Meanwhile, the incidence of caustive bacterial agents in dead calves was 66.67% as shown in Table (1). It is clear that the incidence of respiratory disease was somewhat higher in nasopharyngeal swabs than other collected samples, so the nasopharyngeal swabs may be used as a sufficient guide to indicate bacterial respiratory disease especially in life animals. In this

concern, **Vaissaire et al. (1988)** recorded that the incidence of bacterial affections in pneumonic lungs of bovine reached 94.68%. **Riad (1989)** isolated 90.1% bacteria causing respiratory disease from dead buffalo calves. On the other hand, **El Batrawy et al. (1992)** found that the incidence of bacteria was 91.7% and 91.5% in the nasopharyngeal swabs and lung, respectively of buffalo calves with respiratory syndromes. **Moreover, Saleh and El Bably (1998)** recorded that the incidence of respiratory disease from nasopharyngeal swabs in apparently healthy buffalo calves was 29.5%. these results were higher than that in Table (1), it was 22.22%.

The data presented in Table (2) indicated that there were a wide range of gram positive and gram negative bacteria isolated from nasopharyngeal swabs, laryngeal swabs and lung tissue of apparently healthy and diseased buffaloes and calves and in dead calves including *A. pyogenes*, *C. bovis*, *Micrococcus* spp., *S. aureus*, *S. epidermidis*, *S. pneumoniae*, *E. coli*, *K. pneumoniae*, *M. haemolytica*, *P. multocida*, *P. vulgaris*, *P. aeruginosa*, and *Y. enterocolitica* in different incidences, and it was noticed that *S. aureus* was the highest incidence (10.32%) . One point of interest in this work was the isolation of *Y. enterocolitica* from 3.19% of respiratory affections of buffaloes and calves although it is mainly an enteropathogen.

The obtained results coincided to some extent with **Brylin (1986)** who isolated *Staphylococcus* spp. and *Micrococcus* spp. from lungs of apparently healthy calves. Also, *Staphylococcus* spp. and *Streptococcus* spp. were isolated from pneumonic lungs of calves by **Suleimanov et al.**

Table (1) Prevalence of bacteria causing respiratory disease in buffaloes and calves .

| Samples              | Buffaloes          |     |       |          |     |       | Calves             |     |       |          |     |       |      |     |       | Total |     |       |
|----------------------|--------------------|-----|-------|----------|-----|-------|--------------------|-----|-------|----------|-----|-------|------|-----|-------|-------|-----|-------|
|                      | Apparently healthy |     |       | Diseased |     |       | Apparently healthy |     |       | Diseased |     |       | Dead |     |       |       |     |       |
|                      | No.                | +ve | %     | No.      | +ve | %     | No.                | +ve | %     | No.      | +ve | %     | No.  | +ve | %     | No.   | +ve | %     |
| Nasopharyngeal Swabs | 50                 | 13  | 26    | 20       | 17  | 85    | 90                 | 20  | 22.22 | 70       | 65  | 92.86 | 7    | 4   | 57.14 | 237   | 119 | 50.21 |
| Laryngeal swabs      | 30                 | 10  | 33.33 | 11       | 10  | 90.91 | 40                 | 10  | 25    | 60       | 45  | 75    | 7    | 5   | 71.43 | 148   | 80  | 54.05 |
| Lung tissue          | 30                 | 5   | 16.67 | 11       | 8   | 72.73 | 40                 | 9   | 22.5  | 60       | 41  | 68.33 | 7    | 5   | 71.43 | 148   | 68  | 45.95 |
| Total                | 110                | 28  | 25.45 | 42       | 35  | 83.33 | 170                | 39  | 22.94 | 190      | 151 | 79.47 | 21   | 14  | 66.67 | 533   | 267 | 50.09 |

The percent was calculated according to the number of examined samples.



