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SURVEY ON BACTERIAL CAUSES OF ENTERIC DISEASES IN YOUNG RABBITS IN ALEXANDRIA GOVERNORATE

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ABSTRACT

A total number of 50 samples from faecal material of rabbits suffering from enteritis were collected from different localities at Alexandria Governorate . Samples were collected as faecal swabs or intestinal content of freshly dead or slaughtetred rabbits aged 1-4 months. Bacteriological examination was done for isolation and identification of bacterial strains causing enteric affections in rabbits ; the isolated microorganisms were E. coli, Proteus, Klebsiella, Pseudomonas aeruginosa, Clostridium perfringens, and Staph. aureus at 80, 18, 12, 6,6,4 % respectivily .Serotyping of isolated E. coli were: O127, O169, O86, O1. Pathogenicity tests of isolated strains revealed that E.coli O127, O169 and Pseudomonas aeruginosa were the most pathogenic causing 100% mortality while E.coli_O1, Proteus, Klebsiella, and Clostredium perfringens caused 66.6 % mortality, E.coli O1 and Staph. aureus caused mortality at 33.3%. The inoculated rabbits showed general signs of enteric disease and trials for reisolation of inoculated strains were conducted. The sensitivity tests of the isolates revealed that Ciprofloxacin was the drug of choice for many of the isolates in this study.

INTRODUCTION

In Egypt, the industry of fattening rabbits plays an important role in facing the major public needs of animal proteins as rabbit's meat is characterized by its palatability and popularity. But this industry faces the enteric diseases which are responsible for high economic losses in rabbit's farms especially in the new born and weaned rabbits ***(Blance et al., 1991)*** . So, interest has been focused on enteric bacteria as a cause of disease and mortality in young rabbits especially at 5 – 10 weeks of age ***(Nikkels et al., 1976)***.

Many species of bacteria were incriminated as a cause of enteritis in rabbits but, *E.coli* was the most prevalent microorganism isolated from the rectum of rabbits suffering from diarrhea ***(Azab, 1992)*** and the strains from diarrhetic weaned rabbits, belonged to at least eight different serotypes of *E.coli* ***(Peeters et al., 1984)***. It was mentioned that, enterotoxigenic *E.coli* lead to infectious diarrhea in the world wide; while strains of *E.coli* which cause diarrhea in rabbits are the classical enteropathogenic *E.coli* ***(walf, 1997)***. In addition , *proteus* species were isolated from rabbits suffering from digestive diseases ***(Ali, 1983)*** and *Klebsiella* could be isolated from diseased or freshly dead rabbits with history of high mortality, diarrhea and lesions of septicemia ***(Hegazy et al., 1992)***. *Pseudomonas aeruginosa* was reported as a cause of infectious diseases of multiple aetiology in farmed rabbits ***(Loliger and Matthes 1989)***. In addition, toxins of *Staph. aureus* play a role of enteritis in rabbits as it is one of the most common causes of intestinal diseases of microbial origin in developing countries ***(Kumar et al., 1997)***. *Cl.perfringens* plays a role in rabbit enteritis depending on

production of toxins type E which considered the etiological agent of rabbit enteritis even if various authors failed to isolate it from the caecum of diseased rabbits ***(Cocchi ,M.et al., 2008)***.

The aim of this study was planned to investigate the bacterial causes of diarrhoea in young rabbits to help farmers to avoid troubles that may face and the economic losses that may arise and the sensitivity tests to different antibiotics drugs.

MATERIAL AND METHODS

1- Collection of samples:

All samples were collected from rabbit's aged 1 – 4 months from farms of different localities at Alexandria governorate . A total number of 50 sample of faecal material (Table ,1) were collected either from intestinal content of freshly slaughtered or freshly dead rabbits or swabs from rabbits with enteric disorder as the symptoms of digestive problems or enteritis in rabbits are simple and constant manifested by decrease in food intake, skin dehydration and quantities of liquid feaces soiling the hind quartes of the rabbit ***(Jones and Duff, 2001)***.

Table (1): Faecal samples collected from different localities at Alexandria Governorate.

Origin	Types of samples	No. of samples
Abbis	Faecal swab	12
Elamria	Faecal material	7
Elmaamora	Faecal material	10
Nubaria	Faecal material	8
Elmalaha	Faecal swabs	13
Total		**50**

2- Bacteriological examination:-

All samples were transferred to the lab where the faecal swabs were kept in tubes with transporting media or about 1-2 gm of the faecal material from diarrhoeic slaughtered rabbits or freshly dead rabbits were obtained from intestinal content.

Each sample was divided into 2 parts. The first part was inoculated into trypticase broth and tetrathionate broth then incubated aerobically at 37°c for 24 – 48 hour. The second part was inoculated into thioglycolate broth and then incubated anarebically in an anaerobic Gas pack jar at 37°c for 24 – 48 hrs.

Aerobically incubated broth were streaked onto nutrient agar, macConkey's agar, eosin methelene blue agar, mannitol salt agar, and S.S.agar to be incubated at 37°c for 24 -48 hours, also subculturing from thioglycolate broth onto TSC agar (Tryptcase sulphate cycloserine) was performed and incubated anarobically in anaerobic Gas pack jar at 37°c for 24 – 48 hours.

