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SOME STUDIES ON THE BACTERIAL CAUSES OF MORTALITY IN NEW BORN RABBITS

(With 4 Tables)

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بعض الدراسات عن الأسباب البكتيرية للنفوق فى الأرانب حديثه الولادة

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فى هذه الدراسة تم تحديد المسببات البكتيرية المصاحبة للنفوق فى الأرانب حديثه الولادة بالاضافه للأعراض والآفات المرضية المصاحبة لها وخلال تلك الدراسة تم فحص عدد ٢٠٠ عينه (١٢٠ حاله نفوق و٤٠ حاله مرضيه و٤٠ حاله سليمة ظاهريا) تم جمعها من المزارع الخاصة بمحافظه الدقهليه. وقد تبين من الفحص الظاهرى للأرانب المريضة وجود خمول وضعف عام مع إسهال واضطرابات تنفسية وبإجراء الصفة التشريحية وجد تضخم بالكبد والطحال مع وجود أنزفه على الأعضاء الداخلية واحتقان بالرئتين. وقد أظهرت نتائج الفحص البكتريولوجى وجود ١٢٦(٦٣%) حاله ايجابيه للعزل البكتيرى حيث تبين أصابه بعض الحالات ٦٠(٤٧.٦٢%) بنوع واحد من البكتيريا (عدوى فرديه) بينما ٦٦(٥٢.٣٨%) عدوى مختلطة. وقد تم عزل كل من الميكروب القولونى ٣٤ (١٧.٧١%) وكل من ميكروب الباستيريلا مالتوسيدا والسالمونيلا ٢٨(١٤.٥٨%) وكليبسيلا نيمونى ٢٧(١٤.٠٦%) والميكروب العنقودى الذهبى ٣٢(١٦.٦٧%) وميكروب الاستربتوكوكس بيوجين ٢٥(١٣.٠٢%) وميكروب السودوموناس ارجينوزا ١٨(٩.٣٨%). وقد تم تصنيف معزولات E.Coli سيرولوجيا الى١٢عتره ($O_{126}K_{71}$(B16)) و٧ عترات من كل من ($O_{26}K_{60}$(B6)) و($O_{59}K_{59}$(B1)). وكذلك معزولات السالمونيلا الى ١٤ عتره سالمونيلا تيفيموريم و٨ عترات سالمونيلا انترتيديس. كما تم عمل اختبار حساسية للميكروبات المعزولة حيث كانت معظم المعزولات حساسة لكل من الانروفلوكساسين والجنتاميسين. هذا وقد تم مناقشه النتائج والتوصيات الواجب اتباعها للمحافظه على الأرانب حديثه الولادة وكذلك الثروة القومية.

SUMMARY

The present study aimed to investigate the bacterial causes of mortality in newly born rabbits and symptoms and pathological lesions. A total of 200 kits (120 freshly dead kits, 40 diseased kits and 40 apparently healthy kits) were collected from private farms at Dakahlia Governorate. The symptoms of diseased rabbits were depresion, weakness, diarrhoea and respiratory distress, while P.M. were enlarged liver and spleen, hemorrhage on the internal organs and congested lungs. The

bacteriological examination revealed 60 cases (47.62%) and 66(52.38%) were single and mixed infection respectively. E.coli was isolated at incidence percentage 34 (17.71%), each of Pasteurella multocida and Salmonella 28(14.58%), Klebsiella pneumoniae 27(14.06%), Staphylococcus aureus 32(16.67%), Streptococcus pyogenes 25(13.02%) and Pseudomonas aeruginosa 18 (9.38 %). E.coli isolates were identified serologically into 12($O_{126}K_{71}$(B16) and 7each from ($O_{26}K_{60}$(B6), ($O_{59}K_{59}$(B1). Also Salmonella spp. was identified as 14 Salmonella typhimurium and 8 Salmonella enteritidis. In vitro sensitivity pattern of isolated strains proved that Enrofloxacin and Gentamycin were the most effective drugs for most isolates.

Key words: *Rabbits, mortality, bacterial diseases*

INTRODUCTION

Rabbit production is a growing industry in Egypt, which proved economically profitable. Mortalities in new born rabbits was represented a high percentage duo to many different causes, it has been reported that some bacterial agents play a very important role in mortalities which lead to severe economic losses (Okerman, 1987).

