Bacterial Isolates from Calves Slaughtered at Abattoir Suffering from Respiratory Problems in Sharkia Governorate

Abd-El-Kaliek, A.A.[1]; Mokhtar, A. Selim[1] and Medhat, K. Rizk[1, 2]

[1]Animal Health Research Institute Zagazig Provincial Laboratory*
[2]Animal Health Research Institute Mansoura Provincial Laboratory**.
ahmedabdelkaleik@yahoo.com

Abstract: This study was carried out on 300 samples from slaughtered calves (150 heart blood and 150 pieces pneumonic lungs), at different ages and different breed at Sharkia Governorate and subjected to bacteriological examination. Results showed that, 53.3% of blood samples were positive for bacterial examination as 81.2%, 18.8% harbour single and mixed isolates respectively, meanwhile 86.7% of lung samples were positive for bacterial isolation of which 78.5% single isolate and 21.5% mixed isolates. The total number of isolates were 210, of which 90 *Pasteurella multocida* type A (35 heart blood and 40 pneumonic lung and 15 mixed infection), 41 (5 heart blood and 22 lung tissue and 14 mixed infection), 79 *Mannhaemia haemolytica* type A species (25 heart blood, 40 lung and 14 mixed infection). Sensitivity test was carried out using different types of antibiotic most isolated bacteria were highly sensitive to Enerofloxacin, Tetracycillin and florofinicol. The total bacterial count of 10% of samples had count ranging from 10 – 10^8; while the most samples showed count 10^8-10^{11}.
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1. Introduction

Calf pneumonia is caused by many pathogens and several contributory factors such as warm wet weather, mixing age groups, poor ventaliation, stress and high densities ***(Hartel et al., 2004)***.

Pneumonia is a major cause of economic losses because of decreased production, high levels of mortality and morbidity and increased veterinary and labour costs ***(Genaba, et al., 1995)***, moreover, calves experienced pneumonia at early age might have severe depression in the production capabilities in the future ***(Sayed and Zaitun, 2009)***.

Pasteurellosis broadly refers to any of the disease condition caused by species of the genus pasteurella ***(Davies et al. 2003)***. *Mannheimia haemolytica* formerly known as *Pasteurella haemolytica* ***(Christensen et al., 2003)***, *Mannheimia haemolytica* is the etiological agent of both bovine and ovine pneumonic pasteurellosis ***(Falada, 2002)***. *Mannheimia haemolytica* and *Pasteurella multocida* are often associated with bovine respiratory disease in cattle; these two bacteria are considered as part of the normal bacterial flora found in the upper respiratory tract of most cattle but are not considered as normal flora of the lungs ***(MaxIrsik, 2010)***.

Respiratory troubles to all domestic animals attributed mixed infection with different bacteria isolates (*Mycoplasma bovis*, Pasteurella species, Streptococcus species, *Staphylococcus aureus*, Kelbesella species and *E. coli*) and their toxins ***(Yehia, 2000)***.

The aim of this work planned to isolation and identification of bacteria with clinical signs of pneumonia in slaughtered calves, determine the effect of antibiotic on the isolated bacteria to reach specific treatment, total bacterial count in lung and.

2. Material and Methods

A total of 300 samples were collected from slaughtered calves of different ages and different breeds, from different abattoirs at Sharkia Governorate, at period from March 2012 to June 2012.

30 white mice, each was 3 weeks age and about 18 – 22 gm body weight were used for purification of Pasteurella isolates.

The samples were 150 swabs from heart blood, the samples collected by sterile cotton swabs and transferred immediately to laboratory for bacteriological examination; and 150 lungs were collected from congested lungs area of slaughtered calves, in sterile plastic bages and transported on ice box for bacteriological examination. Each heart blood swabs and congested lungs were cultured into nutrient broth for 24 hours at 37°C and then loopful was taken and sub-cultured into each of the different following media: nutrient agar, 7% sheep blood agar and MacConkey's agar.

The inoculated plates were incubated at 37°C for at least 24 - 72 hours. The growing colonies were picked up and purified by inoculation on nutrient broth and then sub-cultured on selective media and the isolates were identified morphologically by Gram's stain and colonial appearance according to ***Finegold and Martin***

(1982), the pure colonies were identified biochemically according to ***Koneman, et al. (1983)*** and ***Krieg and Holt (1984)***.

On the other hand coccobacilli were inoculated in laboratory mice for pathogenicity test and virulence of some isolated strains of pasteurella were determined according to ***Wilson and Miles (1975)***, the serological identification of pasteurella organisms using pasteurella antisera (Vet. Serum and Vaccine Research Institute, Abbasia, Cairo) were carried out ***(Carter and Rappy, 1962)*** for capsular typing with indirect haemagglutination test and somatic typing as carried ***(Heddleston et al., 1972)*** using gel diffusion precipitation test.

Purification of *Pasteurella haemolytica* in mice carried out according to ***Wessman (1964)***.

Sensitivity test:

The susceptibility of the most predominant pathogenic isolates to different chemotherapeutic agents was tested by using disc diffusion method according to ***Finegold and Martin (1982)*** and ***Cruickshank et al., 1975)***.

