Animal Health Research Institute
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SOME STUDIES ON BACTERIAL CAUSES OF PNEUMONIA IN CATTLE IN ASSIUT GOVERNORATE

(With 4 Tables)

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(Received at 24/6/2001)

بعض الدراسات عن الأسباب البكتيرية للالتهابات الرئوية في الأبقار في محافظة أسيوط

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لقد نفذت هذه الدراسة على ٨٢ عينة , ٦٠ عينة عبارة عن مسحات أنفية من أبقار تعانى من أعراض التهاب رئوي و٢٢ عينة من رئة حيوان مذبوحة اضطراريا وبها أعراض التهابات رئوية في بعض المزارع الحكومية وأماكن متفرقة في محافظة أسيوط. وقد زرعت هذه العينات على أوساط غذائية مختلفة للعزل البكتريولوجى وقد أوضحت نتائج زرع هذه العينات على الحصول على ٩٢ عترة بكتيرية منهم ٧٢ من عينات المسحات الأنفية و٢٠ عترة من عينات الرئة وكانوا كالتالي: الميكروب العنقودي الذهبي ١٨ عزلة بنسبة ٢٥% ,٦(٣٠%) وميكروب الكلبسيلا نيمونى ١٠ (١٣.٩٥%) , ٢ (١٠.٠%) وميكروب السيدوموناس الصديدي ٨ (١١.١٠%) , (صفر%) وميكروب المتكور السبحى الصديدي ٨ (١٠,١٠%) , ٣ (١٥%) والميكروب العصوى القولونى ٧ (٩.٧%) , ١ (٥%) والميكروب المتكور السبحى نيمونى ٦ (٨,٣%) , ٤ (٢٠%) وميكروب الباستيرلا مالتوسيدا ٦ (٨.٣٠%) ,(صفر %) وميكروب الميكروكوكس ليوتس ٤ (٥.٦%) , ٢ (١٠%) وميكروب بروتيس فولجاريس ٢ (٢.٨%) , (صفر%) وميكروب الميكروكوكس فاريتز ١ (١.٤%) , ٢(١٠%) وميكروب الستروباكتر ١ (١.٤%) , (صفر%) وميكروب سارسينيا ١ (١.٤%) , (صفر%). ولقد أجريت اختبارات الحساسية للميكروبات المعزولة وذلك لمعرفة المضادات الحيوية الاكثر فعالية ولقد وجد أن الميكروبات المعزولة كانت شديدة الحساسية لكل من السبكتراما وكانت مقاومة لكل من البنسلين والاوكسىتتراسيكلين وكذلك السيفالوكسين.

SUMMARY

This study was performed on 82 samples, 60 of them were from nasal swabs of cattle suffering from clinical signs of pneumonia and 22 samples were from pneumonic lung from emergancy salughterd cattle in some governmental farms and different localities at Assiut Governorate. The samples were cultivated on different media for bacterilogical isolation. The number of isolates were 92 isolates, nasal isolates were 72 isolates and lung isolates were 20. The isolates represented by *Staph. aureus* 18 (25%) and 6 (30%), *Kelbseila pneumoniae* 10 (13. 90%) and 2 (10.0%), *Pseudomonas aeruginosa* 8 (11.10%) and (0.0%), *Strept. pyogenes* 8 (11.10%) and 3 (15.0%), *E. coli* 7 (9.7%) and 1 (5.0%), *Strept. pneumoniae* 6 (8.30%) and 4 (20%), *Past. multocida* 6 (8.30%) and (0.0%), *Microluteus* 4 (5.6%) and 2 (10%) *Proteus vulgaris* 2 (2.8%) and (0.0%), *Micrococcus varians* 1 (1.4%) and 2 (10.0%),*Citrobacter spp.* 1 (1.4%) and (0.0%) and *Sarcina* spp. 1 (1.4%) and (0.0%) and they represent the main causative agents affect the nasal cavity and lung of infected cattle respectively. Also these bacterial isolates were highly sensitive to Spectrama and resistant to Penicillin, Oxytetacycline and Cephaloxin.

Key Words: *Pneumonia, Cattle.*

INTRODUCTION

Respiratory system affections are considered one of the most serious problems which affect animals in all ages as well as the widest spread all over the world consequently the cause great economic losses in dairy farm animals (cattle).

