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STUDY ON SOME BACTERIAL CAUSES OF RESPIRATORY, ALIMENTARY AND MORTALITY DISEASES IN NEONATE LAMBS

(With 4 Tables)

By

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(Received at 5/4/2009)

دراسة عن بعض المسببات البكتيرية للأمراض التنفسية والهضمية والنفوق في الحملان حديثه الولادة

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أجريت هذه الدراسة لمعرفة بعض المسببات البكتيرية المصاحبة للنفوق المبكر فى الحملان حديثة الولادة. من خلال تلك الدراسة تم جمع ٧٠ عينة من الحملان (السليمة ظاهريا والمريضة والنافقة حديثا) من عمر يوم حتى ٣ شهور فى إحدى المزارع الخاصة بمحافظه الدقهلية. وقد أظهر الفحص الإكلينيكي فقدان الشهية وضعف عام وإسهال بالاضافه إلى اضطرابات تنفسية على الحملان المريضة. وتم فحص العينات بكتيريولوجيا وذلك بزرعها على أوساط غذائية مختلفة. وأظهرت نتائج الفحص البكتريولوجي وجود ٤٨ (٦٨,٦%) حالة إيجابية للعزل البكتيري حيث تبين إصابة بعض الحالات ٣٠(٦٢,٥%) عدوى فردية بينما ١٨ (٣٧,٥%) عدوى مختلطة. وقد تم عزل كل من الميكروب القولوني ٢٣(٣١,٩%) ، باستريلا ١٦(٢٢,٢%)، سالمونيلا ١٣(١٨,١%)، كليبسيلا ٨(١١,١%)، الميكروب العنقودي الذهبي ٦(٨,٣%)، مكورات سبحية ٤(٥,٦ %) وسيدوموناس ٢(٢,٨%). وقد تم تصنيف معزولات الميكروب القولوني سيرولوجيا إلى ٥ عترات (O_{26})، ٧ (O_{55})، ٤ (O_{101}) و٧ عترات غير مصنفة وكذلك معزولات السالمونيلا إلى ٦ عترات سالمونيلا تيفيموريم و٤ عترات سالمونيلا مونتيفيديو و٣ عترات غير مصنفة. كما تم عمل اختبار الحساسية للميكروبات المعزولة السائدة حيث كانت معظم المعزولات حساسة لكل من الإنروفلوكساسين والسيفوتاكسيم والسلفاميثوكسازول تراى ميثوبريم. وقد تم مناقشة النتائج والتوصيات الواجب إتباعها لتجنب الإصابة بهذه الميكروبات وللمحافظة على الحالة الصحية للحملان.

SUMMARY

The present study was aimed to investigate the bacterial causes of newly born lamb mortalities. Seventy samples were collected (20 apparently healthy, 40 diseased and 10 dead lambs) their age ranged from one day up to 3 months old. The clinical symptoms of diseased lambs were weakness, partial loss of appetite, diarrhoea and respiratory signs. The bacteriological examination revealed that 48 samples (68.6%) were positive for bacterial isolation and 30 (62.5%) and 18 (37.5%) were single and mixed infection respectively. *E. coli* was isolated at incidence percentage 23(31.9%), *Pasturella* spp. 16(22.2%), *Salmonella* spp. 13 (18.1%), *Klebsiella* spp. 8(11.1%), *Staphylococcus aureus* 6(8.3%), *Streptococcus* spp. 4(5.6%) and *Pseudomonas* spp. 2(2.8%). *E.coli* isolates were identified serologically into 5 (O_{26}), 7 (O_{55}), 4 (O_{101}) and 7 untyped strains. Also, *Salmonella* spp. was identified as 6 *S. typhimurium,* 4 *S. montividio* and 3 untyped strains. In vitro sensitivity test of isolated strains revealed that the most effective antibiotic were sensitive for Enrofloxacin, Cefotaxim and Trimethoprim sulfamethaxazole and should be used in treatment of diseased cases.