Table (2) Incidence of bacteria causing respiratory disease in buffaloes and calves.

| Bacteria                 | Buffaloes          |   |           |      |           |      |           |    |           |       |           |       | Calves             |      |           |          |           |     |           |       |           | Total (533) |           |       |          |       |          |       |          |       |      |       |
|--------------------------|--------------------|---|-----------|------|-----------|------|-----------|----|-----------|-------|-----------|-------|--------------------|------|-----------|----------|-----------|-----|-----------|-------|-----------|-------------|-----------|-------|----------|-------|----------|-------|----------|-------|------|-------|
|                          | Apparently healthy |   |           |      |           |      | Diseased  |    |           |       |           |       | Apparently healthy |      |           | Diseased |           |     | Dead      |       |           |             |           |       |          |       |          |       |          |       |      |       |
|                          | N.S. (50)          |   | L.S. (30) |      | Lung (30) |      | N.S. (20) |    | L.S. (11) |       | Lung (11) |       | N.S. (90)          |      | L.S. (40) |          | Lung (40) |     | N.S. (70) |       | L.S. (60) |             | Lung (60) |       | N.S. (7) |       | L.S. (7) |       | Lung (7) |       |      |       |
|                          | No                 | % | No        | %    | No        | %    | No        | %  | No        | %     | No        | %     | No                 | %    | No        | %        | No        | %   | No        | %     | No        | %           | No        | %     | No       | %     | No       | %     | No       | %     |      |       |
| <i>A. pyogenes</i>       | 0                  | 0 | 0         | 0    | 1         | 3.33 | 0         | 0  | 1         | 9.09  | 1         | 9.09  | 0                  | 0    | 0         | 0        | 1         | 2.5 | 2         | 2.86  | 1         | 1.67        | 1         | 1.67  | 0        | 0     | 0        | 0     | 1        | 14.29 | 9    | 1.69  |
| <i>C. bovis</i>          | 0                  | 0 | 0         | 0    | 0         | 0    | 0         | 0  | 0         | 1     | 9.09      | 0     | 0                  | 0    | 0         | 0        | 0         | 0   | 3         | 4.29  | 4         | 6.67        | 6         | 10    | 0        | 0     | 0        | 0     | 0        | 0     | 14   | 2.63  |
| <i>Micrococcus spp.</i>  | 0                  | 0 | 0         | 0    | 0         | 0    | 0         | 0  | 0         | 1     | 9.09      | 1     | 1.11               | 0    | 0         | 0        | 0         | 2   | 2.86      | 2     | 3.33      | 4           | 6.67      | 0     | 0        | 0     | 0        | 0     | 0        | 10    | 1.88 |       |
| <i>S. aureus</i>         | 0                  | 0 | 0         | 0    | 1         | 3.33 | 1         | 5  | 1         | 9.09  | 2         | 18.18 | 1                  | 1.11 | 1         | 2.5      | 1         | 2.5 | 16        | 22.86 | 16        | 23.33       | 13        | 21.67 | 1        | 14.29 | 1        | 14.29 | 2        | 28.57 | 55   | 10.32 |