In studies on bacterial causes of respiratory infection of dairy cattle Abdel-Kader (1992) isolated *Staph.aureus, Streptococcus pyogenes, Sterpt. pneumniae, Corynebacterium pyogenes, Pasteurella multocida, E. coli, Citrobacter spp., Kelbsiella pneumoniae and Salmonella spp.* Also in other investigations Lim, et al. (1995) mentioned that *Kelbsiella pneumoniae, Pseudomonas aeruginosa, E. coli* and *Pasteurella multocida* were the most prevalent cause of lung infection in bovine animals.

Barbour, et al (1997) mentioned that 18 out of 28 (64.3%) of identified bacterial species in upper respiratory tract of Halstein cattle

were more prevalent in the nasal cavity with respiratory signs than apparently healthy animals. Also they added that the isolated bacteria were *corynebacterium pyogenes, Erysipelothrix spp.; Pasteurella sp.. Staph aureus, Staph epidermidis* and *Pseudomonas aeruginosa.*

Dabo, et al. (1999) and Derosa, et al. (2000) isolated 81 isolates of *Pasteurella multocida* and *E. coil* from nasal cavity of healthy and diseased Holstein dairy cattle from various geographical location of USA.

Sortz, et al. (2000) reported that (RBCV) respiratory bovine corona virus may play a role in outbreak of shipping fever in cattle and spreading infection of *Pasteurella* spp. among cattle and added that they could isolated *Pasteurella* spp. from 10 cattle suffering from respiratory infection.

The aim of this work was to detect the main bacteriological causes of an out break of cattle pneumonia in Assiut Governorate and determine the antibiogram of isolated bacteria to reach an avialable and specific treatment.

MATERIAL and METHODS

Materials

A total of 60 nasal swabs from cattle suffering from signs of pneumonia and 22 samples of pneumonic lung were collected from 42 cases of Bani Mor Freizian farm and 15 cases from Agriculture Secondary School Farm and 25 fom native breed of different localities of Assiut Governorate centers during the four months of winter of (2000-2001). The age of animals ranged from 2-6 years.

Methods

The nasal swabs and lesion part from infected pneumonic lung were inoculated into nutrient broth and incubated at 37°C for 24 hours and then the broth was cultured and used for further bacteriological investigation by inoculation in nutrient agar, blood agar and McConkey agar media. The inoculated plates were incubated at 37°C for 24-48 hours.

Identification of isolated bacteria depending upon culture character, pigment production and microbial examination of Gram stained smears of colonies according to Baily and Scott (1974); Cruickshank et al. (1975): Carter (1984) and Wilson and Miles (1984).

Antibiotic sensitivity test for bacterial isolates was done by the diffusion method using antibiotic discs, Spectrama (10µg), Kanamycin (30µg), Chloramphenicol (30µg), Lincomycin (20µg), Tetracycline (30µg), Penicillin (10 IU), Oxytetracycline (30µg) and Cephaloxin (30µg).

RESULTS

The main clinical signs of infected cattle were rise of body temperarure (39.5-40 °C), depression, increased eye and nasal discharge, loss of appetite, acceleration of respiration (40-50/min.) and congestion of ocular mucous membrane.

By auscultation, vesicular sounds, moist rales were evident with frictional sounds in some cases. The post-mortum findings included congestion, red hepatization and grey hepatization. The positive samples, number of isolates, main clinical signs, main causative agent and antibiogram of the bacterial isolates were demonstrated in tables 1,2,3 and 4.

DISCUSSION

Respiratory disease in cattle particular pneumonia is the result of interaction of more infectious agents under the influence of physical stress (Martin, 1983). The recorded results revealed that the main clinical signs of infected animals were rise in body temperature (39-40°C), accleration of respiration (40-50/min), abdominal respiration, ear drop, increased eye and nasal discharge, congestion of ocular mucous membrane and abnormal sounds on lung auscultation. The post mortem findings of lung infection were varied from severe congestion, reddish greyish exudate within the bronchi to red hepatization (Table 1). These findings are in agreement with Thompson, et al. (1998).