***Key words:** Lambs, mortality, respiratory, disorders, enteritis*

INTRODUCTION

Early mortality of lambs is a major constraint on total sheep productivity. This problem is not a simple one, as it is not related to a specific cause but is the result of many factors attributable to climatic conditions, environmental stress, genetic influences, nutritional and infection causes (Nash *et al.,* 1996).

The major causes of death in lambs were pneumonia, digestive problems (Diarrhoea), endoparasitic infestations, starvation and septicemia (Mukasa- Mugerwel *et al.*, 2000). Bekele *et al.* (1992) and Abdel hadi *et al.* (2006) they observed that pneumonia and enteritis were from the commonest causes of high proportional morbidity and mortality rates between the lambs (Mishra *et al.*, 2000).

Respiratory disorders caused by bacterial infections were considered as leading cause of lamb death among grazing sheep (Rowland *et al.,* 1992; Haziroglu *et al.*, 1994 and Martin 1996). Diarrhoea is still the most common and costly disease affecting neonatal small ruminants (Pugh, 2002 and Andres *et al.*, 2009).

Many organisms were incriminated as causative agents of diarrhoea and death in newly born lambs after natural and experimental infections (Karmy and Ragab, 1983 and Munoz *et al.*, 1996). The most important bacterial causes of pneumonia and and diarrhoea are *E. coli, Salmonella spp., Pasteurella spp.,Klebsiella spp., Staphylococcus, Streptococcus* and *Pseudomonas spp.* (Nesterov, 1981, Shegidevich *et al.*, 1983, Leondidis *et al.*, 1983 and Sharif *et al.*, 2005). These pathogens are responsible for great mortality and various morbidity changes and at the same time contribute a hazard to public health (Orden *et al.*, 2000 and Sharif *et al.*, 2005).

The aim of this work is to isolate and identify some bacterial causes which recovered from both pneumonic, enteric cases and dead lambs. Determination of invitro antibiotic sensitivity of isolated organisms was also aimed.

MATERIALS and METHODS

1- Animals:

A total number of 70 samples newly born lambs (20 clinically healthy, 40 diseased and 10 dead lambs) from birth up to 3 months old of both sexes were located at a private farms in Dakahlia Governorate and subjected for clinical and laboratory investigations (Kelly, 1984).

2- Samples:

Samples from nasal and rectal swabs were collected from all cases. Other samples from liver, heart, lung, spleen, lymph nodes and small intestine were taken from dead ones under a septic conditions and sent without delay to the laboratory for bacteriological examination.

3- Bacteriological examination:-

The obtained samples were inoculated by sterile platinum loop in Nutrient, MacConkey and Selnite "F" broth and incubated at 37°C for 18 hr. and then subcultured into Nutrient agar, 5% Blood sheep agar, S.S agar and MacConkey agar plates, then incubated over night at 37 °C. The produced colonies were prepared and stained with Gram stain, then examined microscopically for detection of morphological appearance, arrangement and staining reactions of the isolates (Cruickshank *et al.*, 1982). Isolates thenafter were identified by biochemical tests (Edwards and Ewing 1972, Koneman *et al.*, 1997 and Qunin *et al.*, 2002).

4- Serological identification of *E.coli* and *Salmonella*:

a- *E.coli*: Serological identification of purified *E.coli* strains using available agglutinating Coli test sera (Behring merk, AG Marburg) was done according to manufacturer's instruction (Lab , Germany).

b- *Salmonella*: The biochemically identified Salmonella strains were subjected for serological identification as described by Edwards and Ewing (1972), and the instruction of the manufacturer (Denken Selken Co. LTD, Tokyo, Japan).

5- Invitro antibiotic sensitivity test: The disc diffusion technique was performed on the isolated bacteria using Muller-Hinton (Oxoid 1998).

