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STUDIES ON SOME BACTERIA ASSOCIATED WITH ABORTION IN RABBITS

(With 4 Tables)

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(Received at 6/3/2006)

دراسات على بعض البكتريا المصاحبة للإجهاض فى الأرانب

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لاستبيان المسببات البكتيرية المصاحبة للإجهاض فى الأرانب تم جمع عدد ٦٠ عينة من (٢٠ أرنبية مجهضة وناقصة حديثا و٣٠ أرنبية مجهضة وحية و١٠ أرانب غير مجهضة مخالطة) من المزارع الخاصة بمحافظة الدقهلية وذلك بهدف فحصها بكتريولوجيا. وأظهر الفحص البكتريولوجى وجود ٤٤ (٧٣,٢%) حالة ايجابية للعزل البكتيرى حيث تبين إصابة بعض الحالات ٢٦ (٥٩,١%) بنوع واحد من البكتيريا (عدوى فردية) بينما تم عزل أجناس ميكروب الليستيريا مونوسيتوجينس وكذلك ميكروب الباستيريل مالتوسيدا فى ٢٠% لكل منها أما العدوى البكتيرية المخالطة فتمثلت فى عزل الميكروب القولونى (E. Coli) مع كل من الميكروب العنقودى الذهبى (Staph. aureus) وميكروب الباستيريل مالتوسيدا فى ١١,٣٦% ، ٩,٠٩% على التوالي كذلك تم عزل ميكروب الباستيريل مالتوسيدا مع ميكروب السالمونيلا فى ١١,٣٦% أيضا ثم عزل الميكروب المكور العنقودى الذهبى مع ميكروب الإستربتوكوكس بيوجين فى ٩,٠٩% وقد تم تصنيف معزولات السالمونيلا إلى ٨ عترات (٨٨,٩%) سالمونيلا تيفى ميوريم. كما تم عمل اختبار حساسية للميكروبات المعزولة حيث كانت معظم المعزولات حساسة لكل من الإنزوفلدكاسين والجنتاميسين بينما كانت معزولات الليستيريا مونوسيتوجينس حساسة لكل من الأمبسلين والأموكسيسيلين والبنسلين. هذا وقد تمت مناقشة النتائج والتوصيات الواجب إتباعها للمحافظة على مزارع الأرانب وكذلك الثروة القومية.

SUMMARY

A total of 60 does (20 freshly & aborted dead, 30 live & aborted and 10 non aborted) were collected from private farms at El-Dakahlia Governorate for bacteriological examination. 44 cases (73.3%) of examined samples were positive for bacterial isolates, which indicated that *Listeria monocytogenes* and *Pasteurella multocida* were the

common bacteria as a single infection in 59.1% of examined samples, however, *E. coli* with *Staph. aureus* and *P. multocida*; *P. multocida* with *Salmonella* and *Staph. aureus* with *Strept. Pyogene* were isolated as mixed infection in 40.9% of examined samples. The majority of *Salmonella* strains were identified as *Salmonella typhimurium* 88.9%. In – vitro sensitivity pattern of isolated strains proved that enrofloxacin and gentamycin were the most effective drugs for most isolates, except *Listeria monocytogenes* was sensitive to ampicillin, amoxicillin and penicillin.

Key words: Rabbits, abortion, *Salmonella*, *P. multocida*, antibiotic sensitivity.

INTRODUCTION

In Egypt rabbit breeding is considered as an important source of good quality and economic animal protein because it needs simple requirements to start as well as its short production cycle and large number of off springs. Rabbits are more sensitive to diseases of the reproductive tract. Interest has been focused on abortion in rabbits, since it is responsible for high economic losses.

Many microorganisms are commonly associated with infectious abortion in rabbits, *Salmonella* species, *Pasteruella multocida*, *Listeria monocytogenes*, *E. coli* and *Staphylococcus aureus* (Harwood, 1989; Boucher and Nouaille, 1996; Peters and Scheele, 1996 and Rosell, 2000).

