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CONTRIBUTION ON THE BACTERIAL CAUSES OF LUNG SEPTIC FOCCI IN SHEEP IN GHARBIA PROVIENCE

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ABSTRACT

This study was carried out on 120 sheep clinically have respiratory disorder and were slaughtered in different abattoirs and private farms in Gharbia governorate.Samples were taken from nasal discharges,tracheal swabs, lung tissue and lung abscess. Bacteriological examination revealed that 103,36, 56 and 19 positive for bacterial growth with percentages (85.83%) (30.0%), (46.66%) and (51.83%) respectively from previously affected sites of sampels. In total out of 259 bacterial isolates. Concerning G+ve bacteria, *Staphylococcus aureus* was 51 (19.6%),*Streptococcus pneumonae* 14 (5.4%), *Corynebacterium pyogens* 9 (3.5%), *Streptococcus pyogens 8 (3.1%), Micrococcus luteus 5 (1.9%), Staphylococcus epidermidis 4 (1.5%), Enterococcus fecalis 3 (1.1%) and Streptococcus durans.* 2 (0.8%) while the G-ve bacteria were identefied as *E.coli* 63 (24.3*%), Pasteurella multocida 43 (16.6%), Klbsiella pneumonae 25 (9.7%), Mannheimia heamolytica 13 (5.1%), Proteus.vulgaris 9 (3.4%), Pseudomonos aeruginosa 5 (1.9%), Citrobacter.spps 11 (1.5%) and Enterobacter aerogens. 1 (0.4%)*,some samples give 38 mixed infection with a percentage of (7.91%) .the mixed organisms were 8 (15%), 5 (1.1%) 9 (7.5%) and 6 (5%) respectively from the different examined collected sampels.The serological identification of *Pasteurella.multocida* revealed that 27 (62.6%) were had capsular serotyped A, out of 43 samples +ve of *Past. multocida.* The Pathogenicity test for recovered *Past. multocida* isolates in mice indicated that most isolates were pathogenic (60 – 100%). Sensitivity test revealed that the most isolates were highly sensitive to Enrofloxacin, and Gentamicin, and were resistant to Streptomycin and Ampicillin. (Key words : Bacteria – lung septic focci – antibiotics)

INTRODUCTION

Sheep play a vital socioeconomic role and support the survival of thousands of people in our country, it is used as a source of meat, milk, wool as well as quite and effective means of money.Respiratory disorder is still serious problem facing sheep rearing.(**Hatem ;et.al.2003**) The main causes of pneumonia are bacteria, fungi and viruses where poor hygienic measurement and climatic disorders are the most various predisponing factors to infection.(**Radwan et al. 2002**). Commencal bacteria were isolated with a percentage (20%) from clinically healthy sheep (**Ibrahim and Mokhtar 2003**) .also

(**Abdel.latif and El-dossouky.,2006**) isolated ***Pasteurella multocida (5.71%),E.coli (2.86%),Klbsiella pneumoniae (2.86%) and Pseudomonas aeruginosa (5.71%)*** from clinically healthy living lambs. Moreover (**Elyas.1993**) recovered ***Staphylococcus aureus(26%) ,E.coli(16%) and Pasteurella multocida(3%)*** from clinically normal lambs. ***Mannheimia heamolytica*** were culturelly isolated from the nasal secretion and lung of diseased pneumonic sheep (**Quinn et al., 1994**). Both ***Mannheimia hemolytica*** and ***Pasteurella multocida*** were associated with pneumonia in sheep and goats **(Davies ; 1985).**Moreover**, (Radwan, et al., 2002)** reported that ***Pasteurella multocida*** was the most important cause of pneumonia in goats. Also,**(Elyas. 1993)** recovered from pneumonic lung of sheep and goat ***Mannheimia heamolytica***, ***E. coli and Klebsiella pneumonia***. The clinical course of ***Klebsiella pneumonia*** infection is usually rapid with lung tissue necrosis and frequent formation of abscess **(Reed, 1973*). Pasteurella spps. Klebsiella spps. Pseudomonas spps.*** and ***Staphylococcus aureus*** are the most bacterial cause of sheep pneumonia **(Martin. 1996)**.He added that these organisms are indogenous to the upper respiratory tract, the female genital tract and the gastrointestinal tract whereas infection with these organisms usually result from spread of the organisms outside their normal habit. Purulent pneumonia also caused by ***Mannheimia heamolytica*** and ***Staphylcoccus aureus*** with abscess formation **(Ibrhaim and Mokhtar., 2003)**.