The recorded results in table (2) and (3) revealed that the positive culture sample of nasal swabs were 95%, infected lungs were 95.23%, and the main causative bacterial agents of nasal cavity and lung infection of dairy cattle were *Staph. aureus* 8 (25%), and 6 (30.0%), *Klebsiella pneumoniae* 10 (13.90%) and 2 (10.0%), *Pseudomonas aeruginosa* 8 (11.10%) and (0.0%), *Strpt. pyogenes* 8 (11.10%) and 3 (15.0%), *E.coli* 7 (9.7%) and 1 (5.5%), *Strep.pneumoniae* 6 (8.30%) and 4 (20.00%), *Past.multocida* 6 (8.30%) and (0.0%), *Micro.luteus* 4 (5.6%) and 2

(10.00%), *Proteus vulgaris* 2 (2.8%) and (0.0%), *Micrococcus varians* 1 (1.4%) and 2 (10.0%), *Citrobacter* spp. 1 (1.4%) and *Sarcina* spp. 1 (1.4%) respectively (Table 3). Also (78.3%) of these bacterial isolates were isolated from nasal cavity of infected animals and (21.70%) of the isolates from the lung of affected animals (Table 1).

In refereing to bacterial isolates from nasal cavity our obtained results were in agreement with Abd El-Kader (1992) who isolated *Strept.pneumoniae* (12.85%) and *E.coli* (10.70%) from nasal cavity of infected cattle. On other hand he isolated *Past.multocida* and *Citrobacter* in higher incidence (17.35%) and (3.57%) and *Strept. pyogenes* (7.14%), *Staph.aureus* (7.14%) and *Klebseilla pneumoniae* (3.57%) in a lower incidence.

In another study of upper and lower respiratory tract infection of dairy cattle, Thomas et al. (1980) mentioned that the most prevalent microorganisms isolated from nasal cavity of native bovine, Holstein cattle and dairy calves were *Staph aureus, Strept.*spp., *Pseudmonas aeruginosa, E. coli* and *Pasteurella* spp. Those recorded results in general agree to large extent to these recorded in our study.

Our obtained results partialy in accordance with the results which obtained by Lim et al. (1995), Wills et al. (1995) and Barbour et al. (1997) who isolated *Klebseilla pneumoniae, Past. multocida, Pseudomonas aeruginosa, Staph aureus* and *Strep. pneumoniae* from upper respiratory tract of dairy calves, the authors mentioned that those bacterial isolats were most prevalent causative agent of upper respiratory infection. Also Barbour et al. (1997) and Fatma et al. (2001) added that 64% of bacterial isolates more prevalent in nasal cavity than lung infection, this percentage more lower than that obtained in our study, it may be due to bad hygienic measures (over crowded and long house standing) and nutritional deficiencies in farms which samples were collected.

In a bacteriological study Wills et al. (1995) isolated *Pseudomonas aeruginosa* in a higher incidence (15%) from nasal cavity of human and bovine animal than that recorded (11.10%) in our study, while Thabet (1993) and Sayed (1996) isolated *Pseudomonas aeruginosa* with a lower incidence (8.3%) and (6.2%) from nasal cavity of infected animals. The high level of *Pseudomonas aeruginosa* in our study may be due to this organism is known as environmental pathogen frequently encountered in upper respiratory tract. (Quinn, et al. 1994).

In our results *Past.multocida* was isolated in an incidence (8.3%) from nasal swabs of infected cattle, this is nearly similar to that obtained by Kim et al. (1973) who isolated *Past. multocida* in an incidence (7.78%) from cattle with respiratory infection, while Abd El-Kader (1992) and Smiko and Lehocky (1993) isolated *Past. multocida* from nasal cavity of infected cattle in a higher incidence (17.85%) and (14.1%), the higher incidence of *Past.multocida* may be attributed to hygienic measures, changes in managment, stress factors, immune defensive mechanism and seasonal variation, such openion was supported by Wary and Thampson (1973) who mentioned that *Past.multocida* was more prevalent between 2 months Februrary and April and more prodomonant prevalence due to change in management during a period of 6-9 th of the year.

In an out break of enzootic bronchopneumonia, El-Sabaie, et al. (1984) isolated a virulent strain of *Past.multocida* from nasal cavity of affected cattle, also Dabo et al. (1999) and Derosa et al. (2000) isolated *Past.multocida* and *E. coli*. from nasal swabs of infected Holestein cattle and dairy cross breed beef cattle.