Total colony count: were examined for 30 specimens according to **American Public Health Association "APHA" (2001).**

3. Results

Table (1): Results of bacterial examination of examined blood and tissue samples (n = 300)

Sources and type of samples	*Total number of samples*	*Positive*		*Negative*	
		No.	**%**	**No.**	**%**
Heart blood	150	80	53.3	70	46.7
Lung tissue	150	130	86.7	20	13.3
Total	300	210	70	90	30

Table (2): Incidence of bacteria isolated bacterial strains from heart blood and pneumonic lungs of slaughtered calves

Types of samples	*Single infection*		*Mixed infection*	
	No.	**%**	**No.**	**%**
Heart blood	65	81.2	15	18.8
Pneumonic lungs	102	78.5	28	21.5
Total	167	79.4	43	20.6

No.= number %= percentage

Table (3): The prevalence of pathogenic bacteria isolated from examined slaughtered calves samples

Type of infection	*Type of bacteria*	*Heart blood*		*Pneumonic lungs*	
		No.	**%**	**No.**	**%**
Single infection	*E. coli*	5	7.7	22	21.6
	P. multocida (m) serotype A*	35	53.8	40	39.2
	P. (Mannheimia) *haemolytica* serotype A**	25	38.5	40	39.2
Mixed infection	*E. coli* with *P. multocida* serotype A*	7	46.7	15	53.6
	P. multocida type A with *Mannheimia haemolytica* serotype A	8	53.3	13	46.4

**P. multocida*: Indole +ve

***P.* (Mannheimia) *haemolytica*: growth on MacCkonky – fermentation arginose sugar

Table (4): Passive mouse for purification of Pasteurella isolates (0.1 ml x 10^8 S/C)

Group	No. of mouse	1st day		2nd day		3rd day		7th day	
		Dead	**Live**	**Dead**	**Live**	**Dead**	**Live**	**Dead**	**Live**
Control	15	0	15	0	15	0	15	0	15
Infected mouse	15	12	3	2	1	1	0	0	0

Table (5): Results of antibiotic sensitivity test to some pathogenic representative bacteria isolates

Bacteria isolates / *antibiotic disc*	*P. multocida Type A (82)*		*E. coli (30)*		*P. (Mannhemia) haemolytica type A (75)*	
	No.	**%**	**No.**	**%**	**No.**	**%**
Enrofloxcin 10ug	71	86	27	90	65	87
Gentamycin 10ug	35	42	25	83	60	80
Tetracycline 30ug	74	90	26	87	67	89
Erythromycin 10ug	44	53	11	37	38	51
Amoxicillin 30ug	59	72	00	00	27	36
Florofincol 10ug	68	83	17	57	63	84
Penicillin 100iu	25	30	00	00	10	13

Table (6): frequency distribution of examined samples based on their total colony count

Specimens	*Intervals*	*Frequency*	
		No.	**%**
Lung	**$10^6 – 10^8$**	3	10
Lung	**$10^8 – 10^{10}$**	12	40
Lung	**$10^{10} - 10^{11}$**	15	50

4. Discussion

Commensal bacteria present in the respiratory system may cause diseases when the animals subjected to stress factors ***(Palatary and Newhall, 1985)***. In the present study 300 samples (150 heart blood and 150 lung tissue) from slaughtered calves were examined and results obtained revealed that 53.3% and 86.7% respectively were pathogenic bacteria from heart blood and lungs tissue (Table 1), such high incidence of isolation was also reported by ***Barbour et al. (1997) and Aba-Alkhail and El-Naenaeey (2003)***.

Due to respiratory defence mechanism depressed due to immunosuppression associated with virus ***(MaxIrisk, 2010)***. Prevalence of pneumonia among slaughtered cattle had the highest number of bacteria ***(Adamu, and Ameh, 2007)***.

Results in table (2) indicate high percentage of isolates refer single infection in heart blood and lung tissue were 81.2% and 78.5% respectively, while mixed isolates in heart blood and lungs tissue were 18.8% and 21.5% respectively. Nearly results agreement with ***Genedy et al., (2008)***.

There are several bacteria capable of inducing pneumonia, but the most common organisms are *Pasteurella multocida*, *Pasteurella hemolytica*, and *Hemophilus somnus* ***(William and Rebhun, 1982)***.

Tables (3 & 4) showed bacteria isolated *E. coli* from heart blood and pneumonic lung were 7.7% and 21.6% or mixed with *Pasteurella multocida serotype A* were 46.7% and 53.6% respectively. These results reported by ***Quinn et al. (1994); Sayed (1996) and Sedeek and Thabat (2001)***; also isolated *P. multocida* serotype A from heart blood and pneumonic lungs were 53.8% and 39.2% or mixed with *Mannheimia haemolytica* serotype A were 53.3% and 46.4% respectively. At the same time isolated *Mannheimia haemolytica* serotype A as pure colonies from heart blood and pneumonic lungs were 38.5% and 39.2% respectively. These results were in agreement with ***El-Sangary et al. (2008)*** and ***Genedy et al. (2008)***.

Antibiotic treatment of bacterial pneumonia must be sufficient in duration and, most crucially, early enough to prevent lesions forming that may resist both therapy and regeneration of normal lung parenchyma ***(Ingrid Lorenz et al., 2011).***

In table (5), sensitivity test of the antibiotic was carried on 3 different types of bacterial isolates which represent the main causative agents of respiratory manifestation in slaughtered calves, the most of the bacterial isolates were highly sensitive to Enrofloxacin and florofincol and less sensitive to Erythromycin, the results the previous findings of ***Roberson, et al. (1994) and Singer et al. (1998)***.

Obtained result recorded in table (6) showed that 10% of samples had count ranging from $10^6 – 10^8$, while most samples showed count ranging from $10^8 – 10^{11}$. These results are nearly similar to that obtained by ***Vanderline et al. (1999) and Paulsen et al. (2006)***.

In conclusion; stress factors as bad hygiene and rise in environmental temperature and increase in

humidity in Egypt lead to activation non pathogenic bacteria which are normal inhabitant of the upper respiratory tract, increase in number and become pathogenic and cause respiratory manifestation characterized by bronchopneumonia which is fibrinous and pleurisy and death occurs due to toxaemia and anoxia.

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