The aim of this study was directed mainly to: • evaluate the role played by pathogenic bacteria either single or mixed infection on affected respiratory organs, and that extend and cause lung septic focci in sheep .• Determine the sensitivity of the isolated bacteria to different drugs to aid in the choise of drug.

MATERIAL & METHODS

Material:

1- Samples:

A total of 480 samples including 120 each of nasal swabs, tracheal swabs, lung tissue and lung abscesses were collected under septic condition from 120 clinically diseased sheep suffered from respiratory disorders. Manifested by rapid breathing, moist rales, congested mucous membranes, mucopurulent nasal discharges, severe dyspenea and pyrexia (Rectal. Temp 40-41^{o}C) (**Quinn et al., 1994**). Samples from both males and females 1-2 years old which were ready for slaughter at abattoirs and some private farms at Gharbia governorate during the period from May 2007 till April 2008 were collected.

2- Media:

The following media were used according to (**Finegold and Martin. 1982**).: Nutrient agar, Blood agar, Tryptose soya agar, MacCkonkey agar, Mannitol salt agar , Dextrose starch agar, Nutrient broth, Brain heart infusion broth, Mullar Hinton broth, Mullar Hinton agar.and were obtained from(Oxoid.Ltd) **Antibiotic sensitivity test** (Amoxycillin(10ug),Ampicillin(10ug)

Cephaloxin(10mg).Enrofloxacin (10 mg) Erythromycin(10 mg), Gentamycin(10 ug) Oxytetracyclin (30 ug)Pencillin(10 ug)and Streptomycin (15 ug) were used according to the instruction of the manufactures on random isolates from each species and obtaind from(Oxoid.Ltd).

Stains:- gram stain –gemsa stain (**Finegold and Martin. 1982**)

Methods:

1. Collection of samples:

1.1 Nasal swabs**:** were collected from clinical cases before slaughter, the cotton swabs, were pushed as for as possible into one nostril then transferred to sterile nutrient broth in tubes (**woldehiwt. et al., 1990**)

1.2. Tracheal swabs: were collected from slaughtered sheep then trasnfered as before.

1.3 Lung tissues and abscesses from slaughtered sheep.

The sterilization of trachea, lung tissues and abscesses were done by flamed spatula and cut ,take swabs or apiece of lung or abscess to culture into peptone water. The samples were taken under aseptic condition and sent without delay to Tanta. Vet. Lab for bacteriological examination. All media Previously melted and cooled till 40^{o}c inoculated and control plates were incubated at 37^{o}c for 24- 48 hours **(Cruickshank. et al., 1975).**

2- Isolation and identification of the isolates according to (**Quinn et al., 1994**):

All previously collected samples were inoculated into the following solid media: nutrient agar, MacCkonky agar, Mannitol salt agar 7.5% , tryptose soya agar and Dextrose starch agar. Incubated aerobically at 37^{o}C for 24-48 hours, the isolated colonies were streaked on new sterile pure culture of nutrient agar and subcultured on slope agar for further identification by study the culture characters, pigment production. staining reaction and cell morphology as well as aggregation, The biochemical activities of pure isolates were carried out according to (**Finegold and Martin. 1982**).

3-Serological identification :

Standard ***Pasteurella multocida*** capsular typing antisera were obtained from National animal Disease center (NADC), Ames, Iowa, U.S.A through Aerobic Bacterial vaccine Dept. Vet. Serum vaccine Research Institute, Abassia, Cairo, Egypt.

Rapid plate aggluation test (RPA) was used for capsular serotyping of Pasteurella isolates **(Fricker,et al., 1986).**

4- Pathogenicity and virulence of the isolated *Pasterulla multocida*; was done according to **(Quinn.et .al, 1994**), where 40 mice was injected with the tested isolates interaperitoneally by 0.1 ml of bacterial suspension (1.5x10^{8}) C.F.U /ml.All dead mice were examined for post mortum changes, and reisolation of inoculated isolates from heart blood. Also a film from heart blood was prepared and dried with methyle alcohol and stained with Gemsa stain for showing characteristics features of ***Pasteurella multoacida*** organisms (bipolarity and capsules). Mice of the control group were inoculated with 0.1 ml normal broth for each animal.