Rontaved et al. (2000) and Fulton et al. (2000) found that *Past.multocida* more virulent and prevalence among cattle with certain respiratory disease virus infection as reovirus herpes virus (BH V1 and BH V2), BVDV and (BRSV) bovine respiratory syncytial virus. In other investigation, Thomson et al. (1964) observed a higher frequency of *Past.* spp. in the nasal flora of cattle. The authors suggested that there may be a relationship between the high number of microorganisms isolated from the nasal passages and infection with these organisms in the lower respiratory tract particularl in the lung, this suggestion supported our investigation results where no isolates of *Past.multocida* from lung and the frequency of *Past multocida.* isolates were from nasal swabs of infected cattle.

In concerning to lung infection Hordagoda et al. (1980) and Thomas et al (1981) in performed bacteriological studies in normal; and pneumonic lung of animals in Srilanka found that the prodominant bacterial isolates were *Strept.* spp., *E. coli* and *Micrococcus luteus,* this performed studies were partialy in agreement to the results in Table (3).

The revealed results in this investigation showed that *E.coli* and *strept.pyogenes* were isolated in an incidence (9.70%) and (11.10%) which were in agreement to that obtained by Abd El-Kader (1992) who isolated *E.coli* and *Strept. pyogenes* from pnumonic lung of cattle in an

incidence (8.69%) and (13.04%), the same author isolated *Past.multocida* and *strept.pneumoniae* in a higher incidence (13.04) and (39.13%) while he isolated *Staph.aureus* in a lower incidence (13.39%). The variation in isolation percentage may be attributed to bad hygienic measures, change in managment, seasonal variations and immune status of infected cattle (Allan et al., 1985 and Tegtmeiere et al., 1999).

Smiko and Lehocky (1993) found that the main causative bacterial agents of calves died from respiratory infection were *Strept. pneumoniae* in an incidence (18.7%) nearly similar to the results obtained in our study, on the other hand the same authors isolated *E. coli* (7.9%) and *Staph.aureus* (13.30%) in lower incidence than that recorded in this investigation. Pboan et al. (1999) isolated *Staph.* spp. and *Strept.* spp. with lower incidence (7%) and (12%) in contrast to our results.

Lim et al. (1995) mentioned that the main prevalent Gram negative bacteria of lung infections of native bovine were *E.coli* and *Klebsiella pneumoniae*, these support our obtained results in this investigation. Also Abd El-Kader (1992) isolated *E. coli* and *Klebsiella pneumoniae* from pneumonic lung of infected cattle but he failed to isolate *Pseudomonas aeruginos* from infected lung, such results are in agreement with ours.

The high incidence of *Staph. aureus* in nasal cavity and infected lung (25%) and (30%) in our study may be due to *Staph.aureus* has wide spreading among all seasonal year (Roberson, et al. 1994). Also the difference of bacterial isolates in this investigation in contrast to that reported by some authors attributed to management change and immune defensive mechanism of infected animals (Allan et al., 1985 and Tegtmeiere et al., 1999).

Mixed infection in this investigation occurred in 12 samples, as showed in table (2) double mixed infection in 9 samples and 3 mixed infection in 3 samples, the mixed infection mainly *Staph.aureus* with *E.coli, Klebseilla pneumoniae* and *Pseudomonas aeruginosa,* where *E.coli, Pseudomonas aeruginosa* and *Klebseilla pneumoniae* known as environmental pathogenic microorganisms and they are frequently encountered in both upper and lower respiratory tract specially in animal housed at bad hygienic condition (Quinn, et al., 1994 and Sayed, 1996).

The presence of mixed infection mainly *Staph.aureus* with *E. coli* and other organisms demonstrate the complexity of the disease

where the *Staph.aureus* may predispose the dairy herd to infection by coliform organisms or other pathogens (Roberson et al. 1994)

Concerning to antibiotic sensitivity the study of antibiogram of isolated bacterial species for the antibiotic of choice of proper treatment. In this investigation, most of the bacterial isolates were highly sensitive to Gentamycin, and Spectrama, and slightly sensitive to Kanamycin and resistant to lincomycin, Chloramphenicol, Tetracyline, Cephlaoxin, Oxytetracycline and pencillin. These recorded results agree to that obtained by Thabet (1993) and Sayed (1996).

