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AEROBIC BACTERIAL PATHOGENS OF PNEUMONIC FEEDLOT BUFFALO-CALVES, IN ASSIUT GOVERNORATE, EGYPT

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ABSTRACT:

The current study conducted on 68 lung samples from 1–3 years old buffalo-calves slaughtered at Assiut abattoirs. The samples showed variable gross lesions of pneumonia in particular the grey and red hepatization. The bacteriological examinations indicated that 66 (97.06%) samples of the examined lungs were positive for mixed bacterial isolation, while the other two samples (2.94%) found to be bacteriologically negative. *Staphylococcus aureus* (22.43%); *Escherichia coli* (18.22%) and *Pasteurella multocida* (15.89%) were the predominant isolated bacterial pathogens. However, *Proteus vulgaris* (7.01%); *Streptococcus pyogenes* (5.61%); *Actinomyces pyogenes* (3.74%); *Klebsiella pneumoniae* (3.27%) and *Corynebacterium bovis* (2.8%) were also isolated. *Pasteurella multocida* were isolated from pulmonary tissues and their virulence and pathogenicity test revealed that all injected mice were died at various time-intervals, from less than 24 hours up to 48 hours, with 100% mortality rates.

INTRODUCTION:

Pneumonia is a leading cause of loss to ruminants throughout the world, where several causative agents and contributory factors appear to be involved (Novert, 2002 and Yener *et al.*, 2005). Pneumonia is the main cause of morbidity and mortality in buffalo-calves especially in intensive rearing system (Schmoltdt, *et al.*, 1979). Rather than, calves which having early age pneumonia might have in their future severe depression in the production capabilities rendering their keeping economically unprofitable (Mona, 2005). Mortality rate due to pneumonia among necropsies dairy cattle was 26% (Müller, *et al.*, 2005).

Pneumonia is the most frequently occurring respiratory affections in domestic animals, since the etiologic agents are bacteria, viruses or viruses complicated with bacteria (Allan, *et al.*, 1991).

Among various respiratory affections, pneumonic pasteurellosis is the most common cattle respiratory disease caused by *Pasteurella multocida* which is one of the nasopharyngeal commensally and commonly isolated from bovine respiratory diseases (Dabo, *et al.*, 2007). Other pneumonic pathogens-but less frequently-could be recovered from pneumonic lungs are *Staph. aureus*; *Strept. pneumoniae* (Beiter, *et al.*, 2006) and *E. coli spp.* (Wessely *et al.*, 2005). The present work aimed to isolate the etiologic pathogens from pneumonic lungs of

slaughtered feedlot buffalo-calves as well as pathogenicity of the isolates to laboratory animals.

MATERIAL AND METHODS:

Samples:

Sixty-eight lung samples showing gross lesion at different degrees of pneumonic features were collected from slaughtered buffalo-calves 1-3 years old. The samples were collected from abattoirs at Assiut Governorate. All samples were collected in sterile polyethylene bags for bacteriologic examination.

Bacteriological examination:

Under aseptic conditions from the entire of the collected samples, a loop full inoculated into brain heart infusion broth supplemented with 5% inactivated horse serum (Algoza Research Institute, Giza) and incubated aerobically for 24 hours at 37°C. Then, a loopfull of the inoculated broth was directly streaked onto blood agar and MacConkey agar plates, aerobically incubated at 37°C for 24 hrs. All isolates were morphologically and biochemically identified according to the criteria prescribed by Quinn *et al.* (1994).

Pathogenicity and virulence of isolated *Pasteurella multocida* according to Wessman, (1964):

Four Swiss Webster white mice weighting about 18-22 g. were used for each isolate. The all mice were injected intrapritoneally (I/P) by 0.1 ml of bacterial suspension of $(1.5 \times 10^8 \text{ cfu})$. One mouse was kept as a control for each isolate and be injected I/P with 0.1 ml sterile normal saline. The mortality rates and post mortem changes were recorded. From heart blood of the dead mice, reisolation of inoculated strains was carried out and blood films were prepared and stained with Leshiman's stain for showing the characteristic features of *Pasteurella multocida* organisms.