Ten chemotherapeutic discs were kindly supplied by Oxoid and namely: Enrofloxacin, Amoxycillin, Ampicillin, Gentamycin, Strepomycin, Cefotaxim, Erythromycin, Colistin sulphate, Oxytetracycline and Trimethoprime sulphamethexazole.

RESULTS

Clinical signs: The main clinical signs encountered for diseased lambs were depression, off food, emaciation and diarrhoea (either mucoid or bloody). In some cases difficult breathing, sneezing and nasal discharge.

Postmortem lesion: Dead lambs showed the following lesions: congestion of small and large intestines, congestion liver, kidneys and mesenteric lymph nods. In some cases there was congested lungs.

The results of bacteriological examination were recorded in Table 1, 2, 3 and 4.

Table 1: Results of bacteriological examination of obtained samples

Source of samples	Total No. of examined samples	Positive samples		Single isolates		Mixed isolates		Total No. of isolates
		No	%	No	%	No	%	
1-Apparently healthy lambs	20	6	30.0	4	66.7	2	33.3	6
2-Diseased lambs	40	32	80.0	22	68.8	10	31.2	46
3- Dead lambs	10	10	100.0	4	40.0	6	60.0	20
Total	70	48	68.6	30	62.5	18	37.5	72

Table2: Types of pathogenic bacteria recovered from examined samples

Bacterial isolates	Condition of lambs						Total	
	Apparently healthy lambs (20*)		Diseased lambs (40*)		Dead lambs (10*)			
	No.	%	No.	%	No	%	No.	%
E. coli	2	10.0	14	35.0	7	70.0	23	31.9
Salmonella spp.	0	0.0	8	20.0	5	50.0	13	18.1
Pasteurella spp.	2	10.0	11	27.5	3	30.0	16	22.2
Klebsiella spp.	0	0.0	6	15.0	2	20.0	8	11.1
Staph.aureus	1	5.0	3	7.5	2	20.0	6	8.3
Streptococcus	1	5.0	2	5.0	1	10.0	4	5.6
Pseudomonas spp.	0	0.0	2	5.0	0	00.0	2	2.8
	6		46		20		72	100.0

* No. of examined samples.

Table 3: Serological identification of isolated *E. coli* and *Salmonella* strains.

Source of Samples	(23) *E. coli*									(13) *Salmonella*						
	O_{26}		O_{55}		O_{101}		Untyped		Total	*S. typhimurium*		*S. montividio*		untyped		Total
	No.	%	No.	%	No.	%	No.	%		No.	%	No.	%	N0.	%	
App. Healthy lambs	1	4.3	0	0.0	0	0.0	1	4.3	2	0	0.0	0	0.0	0	0.0	0
Diseased lambs	2	8.7	5	21.7	3	13.1	4	17.4	14	2	15.4	2	15.4	1	7.7	5
Dead lambs	2	8.7	2	8.7	1	4.3	2	8.7	7	4	30.7	2	15.4	2	15.4	8
Total	5	21.7	7	30.4	4	17.4	7	30.4	23	6	46.1	4	30.8	3	23.1	13

Table 4: Invitro antibiotic susceptibility of important isolated bacteria

Organisms / Antibiotic agent	*E. coli*	*Salmonella*	*Pasteurlla*	*Klebsilla*	*Staph. aureus*
Enrofloxacin 5 ug	+++	+++	++	+++	+++
Amoxycillin 25 ug	R	R	R	R	R
Ampicillin 10 ug	R	R	R	R	R
Gentamycin 10 ug	++	++	R	+++	++
Streptomycin 10 ug	++	++	++	++	++
Cefotaxim 30 ug	+++	+++	+++	+++	+++
Erythromycin 15 ug	R	R	R	R	R
Colistin sulphate 10ug	++	++	R	++	++
Oxytetracyclin 30 ug	++	++	++	R	++
Trimethoprim-sulpha methaxazole 1.25-23.75ug	+++	+++	++	++	+++

+++ = Highly sensitive
++ = Moderately sensitive
R = Resistante

DISCUSSION

Neonatal lamb mortality represents an economic loss and welfare concern. Mortality in lambs can be provoked by a multitude of both infections and non infectious causes. A varieties of infectious pathogens were revealed from diseased and dead lambs mainly bacterial causes. (Mitchell and Link Later, 1983).