Some pathogenic microorganisms, (E.coli, Salmonella, Corynebacterium, Pasteurella, Pseudomonas) could be isolated from cases of mortality in newly born rabbits (Peeters, *et al*., 1984; Fahmy, *et al*., 1985; Okerman, *et al*., 1985; Okerman, 1987; Marlier, *et al*., 2003 and Boucher, 2005).

Little researches have focused on the bacteria associated with mortality in new born rabbits, hence the goal of this study was planned to:

- Isolate, identify and determine the incidence and types of bacteria and serological identification of the isolated pathogens from newly born rabbits and determine their spectrum of antimicrobial activity.

MATERIALS and METHODS

1- Samples:

A total of 200 samples (120 freshly dead kits, 40 diseased kits and 40 apparently healthy kits) of different breeds at age ranged from birth up to four weeks were collected from different private farms at EL- Dakahlia Governorate .

a- Freshly dead and diseased kits.

Samples from liver, spleen, lung, intestine, bone marrow, heart blood and faeces.

b- Apparently healthy kits. Cloacal swabs.

The samples were subjected to clinical and /or post-mortem examination and bacteriological examination.

2- Media:

a- Liquid media: Tryptose broth, Peptone water and Selenite F-broth.

b- Solid media: Blood agar, Tryptose agar, MacConky's agar and Xylose lysine deoxycholate agar (Oxoid).

3- Isolation and identification:

The collected samples were transferred to test tubes containing Tryptose broth and Selenite F-broth and incubate at $37C^0$ for 18-24 hours, followed by subculturing on Blood agar, MacConky's agar and Xylose lysine deoxycholate agar plates and incubated aerobically at $37C^0$ for 18-24 hours.

The growing colonies on various plates were examined morphologically, culturally and biochemically (Indole, Nitrate Reduction, Vogas Proskaure, Citrate utilization, Urease, Sugar fermentation and Coagulase test) according to Edwards and Ewing, (1972); Cruickshank, *et al*., (1982); Finegold & Baron, (1986) and Carter & Cole, (1991).

4- Serological identification of:

a- Salmonella:

The biochemically identified Salmonella strains subjected for serological identification as described by Edwards and Ewing, (1972); Kaufmann (1973) and the instruction of the manufacturer (Denken Selken Co. LTD, Tokyo, Japan).

b- E. coli:

Serological identification of purified E.Coli strains using available agglutinating Coli test sera (Behring werk, AG Marburg) according to mannufacturer's instruction .Labn, Germany).

5- In vitro antibiotic sensitivity test:

The disc diffusion technique was performed on the isolated bacteria using Muller-Hinton media (Oxoid). Ten chemotherapeutic disks kindly supplied by Oxoid and namely Ampicillin, Amoxycillin, Chloramphenicol, Enrofloxacin, Erythromycin, Gentamycin, Streptomycin, Penicillin, Oxytetracycline and Trimethoprim-sulphamethoxazole. The degree of sensitivity was interpreted according to Koneman, *et al*. (1994); Quinn, *et al*. (1994) and Oxoid Manual, (1998).

RESULTS

Clinical signs:

The main clinical signs encountered of diseased kits were ruffled fur, depresion, off food, emaciation, diarrhoea (either mucoid or bloody). In some cases difficult breathing, sneezing, occular and nasal discharges and wetted fur of nose and fore limbs, were observed.

Postmortem Lesion:

Enlargement of liver and spleen, petechial haemorrhages on the internal organs, the overful stomach with undigested milk, the intestinal contents were watery and thin. In some cases there was congested lungs. The clinical signs and postmortem lesion in the present work in agreement with those previously diescribed by (Awaad, 1972; Peeters, 1994 and Okerman, 1999). The results of bacteriological examination were recorded in Table 1, 2, 3 and 4.

Table 1: Results of bacterilogical examination of kits

Source of samples	Total No. of iamples	+ve Samples		Single isolate		Mixed isolates		Total No. of isolates
		No.	%	No.	%	No.	%	
Apparentl y healthy	40	19	47.5	19	47.5	-	-	19
Diseased	40	26	65.0	10	25.0	16	40.0	42
Dead	120	81	67.5	31	25.83	50	41.67	131
Total	200	126	63.00	60	47.62	66	52.38	192

Table 2: Incidence of bacteria isolated from examined kits.