RESULTS:

Out of 68 samples collected from pneumonic tissues of the slaughtered buffalo-calves, 66 samples (97.06%) were positive for mixed bacterial isolation, while the two samples (2.94%) were found to be negative for bacteriological examination. Detailed obtained results were illustrated in tables (1-3).

Table (1): Bacterial species recovered from pneumonic lungs of buffalo-calves

Bacterial species	No.	%
- <i>Staph. aureus</i>	48	22.43
- <i>E.coli</i>	39	18.22
- <i>Pasteurella multocida.</i>	34	15.89
-Coagulase negative staphylococci	21	9.81
- <i>Proteus vulgaris</i>	15	7.01
- <i>Strept. pyogenes</i>	12	5.61
- <i>Proteus mirabilis</i>	9	4.20
- <i>Micrococcus spp.</i>	9	4.20
- <i>Actinomyces pyogenes</i>	8	3.74
- <i>Klebsiella pneumoniae</i>	7	3.27
- <i>Corynebacterium bovis.</i>	6	2.80
- <i>Proteus spp.</i>	3	1.4
- <i>Serratia marcescens</i>	3	1.4
Total	214	100

Table (2): Virulence and pathogenicity of isolated *Pasteurella multocida* in mice

No. of isolates	No. of inoculated mice	Time of death post intraperitoneal inoculation			Mortality Rate
		Within 12 hrs.	24 hrs.	48 hrs.	
34	136	95	26	15	100

Table (3): Coagulase test results for the isolates of *Staph. aureus*

NO. of isolates	Time of coagulation		
	Less than 1 hrs	12 hrs.	24 hrs.
48	32 66.7%	12 25%	4 8.3%

DISCUSSION:

Buffalo pneumonia represents important problems as they affect lung confronting animal production (Radwan, *et al.*, 2002). In the present study, bacteriological examination of pneumonic lung samples revealed that 66 samples (97.06%) were positive for mixed bacterial isolation (table,1). It is commonly to detect pulmonary mixed infection since the bovine respiratory air pathways act as reservoirs for potential pathogenic micro-organisms, which develop pneumonia on the onset under stress factors, decline of hygienic measurements or climatic conditions (Yehia,2000 and Moustafa, 2004), with obvious pneumonic lesions than when a single bacterium was incriminated (Novert, 2002). Pneumonic mixed pathogens are mainly *Staph. aureus*, *E. coli*, *P. multocida* and other organisms demonstrate the complexity of the disease where *Staph. aureus* may predispose the animals to infection by coliform organisms or other pathogens (Roberson, *et al.*, 1994 and Sedeek and Thabet, 2001). Identification of the pneumonic pathogens in the present work, cleared the isolation of *Staph. aureus*, (22.43%), *E. coli* (18.22%) and *P. multocida* (15.89%) as shown in table (1), which were the most common pneumonic bacteria-isolated from lung tissues - widely documented (Blobe, *et al.*, 1985, Erdag *et al.*, 1993, Saleh and El-Bably, 1998,

Zaki *et al.*, 2002, Mona,2005 and Wessely, *et al.*, 2005).

Percentage of *Past. multocida* isolate in the present work from pneumonic lung of buffalo-calves (15.89%) was in accordance with that obtained by Zaki, *et al.* (2002), while higher incidence of *Past. multocida* were 69.8% and 70% obtained by Bloble, *et al.* (1985) and Erdag, *et al.* (1993). The low incidences were recovered by Abd El-Kader (1992), *Past. multocida* (5.71%). Novert (2002) isolated *Past. multocida* (8%). *Staph. aureus* the most common bacteria isolated from buffalo-calves (Ismail, *et al.*,1993). *E.coli* was one the most important causes of early onset infection and frequent causative agent of pneumonia in calves (Mona, 2005). In present study, the percentage of *Staph. aureus* (22.43%) and *E coli* (18.22%) was similar with that obtained by Ismail, *et al.*(1993) and Mona (2005), while the low incidence was 11.42%, *Staph. aureus* and 5.71%, *E. coli* (Abd El-Kader, 1992) and 1.5%, *Staph. aureus* and 7%, *E. coli* (Novert, 2002).The variation in isolation percentage may be attributed to change in hygienic measure, stress factors, change in management and immune status of infected animals (Sedeek and Thabet, 2001). *Past. multocida* is commonly inhibits the upper respiratory tracts as commensally or opportunistic pathogen (Quinn *et al.*, 1994), however, it was isolated from the deep

pulmonary tissues of the examined cases. This may refer to the presence of a risk induces a favorable media in lung facilitating the invasion of *Past. multocida* into deep tissues. Consequently, viral and mycoplasma examinations of the pneumonic lungs are desirable.