| <i>S. epidermidis</i>    | 3                  | 6 | 2         | 6.67 | 2         | 6.67 | 2         | 10 | 1         | 9.09  | 1         | 9.09  | 1                  | 1.11 | 2         | 5        | 2         | 5   | 7         | 10    | 5         | 8.33        | 4         | 6.67  | 0        | 0     | 0        | 0     | 0        | 0     | 34   | 6.38  |
| <i>S. pneumoniae</i>     | 4                  | 8 | 3         | 10   | 2         | 6.67 | 2         | 10 | 0         | 0     | 0         | 0     | 7                  | 7.77 | 3         | 7.5      | 3         | 7.5 | 7         | 10    | 3         | 5           | 3         | 5     | 1        | 14.29 | 0        | 0     | 0        | 0     | 38   | 7.13  |
| <i>E. coli</i>           | 0                  | 0 | 1         | 3.33 | 2         | 6.67 | 1         | 5  | 1         | 9.09  | 1         | 9.09  | 1                  | 1.11 | 1         | 2.5      | 1         | 2.5 | 6         | 8.57  | 5         | 8.33        | 4         | 6.67  | 1        | 14.29 | 1        | 14.29 | 1        | 14.29 | 27   | 5.07  |
| <i>K. pneumoniae</i>     | 0                  | 0 | 0         | 0    | 2         | 6.67 | 1         | 5  | 1         | 9.09  | 1         | 9.09  | 3                  | 3.33 | 0         | 0        | 0         | 0   | 4         | 5.71  | 1         | 1.67        | 2         | 3.33  | 0        | 0     | 0        | 0     | 0        | 0     | 15   | 2.81  |
| <i>P. multocida</i>      | 0                  | 0 | 0         | 0    | 1         | 3.33 | 2         | 10 | 2         | 18.18 | 3         | 27.27 | 0                  | 0    | 0         | 0        | 2         | 5   | 9         | 12.86 | 7         | 11.67       | 7         | 11.67 | 2        | 28.57 | 2        | 28.57 | 3        | 38.57 | 39   | 7.32  |
| <i>M. haemolytica</i>    | 0                  | 0 | 0         | 0    | 0         | 0    | 1         | 5  | 1         | 9.09  | 1         | 9.09  | 1                  | 1.11 | 1         | 2.5      | 1         | 2.5 | 8         | 11.42 | 5         | 8.33        | 4         | 6.67  | 0        | 0     | 1        | 14.29 | 2        | 28.57 | 26   | 4.89  |
| <i>P. vulgaris</i>       | 0                  | 0 | 0         | 0    | 0         | 0    | 1         | 5  | 1         | 9.09  | 0         | 0     | 0                  | 0    | 0         | 0        | 0         | 2   | 2.86      | 2     | 3.33      | 1           | 1.67      | 0     | 0        | 0     | 0        | 0     | 0        | 7     | 1.31 |       |
| <i>P. aeruginosa</i>     | 0                  | 0 | 1         | 3.33 | 1         | 3.33 | 3         | 15 | 1         | 9.09  | 1         | 9.09  | 3                  | 3.33 | 2         | 5        | 1         | 2.5 | 3         | 4.29  | 2         | 3.33        | 2         | 3.33  | 2        | 28.57 | 3        | 38.57 | 1        | 14.29 | 25   | 4.69  |
| <i>Y. enterocolitica</i> | 0                  | 0 | 1         | 3.33 | 1         | 3.33 | 0         | 0  | 1         | 9.09  | 1         | 9.09  | 2                  | 2.22 | 1         | 2.5      | 1         | 2.5 | 2         | 2.86  | 2         | 3.33        | 2         | 3.33  | 1        | 14.29 | 1        | 14.29 | 1        | 14.29 | 17   | 3.19  |