Bacterial isolates	Condition of kits						Total	
	Apparently healthy (*40)		Diseased (*40)		Dead (*120)			
	No.	%	No.	%	No.	%	No.	%**
E.coli	4	10.0	6	15.0	24	20.0	34	17.71
Past. multocida.	-	-	8	20.0	20	16.67	28	14.58
Ps.aeruginosa	2	5.0	4	10.0	12	10.0	18	9.38
Salmonella	-	-	8	20.0	20	16.67	28	14.58
Kl.pneumoniae	3	7.5	4	10.0	20	16.67	27	14.06
Strep.pyogenes	4	10.0	6	15.0	15	12.50	25	13.02
Staph.aureus	6	15.0	6	15.0	20	16.67	32	16.67
Total	19		42		131		192	100.00

* The number of examined kits

**The percentage was calculated according to the total isolates (192)

Table 3: Isolated E.coli and Salmonella serotype.

Source of Samples	E. Coli									Salmonella						
	$O_{26}K_{60}$(B6)		$O_{59}K_{59}$(B1)		$O_{126}K_{71}$(B16)		Untypable		Total	S. typhimurium		S. enteritiodis		Untypable		Total
	No.	%	No.	%	No.	%	No.	%		No.	%	No.	%	No.	%	
Apparently healthy	2	50.0	1	25.0	-	-	1	25.0	4	-	-	-	-	-	-	-
Diseased	2	33.33	1	16.67	2	33.33	1	16.67	6	4	50.00	2	25.0	2	25.0	8
Dead	3	12.5	5	20.83	10	41.67	6	25.0	24	10	50.0	6	30.0	4	20.0	20
Total	7	20.59	7	20.59	12	35.29	8	23.53	34	14	50.0	8	28.57	6	21.43	28

Table 4: Antibiotic sensitivity test for the bacteria isolated from examined samples of newly born rabbits.

Antibiotic Disc	E.coli	Past. multocida.	Salmonella	Kl. pneumoniae	Staph.aureus
Ampicillin 10ug	R	R	R	R	R
Amoxycillin 25ug	R	R	R	R	R
Chloramphenicol l30ug	++	++	+++	+++	++
Enrofloxacin 5ug	+++	+++	+++	+++	+++
Erythromycin 15ug	R	R	R	R	++
Gentamycin 10ug	+++	+++	+++	+++	+++
Streptomycin 10ug	R	++	R	++	++
Penicillin 10ug	R	R	R	R	++
Oxytetracyclin 30ug	++	++	++	R	++
Trimethoprim-Sulpha Methoxazol 1.25-23.75 ug	++	++	++	R	R

+++ = Highly sensitive
++ = Moderately sensitive
R = Resistance

DISCUSSION

The rapid expansion of rabbit production in recent years in Egypt, is accompained by several problems. Mortalities in baby rabbits have become a problem of utmost concern. These mortalities cause severe economical losses in rabbit production (Okerman, 1987).

The bacterial infections are major health concern of rabbits production, the major causes of mortality in kits were, gastroenteritis, respiratory infection and pneumoenteritis (Rai, *et al*., 1985).

It was found that of 200 cases of examined kits 126 (63%) revealed bacterial infection from which 60 (47.62%) yielded a single pure isolate and 66 (52.38%) yielded a mixed bacterial isolates (Table1). High percentage of mixed cultures were obtained from diseased and freshly dead kits. The incidence of isolation of one organism from apparently healthy, diseased and dead kits were 47.5, 25 and 25.83 % respectively.

Bacteriological examination of samples revealed that isolated bacterial pathogens were, E.coli 34(17.71%), Staphylococcus aureus 32(16.67%), Pasteurella multocida 28(14.58%), Salmonella 28(14.58%), Klebsiella pneumoniae 27(14.06%), Streptococcus pyogenes 25(13.02%) and Pseudomonas aeruginosa 18(9.38 %). Nearly similar pathogens were isolated by (Peeters, *et al*., 1984; Fahmy, *et al*., 1985; Okerman, *et al*., 1985; Okerman, 1987; Marlier, *et al*., 2003 and Boucher, 2005).

Escherichia coli is a gram-negative, lactose-fermenting, indole positive, facultative anaerobe of the human and animal intestinal flora. The organism typically colonizes the infant gastrointestinal tract within hours of life (Brasar and Hill, 1974).

The gastrointestinal tract of most warm-blood animal is colonized by E.coli within hours or a few days after birth, E.Coli can adhere to the mucus overlying the large intestine. From our results in Table (2) the E.coli was the most frequent isolates 34(17.71%) which considered the main causes of mortality in newly born rabbits, the obtained results nearly similar with the result obtained by (Peeters, *et al*., 1984 and Percy, *et al*., 1993) they concluded that E.coli infection is the primary causative agent in most outbreaks of diarrhoea in rabbits.