Other pneumonic pathogens but less frequently were recovered from pneumonic lungs in the present study such as *Proteus vulgaris* (7.01%) *Strept. pyogenes* (5.61%) *Klebsiella pneumoniae*, (3.27%) as shown in table (1) are similar to the findings of Elyas (1982), Abd El-Kader (1992) and Ismail, *et al.* (1993).

Despite of *Pasteurella multocida* being nasopharyngeal commensally (Dabo, *et al.*, 2007), when invade lung tissue under stress factors, its virulence exaggerates and pathogenicity differs (Christensen, *et al.*, 2004), depending upon their outer membrane proteins (Dabo, *et al.*, 2007). The virulence and pathogenicity test of *Pasteurella multocida* in mice (table, 2) revealed that all isolates were highly pathogenic to mice producing acute septicemia an death within 24-48 hours post inoculation. Small doses of *Pasteurella multocida* recovered from pneumonic lungs of buffaloes were highly virulent and sufficient to kill all mice (Sharma, *et al.*, 1979; Sheikh, *et al.*, 1994; Aliaa, 2002; Zaki, *et al.*, 2002; Moustafa, 2004; Abd El-Latif, 2006)

In the present investigation, the achieved results revealed that 66.7% and 25% of the isolated *Staph aureus* gave positive results for tube coagulase test before an hour and up to 12 hours, respectively table, (3) depending upon the activity of coagulase enzyme produced by *Staph aureus* resulted in clot formation in rabbit plasma (Kloos and Schleifer, 1986). Contrariwise, staphylokinase produced by

certain strains of *Staph aureus* has been shown to have profibrinolytic properties and act as plasminogen activator enhancing the activity of endogenous fibrinolytic system (Collen and Lingen, 1991).

In the present study, failure for bacteriological isolation in two lung samples may be due to other cause incriminated as mycoplasma, viruses or fungi. The etiology of pneumonia is complex and multifactorial which are either non-infectious or microbial determinants including bacteria, viruses and fungi (Garoia, *et al.*, 1982). It can be concluded that the obtained results give a focus about the importance of *Pasteurella multocida*; *Staph. aureus* and *E. coli* as the causes of pneumonia in feedlot buffalo-calves.

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المسببات البكتيرية الهوائية الممرضة للالتهاب الرئوي في الجاموس التسمين بمحافظة أسيوط - مصر -

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**أستاذ الأمراض المعدية - كلية الطب البيطرى - جامعة أسيوط

أجريت هذه الدراسة على 68 عينة من رئة جاموس التسمين تتراوح أعمارها من 1-3 سنوات مذبوحة بمجازر أسيوط تظهر عليها الآفات الباثولوجية للالتهابات الرئوية. بالفحص البكتيريولوجى وجد أن 66 عينة رئة بنسبة 97.06% إيجابية للعزل البكتيرى المختلط، ولم يتمكن من العزل البكتيرى لعينتين رئة بنسبة 2.94%. كانت أعلى نسب للعزل البكتيرى لكل من الميكروب العنقودى الذهبى (22.43%)، والأشيرشيا القولونية (18.22%)، والباستيرلا مالتوسيدا (15.89%). كما عزل بعض العترات الأخرى بنسب أقل منها ميكروب بروتيس فولجريس (7.01%)، والمتكور السبحى الصديدى (5.61%)، واكتينوماسيس بيوجينز (3.74%)، والكلبسيلا نيمونى (3.27%)، والكورينى باكتيريم بوفس (2.8%). أظهرت نتائج اختبار الضراوة لجميع عترات الباستيرلا مالتوسيدا نفوق كل الفئران المحقونة خلال أقل من 24 ساعة إلى 48 ساعة.