Several outbreaks and sporadic cases of rabbit abortion were occure in our area at El-Dakahlia, therefore the present work was aimed to study the role of bacteria as a cusative agent of abortion in rabbits, also to determine in vitro the antibiotic sensitivity of the isolated microorganisms.

MATERIALS and METHODS

I- Samples:

A total of 60 does (50 aborted does with aborted foeti and aborted materials in addition to 10 non aborted does), were collected from different private farms at El-Dakahlia Governorate.

a) Aborted and freshly dead does.

Samples from uterus and its content, brain, spleen, liver, heart blood, aborted foeti and aborted materials.

b) Aborted living does:

Samples were vaginal swabs, aborted foeti and aborted materials.

c) Non aborted does:

Samples were vaginal swabs.

All samples were collected and subjected to bacteriological examination.

II-Media:

a) Liquid media: Tryptose broth, peptone water, Selenit F-broth and Listeria enrichment broth.

b) Solid media: Blood agar, Tryptose agar, MacConkey's agar, Xylose Lysine deoxycholate agar and Listeria enrichment agar (Oxoid).

III- Isolation and identification:

The collected samples were transferred to sterile test tubes containing tryptose broth, selenite. F. broth and listeria enrichment broth, and incubated at 37°C for 18 – 24 hours, followed by subculturing on blood agar, MacConky's agar, Xylose Lysine deoxycholate agar and Listeria enrichment agar plates and incubated aerobically at 37°C for 24–48 hours. For isolation of *Pasteurela multocida* the blood agar plates were incubated in candle Jar at 37°C / 48 hours.

The growing colonies on various plates were examined morphologically, culturally and biochemically according to Edward and Ewing, (1972); Cruickshank *et al.*, (1982); Finegold and Baron, (1986) and Carter and Cole (1991).

IV – Serological identification of Salmonella:

The biochemically identified salmonella strains were subjected for serological identification as described by Edward and Ewing (1972), Kauffmann (1973) and the instruction of the manufacturer (Denka Selken Co. LTD, Tokyo, Japan).

V- In vitro antibiotic sensitivity test:

The disc diffusion technique was performed on isolated bacteria using Muller-Hinton medium (Oxoid). Ten chemotherapeutic disks kindly supplied by Oxoid and namely ampicillin, amoxicillin, enrofloxacin, chloramphenicol, gentamycin, streptomycin, pencillin, erythromycin, oxytetracycline and trimethoprim – sulphamethoxazole.

The degree of sensitivity was determined and interpreted according to Koneman *et al.*, (1994); Quinn *et al.*, (1994) and Oxoid Manual (1998).

RESULTS

Table 1: Incidence of single and mixed infection cases with bacteria in examined does.

Condition of does	Single infection cases		Mixed infection cases		Total No. of positive cases
	No.	%	No.	%	
Non. aborted does	3	6.82	2	4.55	5
Aborted & dead does	8	18.18	10	22.73	18
Aborted & live does	15	34.09	6	13.63	21
Total	26	59.09	18	40.91	44

The percentage was calculated in relation to the total number of positive cases (44).

Table 2: Incidence of different mixed bacterial isolated recovered from examined does.

Types of microorganisms	Condition of examined does						Total	
	Non-aborted		Aborted & dead		Aborted & live			
	No.	%	No.	%	No.	%	No.	%
E.Coli + Staph. Aureus	1	2.27	2	4.55	2	4.55	5	11.36
E. coli + P. multocida	0	0	3	6.82	1	2.27	4	9.06
P. multocida + Salmonella	0	0	3	6.82	2	4.55	5	11.36
Staph. aureus + Strept. Pyogenes	1	2.27	2	4.55	1	2.27	4	9.09

Table 3: Incidence of bacteria isolates from examined does.