From Table (3) it is clear that 26 out of 34 identified E.coli strains could be identified serologically into 3 serotypes, 12($O_{126}K_{71}$(B16) and 7($O_{26}K_{60}$(B6), 7($O_{59}K_{59}$(B1) and 8 untypable. Most E.coli serotypes isolated from healthy, diseased and dead newly born rabbits were agreement with those recovered by (Asdrubali, *et al.*, 1977; Bekheet, 1983; Ibrahim, 1985 and Abd-El-Rahman, *et al.*, 2005).

Salmonellosis is a very important disease not only from the economic point of view but also from the public health aspect as it is zoonotic disease, it occurs world wide and its incidence is on increase (Englar, 1988). Salmonellosis in rabbits is characterized by neurological signs and dehydration, septicemia, acute enteritis and rapid death (Sadeck & Moustafa, 1970; Ghoniem *et al.*, 1971; Casaro, *et al.*, 1979 and Boucher, 2005).

The results given in Tables (2, 3) revealed that Salmonella could not be isolated from apparently healthy kits and could be isolated from diseased and dead kits with an incidence 8(20%) and 20(16.67%) respectively. On serotyping of the 28 recovered Salmonella organisms from examined samples, 14(50%) of which were recognized as Salmonella typhimurium; 8(28.57%) were Salmonella enteritidis and 6(21.43%) were untyped. Some authors recorded Salmonella typhimurium and Salmonella enteritidis from newly born rabbits (Pigoury, *et al*., 1959; Saad, 1970; Okerman, 1987; Abdel-Azeem, 1995 and Boucher, 2005).

Pasteurellosis is one of the most important bacterial disease which affects rabbits as it causes severe economic losses in most parts of the world through both high mortality and morbidity rates. Affected rabbits may have signs of rhinits (snuffles), pneumonia and abscesses in different parts of the body (Deeb, *et al*., 1990; Frymus, *et al*., 1991; Sami, *et al.,* 1995 and Sharon, *et al*., 1996). The results achieved from Table(2) revealed that Pasteurella multocida could be isolated from diseased and dead kits with an incidence percentage 20, 16.66% respectively, and could not be detected in apparently healthy kits. Nearly similar results were reported by (Hagen, 1966; Saad, 1970; Fahmy, *et al*., 1985; Okerman, 1987 and Abdel-Azeem, 1995).

Klebsiella pneumoniae is a typical member of enterobacteriaceae that produce endotoxin following penetration through intestinal or respiratory mucosa (Gerlach, 1994). Often infection are not detected until respiratory signs occur lately but systemic infection are also common (Jensen, 1992). The results in Table (2) revealed that Klebsiella pneumoniae was isolated from 3(7.5%), 4(10%) and 20 (16.67%) of examined apparently healthy, diseased and dead kits respectively. Nearly similar results were recorded by Abd-El-Rahman, *et al., (*2005).

Staphylococcosis in rabbits is caused by staphylococcus aureus and characterized by fatal septicemia or suppurative inflamation in nearly any oragan or site.Through the present study staphylococcus aureus was isolated from 6(15%), 6(15%) and 20(16.67%) of examined apparently healthy, diseased and dead kits respectively Table (2).

Pseudomonas aeruginosa was recovered from 2(5%), 4(10%) and 12(10%) of examined apparently healthy, diseased and dead kits respectively Table (2).

Streptococcus has been associated with acute septicemia in rabbit .The results recorded in Table (2) revealed that Streptococcus pyogenes

was isolated from 4(10%), 6(15%) and 15(12.50%) of examined apparently healthy, diseased and dead kits respectively.

From the aforementioned results it was concluded that the major pathogenic bacteria associated with mortalities in newly born rabbits were E.coli, Salmonella, Pasteurella and Klebsiella pneumoniae.

In vitro sensitivity testing of isolates revealed that most isolates were highly sensitive to Enrofloxacin and Gentamycin and resistance to Ampicillin, Amoxycillin and Penicillin Table (4). Nearly similar results were reported by Harwood, 1989; Diker, *et al*., 1994; Abdel-Azeem, 1995; Abd-El-Rahman, *et al*., 2005 and Hatab & Abdel-Latif, 2006.

Finally, efforts should be paid to prevent this problem in the furture or its continuation through, good management with complete hygienic measures and avoid the misuse of antibiotics.

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