N.S.: nasopharyngeal swabs.

L.S.: laryngeal swabs.

The percent was calculated according to the number of each of examined samples.

(1986). Furthermore, Sandhu et al. (1987) isolated *Staphylococcus* species from pneumonic lesions in buffalo's calves. Moreover, Ahmed (1994) isolated *S. epidermidis* and *S. pneumoniae* in incidence of 8.2% and 13.8%, respectively from apparently healthy calves. Furthermore, Brylin (1986) who isolated *E. coli* from lungs of apparently healthy calves. On the other hand. Ahmed (1994), Mosharaf (1997) and Mohamed (2002) who noted that *E. coli* was associated with respiratory disorders in calves. Otherwise, *A. pyogenes* isolated by El Enbaawy (1986) and Vaissaire et al.(1988) who found that the incidences of *A. pyogenes* isolated from buffalo's calves with respiratory manifestations were 4.0% and 22% respectively. Meanwhile, Abdel Ghani et al. (1990) isolated *A. pyogenes* from dead buffalo calves with respiratory disorders. *P. multocida* was one of the most prevalent isolates obtained from diseased buffalo calves Mosharaf (1997) and Mohamed (2002). In addition, Vipash et al. (2003) who recorded that the incidence *P. multocida* was 50% in pneumonic lung of died buffaloes in outbreak of haemorrhagic septicemia. Also, *Klebsiella* spp. and *P. aeruginosa* were isolated by El Enbaawy (1986) and Mosharaf (1997). The high incidence of bacterial isolation may be related to environmental contamination and low immunity which may be due to excessive use of antibiotics or hormones used in breeding of the buffaloes. In the present study, it was clear that bacteria causing respiratory disease in buffaloes and calves may occur in a mixed form and concomitant with each other in establishment of disease. It is evident from the results recorded in Table (3) that the incidence of positive samples yielded single bacterial infection (78.28%) was higher than the incidence of samples yielded mixed

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isolates (21.72%). Moreover, there were variations among the incidence of single and mixed infection in different samples.

**Table (3): The incidence of positive samples yielding single and mixed bacterial isolates causing respiratory disease in buffaloes and calves.**

| Condition of animals              | No. of examined samples | No. of +ve samples |       | Single infected samples |       | Mixed infected samples |       |
|-----------------------------------|-------------------------|--------------------|-------|-------------------------|-------|------------------------|-------|
|                                   |                         | No.                | %*    | No.                     | %**   | No.                    | %**   |
| Apparently healthy buffaloes.     | 110                     | 28                 | 25.45 | 23                      | 82.14 | 5                      | 17.86 |
| Diseased buffaloes.               | 42                      | 35                 | 83.33 | 28                      | 80.00 | 7                      | 20.00 |
| Apparently healthy buffalo calves | 170                     | 39                 | 22.94 | 33                      | 84.62 | 6                      | 15.38 |
| Diseased buffalo calves           | 190                     | 151                | 79.47 | 114                     | 75.50 | 37                     | 24.50 |
| Dead buffalo calves               | 21                      | 14                 | 66.67 | 11                      | 78.57 | 3                      | 21.43 |
| Total                             | 533                     | 267                | 50.09 | 209                     | 78.28 | 58                     | 21.72 |

\* The percent was calculated according to the number of each examined sample.

\*\* The percent was calculated according to the number of each positive sample.

Many investigators concerned with single and mixed bacterial infection in buffaloes and their calves as **Ismail et al. (1993)** who mentioned that the rates of mixed and single infection in apparently healthy buffalo calves were 3.5% and 5.9%, respectively, while the incidences were 16.3% and 74.3% in diseased animals, respectively and **Selim et al. (1998)** who

found that the incidence of mixed infection in diseased buffaloes was 16.25% while it was 51.66% in single infection. The combination between many bacteria with each other to establish the respiratory disease in buffaloes and calves occurred mainly between *S. aureus* + *P. aeruginosa* ( 5 samples) followed by *S. aureus* + *S. epidermidis*, *S. aureus* + *Y. enterocolitica*, *S. pneumoniae* + *S. epidermidis*, *S. pneumoniae* + *Y. enterocolitica*, and *M. haemolytica* + *Y. enterocolitica* (each in 4 samples). Meanwhile the lowest combination occurred between *S. aureus* + *K. pneumoniae* and *S. pneumoniae* + *P. aeruginosa* (each in 1 sample).

In addition, other combination between many bacteria presented in Table (4). These results verified that *P. vulgaris* and *Y. enterocolitica* could not induce a disease separately. The obtained results supported to a large extent the idea that combination between bacteria affect the disease condition as reported by Ismail et al. (1993) who isolated *A. pyogenes*, *S. aureus*, *S. pneumoniae*, *K. pneumoniae*, *E. coli*, *P. mirabilis* and *P. aeruginosa* from single and mixed infection in buffalo calves suffering from respiratory disorders and El Haenaey et al. (1994) who recorded that 14% of diseased cattle were infected with *S. aureus* with mixed infection.