CONCLUSION

From this study it can be concluded that the correct diagnosis, isolation and identification of microorganisms and the suitable treatment with effcient hygienic measures are essential to reduce the infection of cattle and limits the human infection that consuming the infected lung of emergancy and slaughtered cattle.

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Table 1: Relationship between isolated microorganisms and clinical signs and post-mortem findings of infected cattle

Types and No. of examined cattle	Age	Locality	Type of samples	Main clinical signs and post-mortem findings of infected cattle	Main isolated organisms
A-Friesian cattle (57) B-Native breed attle (25)	2-6 years	1-Friesian Bani Mor (42) 2-Agri. Culture Sec. School (15) different villages	60 nasal swabs of infected cattle with signs of pneumonia and 22 samples of pnumonic lung	Rise of body temperature (39-40 °C), increased eye and nasal discharge, acceleration of respiration and abdominal respiration, by auscultation vesicular sounds, moist ral;es with frictional sound. The pneumonic lung varies from severe congestion, red hepatization and grey hepatization	*Staph.aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Strept. pyogenes, E. coli, Strept. pneumoniae, Past.multocida* and *Micrococcus luteus.* *Staph.aureus. Klebsiella pneumoniae, Strept.pyogenes, E. coli, Strept. pneumoniae, Micrococcus luteus and Micrococcus varians*

Table (2): The percentage of infection in cattle suffering from pneumonia

Type of samples	Total Samples	positive Samples		Samples with single infection		Samples with mixed infection		isolated strains	
		No	%	No	%	No	%	No.	%
Nasal swabs	60	57	95	49	85.96	*8	14.03	72	78.30
Infected lungs	22	20	95.23	16	80.0	**4	20.0	20	21.70
Total	82	77	96.34	65	84.41	12	15.58	92	100

* *2 Staph.aureus + Strept. pyogenes + Ps. aeruginosa*
1 Staph. aureus + Ps. aeruginosa + Micro varians
3 Staph.aureus + Ps. aeruginosa
2 Ps. aeruginosa + E.coli

** *2 Staph.aureus + Klebseilla*
2 Staph. aureus + E. coli

Table (3): Isolated microorganisms from nasal swabs and infected lungs of cattle

Isolated Microorganisms	Nasal swabs 60		Infected lungs 22	
	No.	%	No.	%
Staph.aureus	18	25	6	30.0
Klebsiella pneumoniae	10	13.90	2	10.0
Pseudomonas aeruginosa	8	11.10	-	-
Stept. Pyogenes	8	11.10	3	15.0
E. coli	7	9.70	1	5.0
Strept. Pneumoniae	6	8.30	4	20.0
Pasteurella multocida	6	8.30	-	-
Micrococcus luteus	4	5.6	2	10.0
Proteus vulgaris	2	2.8	-	-
Micrococcus varians	1	1.4	2	10.0
Citrobacter	1	1.4	-	-
Sarcina Spp.	1	1.4	-	-
Total	72	100	20	100

Table 4: Antibiogram of isolated bacteria from naal swabs and infected lung of cattle

Microorganisms	ENR (10μg)	K (20 μg)	L (10μg)	C (10μg)	CN (30 μg)	Ot (30 μg)	TE (30 μg)	P (10 IU)
Staph.aureus	+++	++	-	-	-	+	+	-
Klebsiella pneumoniae	+++	++	-	-	-	-	-	-
Pseudomonas aeruginosa	+++	++	-	-	-	-	-	-
Stept. Pyogenes	+++	++	-	-	-	-	-	-
E. coli	+++	+	+	+	-	+	-	-
Strept. Pneumoniae	+++	++	-	+	-	+	-	-
Pasteurella multocida	+++	+	-	+	-	+	-	-
Micrococcus luteus	+++	+	-	-	-	-	-	-
Proteus vulgaris	+++	++	-	-	-	-	-	-
Micrococcus varians	++	++	-	-	+	-	-	-
Citrobacter	+++	++	-	-	-	-	-	-
***Sarcina* Spp.**	+++	++	-	-	-	-	-	-

ENR : Spectrama K: Kanamycin L: Lincomycin C: Chloramephenicol CN: Cephaloxin Ot: Oxytetracycline

TE : Tetracycline P: Penicillin