Bacteria isolates	Condition of does						Total	
	Non-aborted (10)*		Aborted & dead (20)*		Aborted & live (30)*			
	No.	%	No.	%	No.	%	No.	%
E.Coli	2	20.00	5	25.00	5	16.66	12	20.00
Listeria monocytogenes	0	0.00	6	30.00	6	20.00	12	20.00
Pasturella multocida	0	0.00	6	30.00	6	20.00	12	20.00
Salmonella species **	1***	10.00	4	20.00	4	13.33	9	15.00
Staphylococcus aureus	2	20.00	4	20.00	4	13.33	10	16.66
Streptococcus pyogenes	2	20.00	3	15.00	2	6.66	7	11.66
Total	7		28		27		62	

* The number of examined does.

** salmonella typhimurium (8), untypable (1) ***

Table 4: In-vitro susceptability of important isolated microorganisms.

Antibiotic disc	E.coli (12)*		L. monocytogenes		P. multocida (12)*		Salmonella (9)*		Staph. aureus (10)*	
	Sensitive isolates	Activity %	Sensitive isolates	Activity %	Sensitive isolates	Activity %	Sensitive isolates	Activity %	Sensitive isolates	Activity %
Ampicillin 10 ug	-	0	12	100.00	-	0	-	0	-	-
Amoxycilin 25 ug	-	0	12	100.00	-	0	-	0	-	-
Enrofloxacin 5 ug	12	100.00	8	66.66	12	100.00	9	100.00	9	90.00
Gentamycin 10 ug	12	100.00	10	33.33	12	100.00	9	100.00	9	90.00
Streptomycin 10 ug	-	0	6	50.00	8	66.66	-	0	-	0
Chloramphenicol 30 ug	10	83.33	5	41.66	8	66.66	8	88.88	4	40.00
Pencillin 10 ug	-	0	10	83.33	-	0	-	0	4	40.00
Erythromycin 15 ug	-	0	0	0	-	0	-	0	3	30.00
Oxytetracyclin 30ug	6	50.00	5	41.66	6	50.00	6	66.66	4	40.00
Trimethoprim-sulphamethoxazol 1.25-23.75 ug	10	83.33	7	58.33	6	50.00	4	44.44	-	0

* The number of tested strains.

DISCUSSION

Nowdays, a great attention was payed toward the diseases caused by bacteria which affected the reproductive tract of rabbits because this group of diseases has been responsible for considerable economic loss to rabbit breeder. Peters and Scheele (1996) concluded that previous infection of the uterus by *Listeria monocytogenes* should be considered as a cause of infertility.

It is clear from the results presented in Table (1) that out of 44 positive cases from which pathogenic bacteria were isolated, 26 (59.09%) revealed single infection, while the remaining cases 18 (40.91%) were due to mixed infection. The isolated bacteria as mixed infections included *E. coli* with *Staphylococcus aureus* in 5 cases (11.36%); *E. coli* with *Pasteurella multocida* in 4 cases (9.09%); *Pasteurella multocida* with *Salmonella* in 5 cases (11.36%) and *Staphylococcus aureus* with *Streptococcus pyogenes* in 4 cases (9.09%). Table (2).

Bacteriological examination of the samples revealed that the isolated bacterial pathogens were, *E. coli*, *Listeria monocytogenes*, *Pasteurella multocida*, *Salmonella spp.*, *Staphylococcus aureus* and *Streptococcus pyogenes* (Table 3). Nearly similar pathogens were isolated by (Harwood 1989; Peter and Scheele 1996; Rosel 2000 and Boucher *et al.* 2001). The results achieved revealed the prevalence of *Listeria monocytogenes* and *Pasteurella multocida* in this work whereas the frequency of isolation from aborted dead and live does were 12 isolates from each as a single cause of abortion. The role of the content

of the pregnant uterus may simply offer a more favorable environment for the organism to grow and thereby induce a more severe disease (Gray and Killinger, 1966).