Results illustrated in Table (5) showed serological identification of some isolates causing respiratory disease in buffaloes and calves. On serotyping of 27 isolates of *E. coli*, the most predominant serogroups were O1, O119 and O126, followed by O44 then O26, O125, and O11. On the other hand,

**Table (4): Mixed bacterial isolates causing respiratory disease in buffaloes and calves.**

| Bacteria  | No. of samples contain mixed bacterial isolates | No. of mixed bacterial isolates |
|---|---|---------------------------------|
| <i>A. pyogenes</i> + <i>Micrococcus</i> spp.                    | 2   | 4                               |
| <i>C. bovis</i> + <i>E. coli</i>                                | 3   | 6                               |
| <i>C. bovis</i> + <i>P. vulgaris</i> + <i>Y. enterocolitica</i> | 3   | 9                               |
| <i>S. aureus</i> + <i>Micrococcus</i> spp.                      | 2   | 4                               |
| <i>S. aureus</i> + <i>S. epidermidis</i>                        | 4   | 8                               |
| <i>S. aureus</i> + <i>S. pneumoniae</i>                         | 2   | 4                               |
| <i>S. aureus</i> + <i>K. pneumoniae</i>                         | 1   | 2                               |
| <i>S. aureus</i> + <i>P. vulgaris</i>                           | 2   | 4                               |
| <i>S. aureus</i> + <i>P. aeruginosa</i>                         | 5   | 10                              |
| <i>S. aureus</i> + <i>Y. enterocolitica</i>                     | 4   | 8                               |
| <i>S. pneumoniae</i> + <i>S. epidermidis</i>                    | 4   | 8                               |
| <i>S. pneumoniae</i> + <i>P. aeruginosa</i>                     | 1   | 2                               |
| <i>S. pneumoniae</i> + <i>Y. enterocolitica</i>                 | 4   | 8                               |
| <i>E. coli</i> + <i>S. epidermidis</i>                          | 4   | 8                               |
| <i>E. coli</i> + <i>P. vulgaris</i>                             | 2   | 4                               |
| <i>E. coli</i> + <i>Y. enterocolitica</i>                       | 2   | 4                               |
| <i>K. pneumoniae</i> + <i>E. coli</i>                           | 3   | 6                               |
| <i>M. haemolytica</i> + <i>Y. enterocolitica</i>                | 4   | 8                               |
| <i>P. multocida</i> + <i>E. coli</i>                            | 3   | 6                               |
| <i>P. multocida</i> + <i>S. epidermidis</i>                     | 3   | 6                               |
| Total   | 58  | 119                             |

**Table (5): Serological identification of some bacterial isolates causing respiratory disease in buffaloes and calves.**

| <i>E.coli</i> |     |      | <i>P. aeruginosa</i> |     |     |
|---------------|-----|------|----------------------|-----|-----|
| Serogroup     | No. | %*   | Serogroup            | No. | %*  |
| O1            | 5   | 18.5 | H                    | 7   | 28  |
| O11           | 1   | 3.7  | I                    | 5   | 20  |
| O26           | 2   | 7.4  | K                    | 9   | 36  |
| O44           | 4   | 14.8 | L                    | 1   | 4   |
| O119          | 5   | 18.5 | M                    | 3   | 12  |
| O125          | 2   | 7.4  |                      |     |     |
| O126          | 5   | 18.5 |                      |     |     |
| Untyped       | 3   | 11.1 |                      |     |     |
| Total         | 27  | 100  |                      | 25  | 100 |

\* The percent was calculated according to the total number of each isolates.

identification of *P. aeruginosa* revealed that serogroup K was the predominant isolates then H also I, L and M were identified. These results come in accordance with that of **El Battrawy et al. (1992)** who isolated *E. coli* O55, O68, O119 and O126 from respiratory diseased in buffaloes. In addition, **Mahmoud (1999)** who identified *E. coli* serogroups O26, O86, O119 and O126 from buffaloes suffering from respiratory diseased. Antibiogram of 11 chemotherapeutic agents on 17 isolates from buffaloes and calves are presented in Table (6) all tested isolates were resistant to streptomycin. There were differences in isolates susceptibilities and zones of inhibition to different chemotherapeutic agents. Amikacin, cefadroxil, chloramphenicol.