5- Sensitivity of microorganims to antibiotics: was determined by using Muller Hinton agar plates and standard disc technique according to (**Quinn.et .al, 1994**).

Table (1) Incidence of positive bacterial samples from different parts from Resp. tract of diseased sheep

Number of examined samples	Site of collection	Result of examined samples				Positive samples			
		+ve		-ve		single		mixed	
120 (53)*+(67)** from each infected part		No.	%	No.	%	No.	%	No.	%
	Nasal swabs	40* 63**	33.33 52.50	13 4	10.83 3.33	35 50	29.16 41.67	5 13	4.17 10.83
	total	103	85.83	17	14.16	85	70.83	18	15.00
	Tracheal swabs	14* 22**	11.67 18.33	39 45	32.50 37.50	13 18	18.83 15.00	1 4	0.83 3.33
	total	36	30.00	84	70.00	31	25.83	5	4.16
	Affected lung tissues	11* 54**	9.16 37.50	42 22	35.00 18.33	9 38	7.50 31.66	2 7	1.67 5.83
	total	56	46.66	64	53.33	47	39.16	9	7.50
	Lung septic focci	7* 12**	50.83 10.00	45 55	38.33 45.83	5 8	4.16 6.67	2 4	1.67 3.33
	total	19	15.83	101	84.16	13	10.83	6	5.00
	Cummulative totals	72* 142**	15.00 29.58	140 126	29.17 26.25	63 114	12.92 23.75	9 29	1.87 6.04
	480	214	44.58	266	55.42	176	36.76	38	7.91(%)

*Samples obtained from Balady sheep (osemi-rhmany).
** Samples obtained from imported sheep (swanky breed).
% according to number of examined diseased sheep (120) .
(%) according to cumulative total examined samples (480) .

Table (2): Types and Incidence of bacterial isolates from different parts from Resp. tract of diseased sheep .

Types of isolates / Sources of isolates	Nasal swabs		Tracheal swabs		Affected Lung tissues		Lung septic focci		Totals	
	No	%	No	%	No	%	No	%	No	%
G + ve organisms										
Micrococcus. Luteus	5	4.1							5	1.9
Staphylacoccus. aureus	22	17.9	8	18.6	16	24.2	5	18.5	51	19.6
Staphylacoccus. epidermidis	3	2.4	1	2.3					4	1.5
Streptococcus. pneumonia	6	4.9	4	9.3	4	6.1			14	5.4
Enterococcus fecalis	3	2.4							3	1.1
Streptococcus. durans	2	1.6							2	0.8
Streptococcus. pyogenes	2	1.6	1	2.3	3	4.5	2	7.4	8	3.1
Coryne-bacterium. pyogenes	2	1.6	1	2.3	5	7.6	1	3.7	9	3.5
Totals	45	36.6	15	34.8	28	42.4	8	29.6	96	37.1
G-ve organisms										
E-coli.	26	21.1	10	23.3	19	28.8	8	29.6	63	24.3
Klbsiella. pneumonae	14	11.4	4	9.3	5	7.6	2	7.4	25	9.7
Proteus. Vulgaris	4	3.2	5	11.6					9	3.4
Mannheimia heamolytica	7	5.7	2	4.7	3	4.5	1	3.7	13	5.1
Pasteurella. multocida	19	15.4	7	16.3	10	15.2	7	25.9	43	16.6
Citrobacter. spps	4	3.3							4	1.5
Entrobacter. aerogenes						1.5			1	0.4
Pseudomonas. aeruginosa	4	3.3					1	3.7	5	1.9
Total	78	63.4	28	65.2	38	57.6	19	70.4	163	62.9
Cummulative Totals	123	100	43	100	66	100	27	100	259	100

% according to total +ve number of bacterial isolates in each affected part .