The bacteriological examination of 70 samples from lambs revealed 48 (68.6%) positive bacterial infection, where 6 (30.0%) from apparently healthy lambs, 32(80.0%) from diseased lambs and 10(100%)from dead lambs Table (1).

On the other hand, Ibrahim and Selim (2003) isolated the pathogenic bacteria from apparently healthy, diseased and dead lambs with percentage of 20 %, 66.7% and 78.3% respectively. Khadr *et al.* (2006) isolated *Campylobacter spp.* with percentage of 12.3% from diseased lambs and 7.5% from apparently healthy lambs. Also Hala and Amany (2007) isolated *Salmonella* spp. from healthy and diseased lambs with percentage 4.7% and 12.7% respectively. Concerning the results in table (2) for the incidence of pathogenic bacteria in examined lambs results indicated that *E.coli* 23 (31.9%), *Pasteurella* spp. 16 (22.2%), *Salmoenlla spp* 13(18.1%), *Klebsiella spp.* 8 (11.1%), *Staphylococcus aureus* 6 (8.3%), *Streptococcus spp.* 4 (5.6%) and *Pseudomonas spp.* 2

(2.8%). It appears that the most prevalent bacteria were *E. coli, Pasteurella spp., Salmonella spp.*

This agreed with results reported by Sharif *et al.* (2005) who recorded that the bacteria responsible for neonatal mortality were *E.coli, Pasteurella* and *Staph. aureus*, also *E. coli* was the most frequent bacteria identified as a cause of neonatal mortality in lambs.

Results in Table (3) revealed that *E. coli* serotypes identified as 5 (21.7%) O_{26}, 7 (30.4%) O_{55}, 4 (17.4%) O_{101} and 7 (30. 4%) untybed strains. This result seems in accordance with those reported by Rao and Char, (1983) who isolated *E. coli* serotypes O_{26} and O_{55} from pneumonic lung of septicemic lambs, Duff and Hunt (1989) also isolated E.coli strain O_{101} from 4 day old dead lambs. Salmonellosis is a very important disease of lambs causing enteritis and septicemia, *Salmonella* is potentially lethal to lambs (Moredun Foundation, 2007). Also it is constitute a hazard to public health as all serovares can produce diseases to human (WHO, 2006). The results given in Table (3) revealed that 13 *Salmonella* strains recovered from examined samples were serotyped as: 6 (46.1%) *S.typhimurium* as the most one, 4 (30.8%) *S.montividio* and 3 (23.1%) untyped strains. This resultis similar with those of Hala and amany (2007) who recorded that *S.typhimurium* was the most predominant isolate from dead lambs. On other hand Soumaya and Fadel (2004) isolated *S.dublin* and *S. enteridis* from dead lambs. The data presented in Table (4) showed that most isolates were highly sensitive to Enrofloxacin, Cefotaxim and Trimethoprim sulphamethaxazole and resistance to Ampicillin, Amoxycillin and Erythromycin.

Hatem *et al.* (2003) mentioned that the bacterial isolates from diseased sheep and goats were sensitive to Ciproflaxocin, Danofloxacin and Enrofloxacin and resistance to Ampicillin,Gentamycin and Trimethoprim -sulphamethaxazole.

Finally we could conclude that the most mortality rate between the newly born lambs due to pneumonia and diarrhoea. Also the most prevalent bacterial isolates are *E.coli, Salmonella* and *Pasteurlla spp.* So adequate hygienic measures and proper management may reduce the degree of animals exposure to disease producing agents.

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