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Tab.6



doxycycline, enrofloxacin, norfloxacin, and trimethoprim + sulphamethoxazole, were effective on most isolates. It was clear that, many bacterial isolates showed resistant to many antibiotics and this may be attributed to wrong dose, duration of drugs and route of administration or plasmid resistance. *A. pyogenes* was sensitive to enrofloxacin, norfloxacin and ciprofloxacin. These results are in contrast to those recorded by **Abou Zaid (1996)** who claimed that *A. pyogenes* isolates were highly sensitive to gentamicin (100%), flumequine (93.33%), erythromycin (66.67%) and nalidixic acid (66.67%). *E. coli* serogroups O1, O119 and O126 were sensitive to amikacin, chloramphenicol and enrofloxacin. This result also comes in contrast with **Aarestrup et al. (1998)** who recorded that *E. coli* isolates were resistant to fluoroquinolone and enrofloxacin. *P. aeruginosa* was sensitive to amikacin, and enrofloxacin. This result disagree with **El Haenaey et al. (1994)** who stated that *P. aeuroginosa* was resistant to all antibiotics tested. The obtained results coincided to large extent with **Selim et al. (1998)** who recognized that the gram negative isolates were sensitive to enrofloxacin, chloramphenicol, gentamicin, streptomycin and trimethoprim + sulphamethoxazole. On the other hand, *P. multocida* was sensitive to amikacin, amoxicillin, ciprofloxacin, doxycycline and enrofloxacin. This result some what disagreed with **Abou Zaid (1996)** who found that *P. multocida* strains were sensitive to gentamicin, erythromycin, trimethoprim + sulphamethoxazole, nalidixic acid and chloramphenicol. *S. aureus* was sensitive to a wide rang of antibiotics. Also, there was some agreement with **Selim et al. (1998)** who recorded that *S. aureus* was highly sensitive to enrofloxacin. The result achieved in Table (7) showed the



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virulence of different bacterial isolates causing respiratory disease in buffaloes and calves, as demonstrated by the sequence of mortality of mice which were injected with tested strain I/P.