Table (3): Types of mixed bact. Isolates

Source of samples		*gp. of mixed isolates*	No.	%
Nasal swabs		*Past.mult+E.coli*	4	8.3
		-Past.mult+Staph.aureus	3	18.5
		-Staph.aureus+E.coli	3	12.5
		Klbs.pn+E.coli+Staph.aureus	2	1.7
		Klbs.pn+Strept. pn	3	2.5
		- Strept . pyogenes + E.coli	2	1.7
		-Enterococcus.fecalis+ Staph.aureus	1	0.8
Total			18	15
Tracheal swabs		*-Past.mult+E.coli*	1	0.8
		-Past.mult+Staph.aureus	1	0.8
		- E.coli+Proteus. vulgaris……..	1	0.8
		E.coli+Staph.aureus+past.mult	1	0.8
		E.coli+Staph.aures+Coryn.pyogenes	1	0.8
Total			5	4.1
Affected Tissuse		*-Past.mult+E.coli*	3	2.5
		E.coli+Staph.aureus.	3	2.5
		-Mannheimia.Heamolytica.+E.coli+ Staph.aureus…..	1	0.8
		Staph.aureus+Strept.pyogen	1	0.8
		-Klbs.pn+Coryn.pyogenes	1	0.8
Total			9	7.5
Lung septic focci	Single focci	*Past.mult+E.coli……*	1	0.8
		-E.coli+Staph.aureus.	1	0.8
	Total		2	1.6
	Multiple focci	*- E. coli + Past. mult + Klbs.pn*	1	0.8
		-Staph.aureus+Past. mult	1	0.8
		-E.coli+Strept .pyogenes……..	1	0.8
		- E.coli + Past .mult+ S.aureus……	1	0.8
	Total		4	3.2
Cummulative totals			38	7.91*

%according to total number of + ve isolates in each part .

*%according to cumulative total of isolates.

Strept: Streptococcus -Staph: Staphylococcus - Klbs: klbsiella - Coryn: Corynebacterium

Past.mult: Pasteurella multocida.

Table(4): Capsular typing of *Past.multocida* recovered from different parts of resp. tract of diseased sheep .

Source of samples	**Pasteurella multocida**			
	No	+ve A	%	untyped
Nasal swabs	19	13	30.2	6
Tracheal swabs	7	5	11.6	2
Lung Tissues	10	6	13.9	4
Lung Septic focci	7	3	6.9	4
Total	43	27	62.6	16

+A: serogroup A

% according to +ve isolate of ***Pasteurella.multocida***.

Table (5): Pathogenicity of isolated *P.multocida* (serogroup A) in mice

Types of isolate	**No. of isolates**	**No Of inoculated mice**	**Less than 24 h**	**24 h**	**48h**	**72 h**	**No of survived**	**No. of dead /no. of inoculated**	**Mortality rate %**
Nasal swabs	5	10	-	1	2	3	4	6/10	60
Tracheal swabs	4	10	-	1	3	3	3	7/10	70
Lung Tissue	4	10	-	2	3	3	2	8/10	80
Lung septic focci	2	10	1	2	4	3	0	10/10	100
Control	-	10	-	-	-	-	10	0/10	-

Table (6): Result of sensitivity of the tested isolates to antibiotic.

Bacterial isolates / Antibiotic	Amoxy cillin	Ampicillin	Cephaloxin	Enrofloxacin	Erythromycin	Gentanicin	Oxytetracyline	Pencillin	Streptomycin
Micrococcus luteus	SS	SS	SSS	SS	SSS	SS	SS	S	SS
Staph.aureus	S	SS	SSS	S	SSS	R	S	R	SS
Staph. epidermidis	S	SS	SSS	S	SSS	R	S	R	S
Strept.pneumonia	S	SS	SSS	SS	SSS	R	S	R	S
Enterococcus. fecalis	SS	SS	SSS	S	SSS	R	R	R	S
Strept. durans	SS	SS	SSS	S	SS	R	R	R	S
Strept. pyogens	S	SS	SSS	SS	SSS	R	S	R	SS
Coryn.pyogens	S	S	SS	S	R	R	R	R	R
E-coli	R	SS	SSS	R	SSS	S	S	R	R
Klbsiella. pneumonae	R	S	SS	S	SS	S	R	R	R
Proteus. vulgaris	R	SSS	SSS	S	SSS	S	S	SS	SS
Mannheimia. heamolytica	R	S	SSS	R	SSS	R	R	R	R
Pasteurella. multocida	R	S	SSS	S	SSS	S	SS	S	S
Citrobacter. spp$_s$	S	SS	SSS	S	SSS	R	SS	R	SS
Entrobacter. aerogenes	R	SSS	SSS	S	SS	R	SS	S	S
Pseudomonas. aeruginosa	R	SS	SSS	R	SS	R	R	R	R