Listeriosis is a septicemic disease of rabbits characterized by sudden death or abortion or both (Flatt, 1974), the disease is caused by *Listeria monocytogenes* which is a facultative intracellular gram - positive, small rod, aerobic and facultatively anaerobic, found in soil, vegetation and faeces (Rebhun, 1987 and Jubb and Huxtable, 1993). Results given in Table (3) point out that *Listeria monocytogenes* could be isolated from 6 of 20 aborted and dead does with an incidence percentage 30%, also it could be isolated from 6 of 30 aborted and live does with an incidence percentage 20% and could not be detected in non aborted does.

The same organism was isolated previously from rabbits (Abd El-Waneas 1985; Abd El-Motelib *et al.*, 1990; Peters and Scheele 1996 and Abd El-Ghaffar and Abd El-Gwad 1997).

Pasteurella multocida can be a virulent pathogen of rabbits, producing fatal septicemia, pneumonia, chronic rhinitis, otitis media as well as multiple abscesses (Lu *et al.*, 1978) and is considered all over the world as one of the most dangerous diseases which affect rabbit industry (Deeb *et al.* 1990). The results achieved from Table (3) revealed that the *Pasteurella multocida* could be isolated from aborted dead and live does with an incidence percentage 30, 20% respectively and could not be detected in non aborted does. Nearly similar results were reported by Boucher and Nouaille 1996, Rosell 2000 and Boucher *et al.* 2001).

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 the increase (Engler, 1988). Salmonellosis in rabbits is characterized by septicemia, acute enteritis and rapid death, while pregnant does commonly aborted (Sadek and Mostufa, 1970; Ghoniem *et al.*; 1971 and Casaro *et al.*, 1979).

The results given in Tables (2, 3) revealed that *Salmonella* could be isolated from one of non aborted does and from 4 each of aborted dead and live does with an incidence percentage 10, 20 and 13.33% respectively. On serotyping of the 9 recovered *Salmonella* organisms from examined samples, 8 (88.9%) of which were recognized as *Salmonella typhimurium* and 1 (11.1%) was untyped. Some authors recorded *Salmonella typhimurium* from aborted rabbits (Harwood, 1989; Boucher and Nouaille, 1996 and Rosell, 2000).

Esherchia coli involved in many health problems in rabbits, they may be direct cause of disease (colibacillosis or colisepticaemia) or associated with other microorganisms. *E. coli* were isolated from 2 (20%), 5 (25%), 5(16.66%) of non aborted does, aborted dead does and aborted live does respectively Table (3). Similar finding were also reported by Boucher *et al.*, (2001).

Staphylococcosis is a commonly occurring disease in both domestic and wild rabbit, this disease is caused by *staphylococcus aureus* and characterized by fatal septicemia or suppurative inflammation in nearly any organ or site. Through the present study, *staphylococcus aureus* was isolated from 2(20%); 4(20%) and 4(13.33%) of examined non-aborted does, aborted dead does and aborted live does respectively Table (3).

Streptococcus has been associated with acute septicemia in rabbits. The results recorded in Table (3) revealed that *Streptococcus pyogenes* was isolated from 2 (20%); 3 (15%) and 2 (6.66%) of examined non-aborted does, aborted dead does and aborted live does respectively.

In vitro sensitivity testing of isolates revealed that most isolates were highly sensitive to enrofloxacin and gentamycin, except *Listeria monocytogenes* was sensitive to ampicillin, amoxicillin and penicillin, nearly similar results were reported by Harwood, 1989; Boisivon *et al.*, 1990, Diker *et al.*, 1994; Abd El-Ghaffar and Abd El-Gwad 1997 and Abd El-Rahman *et al.*, 2005.

Finally, great attention and efforts should be paid to overcome that problem. Such efforts include, medical care of does; eliminating potential sources of infection; special precautions can be instituted to detect and eliminate carrier animals and eliminate vehicle of transmission; complete hygienic measures must be followed in the farms and avoid the misuse of antibiotics.

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