**Table (7): Pathogenicity of bacterial isolates causing respiratory disease in buffaloes and calves.**

| Bacteria                 | No. of dead mice / day |   |   |   |   |   |   | Total |     |
|--------------------------|------------------------|---|---|---|---|---|---|-------|-----|
|                          | 1                      | 2 | 3 | 4 | 5 | 6 | 7 | No.   | %*  |
| <i>A. pyogenes</i>       | 1                      | 2 | 2 | 0 | 0 | 0 | 0 | 5/5   | 100 |
| <i>C. bovis</i>          | 0                      | 0 | 1 | 1 | 2 | 0 | 1 | 5/5   | 100 |
| <i>Micrococcus</i> spp.  | 0                      | 1 | 1 | 0 | 1 | 0 | 0 | 3/5   | 60  |
| <i>S. aureus</i>         | 1                      | 1 | 1 | 0 | 0 | 0 | 0 | 3/5   | 60  |
| <i>S. epidermidis</i>    | 0                      | 1 | 1 | 0 | 0 | 0 | 0 | 2/5   | 40  |
| <i>S. pneumoniae</i>     | 0                      | 2 | 1 | 1 | 1 | 0 | 0 | 5/5   | 100 |
| <i>E. coli</i> O1        | 0                      | 1 | 1 | 1 | 2 | 0 | 0 | 5/5   | 100 |
| <i>E. coli</i> O119      | 0                      | 1 | 1 | 0 | 0 | 1 | 0 | 3/5   | 60  |
| <i>E. coli</i> O126      | 0                      | 1 | 0 | 1 | 0 | 1 | 0 | 3/5   | 60  |
| <i>K. pneumoniae</i>     | 0                      | 1 | 2 | 1 | 1 | 0 | 0 | 5/5   | 100 |
| <i>M. haemolytica</i>    | 1                      | 3 | 1 | 0 | 0 | 0 | 0 | 5/5   | 100 |
| <i>P. multocida</i>      | 3                      | 2 | 0 | 0 | 0 | 0 | 0 | 5/5   | 100 |
| <i>P. vulgaris</i>       | 1                      | 1 | 0 | 1 | 0 | 0 | 0 | 3/5   | 60  |
| <i>P. aeruginosa</i> I   | 1                      | 1 | 1 | 1 | 0 | 0 | 0 | 4/5   | 80  |
| <i>P. aeruginosa</i> H   | 2                      | 1 | 2 | 0 | 0 | 0 | 0 | 5/5   | 100 |
| <i>P. aeruginosa</i> K   | 2                      | 2 | 1 | 0 | 0 | 0 | 0 | 5/5   | 100 |
| <i>Y. enterocolitica</i> | 0                      | 1 | 1 | 1 | 0 | 1 | 0 | 3/5   | 60  |

\* The percent was calculated according to the total number of mice in each group. Examination period for 7 days.

It was clear that *A. pyogenes*, *C. bovis*, *S. pneumoniae*, *E. coli* O1, *K. pneumoniae*, *M. haemolytica*, *P. multocida* *P. aeruginosa* serotypes H and K were highly virulent strains followed by *E. coli* O119 and O126, *Micrococcus* spp., *S. aureus* and *Y. enterocolitica*. While, *S. epidermidis* was lower in its virulence. These may be attributed to pathogenic nature of strain or the presence of virulence associated plasmid or production of endo or exotoxins. These results coincided with the findings of Mahmoud (1999) who found that *P. multocida* was highly pathogenic to mice.

**Table (8): Treatment of some buffalo calves suffering from respiratory disorder.**

| Bacteria   | No. of treated case | Used drug    | Dose             |
|--|---------------------|--------------|------------------|
| <i>A. pyogenes</i>                               | 1                   | Baytril      | 1Cm /10Kg B.W    |
| <i>S. aureus</i>                                 | 1                   | Oxytetrac 5% | 1Cm /10Kg B.W    |
| <i>S. aureus</i> + <i>S. epidermidis</i>         | 1                   | Garamycin    | 1Cm /20Kg B.W    |
| <i>S. pneumoniae</i>                             | 1                   | Vit-biotic   | 1 vial /100Kg BW |
| <i>S. pneumoniae</i> + <i>S. epidermidis</i>     | 1                   | Vit-biotic   | 1 vial /100Kg BW |
| <i>S. pneumoniae</i> + <i>P. aeruginosa</i>      | 1                   | Garamycin    | 1Cm /20Kg B.W    |
| <i>K. pneumoniae</i>                             | 1                   | Oxytetrac 5% | 1Cm /10Kg B.W    |
| <i>K. pneumoniae</i> + <i>E. coli</i>            | 1                   | Garamycin    | 1Cm /20Kg B.W    |
| <i>M. haemolytica</i>                            | 2                   | Oxytetrac 5% | 1Cm /10Kg B.W    |
| <i>M. haemolytica</i> + <i>Y. enterocolitica</i> | 1                   | Oxytetrac 5% | 1Cm /10Kg B.W    |
| <i>P. multocida</i> ,                            | 2                   | Oxytetrac 5% | 1Cm /10Kg B.W    |
| <i>P. aeruginosa</i>                             | 1                   | Baytril      | 1Cm /10Kg B.W    |

Route of administration I / M for 3 -5 days according to degree of animal response

It was evident from Table (8) that all patent preparations used in treatment of some buffalo's calves suffering from respiratory disorder as enrofloxacin (Baytril), gentamicin (Garamycin), doxycycline (Oxytetrac 5%) and amoxycillin (Vit-biotic) gave good results as chemotherapeutic agents selected in the light of sensitivity test and gave to animal in the earliest stage of the disease.