R = resistant strain
S = intermediate sensitivity : inhibition gave 12-18 mm
SS = Sensitive : inhibition gave 19-23 mm
SSS = highly sensitive : inhibition gave more than 23 mm
Strept: Streptococcus -Staph: Staphylococcus - - Coryn: Corynebacterium

RESULTS & DISCUSSION

Bacterial nfection of respiratory tract of sheep represent important problems confronting animal production, due to interaction of more infectious agents under the influence of physical stress **(Martin., 1996)**. Also commensal bacteria present in the respiratory system may cause respiratory disease when animals are subjected to stress factors **(Palatoary and Newhall., 1985)**. So the present study deal with the pathogenic bacteria present in the respiratory tract of slaughtered sheep particularly that cause lung septic focci. Bacteriological investigation of the respiratory tract samples (Nasal swabs, tracheal swabs, lung tissues and lung abscesses) revealed that out of a total 480 collected samples from the previously four affected parts 120 from each diseased clinical cases .(Table -1), showed that 103 were positive from the nasal swabs with percentage of (85.83%) and this is higher than **(Ibrahim and Mokhtar; 2003)**. And lower than **(Abdel- latif and EL-Dossouky ., 2006)** while out of 120 tracheal swabs, 36 positive sample were recorded (30%) which was higher than **(Abd el latif and El-Dossukey 2006)** who detected (20%) only from the diseased slaughtered lambs. The results revealed that 56 positive pneumonic lung sample with a percentage of (46.66%) and this was lower than that recorded by **(Hordagoda, et al., 1981)** (89.5%) **, (Kaya and Erganis., 1991)** 75% **,** and **(Radwan et al., 2002)** (73.3%), and higher than **(Hatem, et al., 2003)** which was (20%) . The lower percentage of treacheal affection and the high incidence of lung affections may be due to dysfunction of the lung clearance mechanisms (alveolar macrophage) which facilitates the upper respiratory tract bacterial flora to invade the lung **(Gilk, et al., 1974)**. For lung abscesses or any other septic focci we found 19 positive samples (15.83%) out of 120 clinical cases, which mean that this percentage was partially similar to that recorded by **(Zaiton., 2001)** who mentioned that out of 61 sheep examined 8 cases had lung abscesses with percentage of (13.1%). While it was higher than that recorded by slaughter house documents during 2006-2007 which nearly reached (0.5-1.5%) in Gharbia Vet. Organization, this may be due to that most samples obtained from imported sheep from Sudan and due to the previous reasons of stress factors during transportation, overcrowding, poor ventilation as well as poor hygienic measure and the difference in climate which may enhance potential microbial infection and consequently lung septic focci occur. The bacteriological incidence of pure cultures (in table 2) yield G+ve isolates of a percentage of (37.1%) where ***Staphylococcus aureus*** was the predominant organisms found in all parts of respiratory tract with percentage (19.6%) and this was higher than that isolated by (**Radwan, et.al; 2002)** which was (11.6%) and lower than that recorded by **(Elyas; 1993)** which was (26%) Also, ***Streptococcus pneumonae*** recovered with a percentage of (5.4%) and this was lower than isolated by (**Radwan, et**