**On conclusion:** We must follow effective preventive measures to Egyptian buffaloes including prevention of stress factor, over crowding, cold and humidity which might lower the resistance of the animals and allow other bacteria to invade the body and produce the disease which need high cost of treatment and lower the productivity. We recommended continuous examination of general health of the animals to identify suspected diseased animals and rapid treatment with drug of choice, in addition to improve managements, good ventilation and separation of the diseased animals to avoid spread of the disease especially among the newly born calves.

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## بعض الدراسات عن الأمراض التنفسية البكتيرية فى الجاموس

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أجريت الدراسة على ٥٢٢ عينة من الجاموس وعجول الجاموس الذى يعانى من أمراض تنفسية والسليم ظاهرياً وأيضاً على بعض العجول التى ماتت من مرض تنفسى بغرض معرفة المسببات البكتيرية للأمراض التنفسية فى الجاموس وكانت العينات عبارة عن مسحات أنفية - مسحات من القصبة الهوائية وعينات من نسيج الرئة. كانت نسبة وجود البكتريا المسببة للأمراض التنفسية فى الجاموس وعجول الجاموس ٥٠.٠٩% من جميع العينات.

كما كانت نسبة وجود المعزولات البكتيرية كالتالى:

٢٥.٤٥% من الجاموس السليم ظاهرياً، ٢٢.٩٤% من عجول الجاموس السليمة ظاهرياً، ٨٢.٣٢% من الجاموس المصاب، ٧٨.٤٨% من عجول الجاموس المصاب و٦٦.٦٧% من العجول الميتة. تم عزل عدد كبير من البكتريا من العينات السابق ذكرها بنسب مختلفة وتشمل أكتينومييسيس بيوجينز، كورينى بوفز، فصيلة الميكروكوكس، العنقودى الذهبى، العنقودى أبيدرميدس، السبجى نيمونى، أى كولاى، كلبسيلانيمونى، مانهاميا هيماوليتيكا، باستريلا مالتوسيدا، بروتيس فولجاريز، سيدومونس أروجينوزا وپرسينيا إنتيروكوليتيكا .

وكانت أعلى نسبة عزل للميكروب العنقودى الذهبى هي ١٠.٣٢%. وكانت نسبة العينات التى عزل منها ميكروب واحد هي ٧٨.٢٨%، أما نسبة العينات التى عزل منها أكثر من ميكروب كانت ٢١.٧٢%. كما تم رصد الميكروبات المختلفة مع بعضها فى عدد العينات.

كما تم إجراء الفحص السيرولوجى لكل من ميكروبات أى كولاى والسيدومونس أروجينوزا. وكانت نتيجة الفحص التعرف على ٧ أنواع من الميكروب القولونى و ٥ أنواع من السيدومونس أروجينوزا. كما تم إجراء إختبار الحساسية على معظم العترات المعزولة ومن الملاحظ مقاومة جميع العترات لعقار الستريبيوميسين. أما بالنسبة لباقى العقاقير فإختلفت درجة الإستجابة من ميكروب إلى آخر. وأيضاً تم إجراء إختبار الضراوة فى الفئران على معظم المعزولات وكان الميكروب العنقودى ابيدرميدس أقل المعزولات ضراوة فى الفئران. وقد تم معالجة بعض الحيوانات المريضة بإستخدام الأنوية التى تحتوى على المركبات التى أظهرت أعلى درجة حساسية لهذه الميكروبات.