al., 2002) which was (10.0%) on the other hand ***Micrococcus luteus, Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus pyogens*** and ***Corynebacterium pyogens*** were isolated with a percentage of 1.9%, 1.1%, 0.8, 3.1% and 3.5% respectively and this partially agree with that mentioned by (**Sayed, 1996)**. The results of the identification of G-ve bacilli were 62.9%. ***E-coli*** was the predominant organism with incidence of (27.3%) and this is higher than **(Elyas,1993)** which was(16%) and partially similar to (**Abd el.latif, and EL-Dessouky., 2006)** which was (22.79%), then ***Pasteurella multocida*** with incidence of (16.6%) and this partially similar to **(Abd el.latif and El-Dessouky, 2006)**which was (17.14%) and lower than **(Viana, et.al, 2007)** who isolated ***Past. multocida*** with a percentage of 27% from naso-pharyngeal swabs from healthy and affected lambs., Also ***klebsiella pneumonae*** percentage was 9.7% which is higher than **(Ansam, 2003)** who isolated it with incidence of (6.9%), and lower than **(Sharma, et. al, 1997)** who recorded the percentage of (12.1%). ***Mannheimia haemolytica*** was (5.1%), sharbly lower to that isolated by **(ELshabiny, et. al; 1996)** was (14.3%) **(Rafek,1998**) (57.1%), and **(Hatem, et. al., 2003)** (25.7%) **.**On the other hand G-ve organisms as ***Pseudomonas aeruginosa, Proteus vulgaris. Citrobacter spps*** and ***Enterobacter aerogenes*** were isolated with percentages 1.9%, 3.4%, 1.5% and 0.4% respectively and this partially agree with to that mentioned by **(Sayed, 1996)**. In table (3) out of 120 clinical cases polymicrobial samples were identified as follow 15%, 4.1% , 7.5% and 3.2% from nasal, tracheal swabs, lung tissues and lung abscesses respectively with cumulative totals 7.91% and this partially similar to **(Radwan et. al; 2002)**, We noticed that ***Pasteurella multocida***, ***E-coli and Staphylococcus aureus*** were the predominant organisms charing with each other to make mixed infection than other pathogens, simillar finding were recorded by **(Kaya and Erganis, 1991)**. Concerning lung septic focci, there were samples have single foccus (1.6%) and multiple focci with incidence of (3.2%), this lower than **(Ziaton, 2001)** which was (13.1%). The multiple abscesses of variable size of central liquefied and were surrounded by inflammatory cells and granulation tissues, these finding agreed with that obtained by **(Ali and Ali, 1993)**. On the other hand Pasteurlla spps was isolated in mixed cultures coupled with other bacteria as ***Staphylococcus aureus*** as mentioned by **(Niang, et. al; 1997)** and with ***E-coli*** as mention by **(Ziaton, 2001)**. Also the nodules in multiple abscesses were surrounded with fibrous connective tissue and contained grayish-white to grayish yellow pasty or liquefied pus **(Soroor, 1999)**. Sepsis which mean presence of pyogenic and / or other pathogenic bacteria in blood and tissues is caused due to exposure to stressors as bad hygiene, thermal environmental changes, malnutrition and concurrent infection, and these cause trouble some challenges to Vets. and may lead to wide spread abscessiation on all over the body **(Hungerford, 1975)** and death,**(**

Rosenberger, 1987). Concerning ***Pasteurella multocida*** serotying (table 4), 43 isolates from which 27 (62.6%) were related to capsular serotypes A and 16 untyped this was lower than that isolated by **(Wafaa, 2003)** who isolated this serotype with incidence of 81.25% , 45.48% and 77.55% from nasopharyngeal swabs, lung of infected sheep and apparently healthy ones, respectively.

The pathogencity of ***Pasteurella multocida*** isolates to white mice revealed (Table 5) that most isolates were pathogenic and mortality rates ranged from (60-100%) post inoculation within 72 hours this finding agreed with the result obtained by **(Sheikh, et al. 1995).** In vitro, the susceptibility distribution of each isolated pathogen to different antibiotics was represented (in table 6). Most of the isolates were highly sensitive to Enrofloxacin, Gentamicin and moderately sensitive to Oxytetracycline, but resistant to Ampicillin and Streptomycin these finding are partially agreement with those mentioned by **(Amany, 2000).** We conclude that respiratory disorders are still serious problems due to its special property that multifactor are responsible and difficulty to determine the definite cause. So more efforts as periodical clinical and bacterological examination and vaccination of apparently healthy sheep to avoid misuse of antibiotics, adequate hygienic measures as well as proper management of sheep would reduce the degree of exposure to disease producing agents. It should also concluded that any case fever should be regarded as sepsis until proven other wise **(Smith, 1996)**.

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