Isolated purified colonies were kept onto slope agar then transfered to be preserved on semisolid nutrient agar for further identifications.

The identification of aerobic cultures colonies was carried out according to ***Edwards and Ewing (1972)***, the cultures were examined morphologically and microscopically using Gram's staining technique then the isolates were subjected to series of biochemical tests. The colonies of the plates of anaerobic incubation were examined morphologically and after Gram staining microscopically suspected strains were subjected to biochemical identification according to ***Cruickshank et al.(1975)*** and ***Koneman et al.(1988)***.

3- Serological Tests:

E.coli were subjected to serological identification according to ***(Edwards and Ewing, 1972)***. Agglutination tests were primarily carried out using glass slide technique. A small drop of polyvalent *E.coli* antiserum was thoroughly mixed onto a clean dry slide with a small part of the suspected colony. The presence of any evidence of clumping or grainy appearance indicate a positive agglutination test. A control suspension was similarly prepared at the other end of the glass slide by using sterile saline solution instead of *E.coli*. When a positive reaction was observed with one of the polyvalent sera, testing of the corresponding monovalent sera was carried out.

4- Pathogencity tests:

Thirty five rabbits of 4-6 weeks old were used in the experimental infection which was performed to study pathogenicity of the isolated microorganisms. The rabbits of choice were apparently healthy obtained from commercial rabbit farms in Alexandria Governorate.

The rabbits were kept in cages and observed for a week. Random samples of 5 rabbits were slaughtered and examined bacteriologically to be sure that they are healthy and free from diseases (control). Other rabbits were classified into 10 groups; each group contain 3 rabbits which were given orally one of the isolated microorganism at a dose of 0.5ml of 3×10^{8} cfu/ml while the last group was given sterile normal saline ***(Cantey and Black, 1977)***. During the observation period (one month) clinical signs, post mortum lesions were recorded and trials for reisolations of inoculated strains were conducted.

5- Antimicrobial sensitivity tests:

Disc diffusion method was done according to ***Finegold and Martin (1982)*** by subculturing from the isolated strains broths on Muller Hinton agar, and using antibiotic discs which include: Nitrofuratoin (300 µg), Gentamycin (10 µg), Amoxycillin(10 µg), Cefotaxime (30 µg), Amoxicillin clavulanic acid (30 µg), Ofloxacin (5 µg), polymixine B (300 µg), Ciprofloxacin (5 µg), Neomycin (300 µg), Erythromycin (15 µg), Trimethoprime (25 µg) and Ampicillin (10 µg). The result were interpreted according to ***Quninn et al., (1994)***.

RESULTS

Table (2): Bacteria associated with Enteritis in Rabbit from Farms at Alexandria Governorate:

(Number of samples = 50)

Isolates	No. of isolates	%
E.Coli	40	80
Proteus	9	18
Klebsiella	6	12
Ps.aeruginosa	3	6
Cl. perfringens	3	6
Staph. aureus	2	4
Salmonella	-	-
Total	**63**	

Table (3): Biochemical Identification of Isolated Bacteria:

Negative gram stain bacteria											
	Motility	**Indol**	**Nitrate**	**H2S prod.**	**Oxidase**	**Catalase**	**citrate**	**MR**	**V.P.**	**urease**	**Lactose ferm.**
E.coli	+	+	+	-	-	+	-	+	-	-	+
Proteus	+	±	+	+	-	+	±	+	±	+	-
Klebsiella	-	-	+	-	-	+	+	-	+	+	+
Ps.aeruginosa	+	-	+	-	+	+	+	-	-	+	-

Positive gram stain rod								
Cl.perfringens	Indol	H2S	urease	Haemolysis	manitol	Glucose	Lactose	Maltose
	-	+	±	+	-	+	+	-

Positive gram stain cocci								
Staph.aureus	Cell arrangement	catalase	coagulase	Growth at 9.6	pigment	Haemolysis	Mannitol Ferm.	O.F. medium
	clusters	+	+	+	White, golden yellow	+	+	+

Table (4): Serotypes of E.coli isolated from rabbits suffered from enteritis:

E.coli serotypes	**No.**	**%**
E.coli O127	5	12.5
E.coli O169	5	12.5
E.coli O86	10	25
E.coli O1	5	12.5
Un typable *E.coli*	15	37.5

Table (5): Experimental infection of 4 – 6 weeks old rabbits with pathogenic enteric microorganisms.

Inoculated M.O	**No. of group**	**No. of injected rabbits**	**Total number of deaths**	**% deaths**
E.coli O127	1	3	3	100
E.coli O169	2	3	3	100
E.coli O86	3	3	1	33.3
E.coli O1	4	3	2	66.6
Proteus	5	3	2	66.6
Klebsiella	6	3	2	66.6
Ps.aeruginosa	7	3	3	100
St. aureus	8	3	1	33.3
Cl.perfringens	9	3	2	66.6
Control	10	3	0	0

Table (6): Interpretation of zones of inhibition in agar diffusion method for antibacterial susceptability:

Antibacterial agent	Inhibition zones of isolated bacteria								
	E.coli O127	E.coli O169	E.coli O86	E.coli O1	proteus	klebsiella	Pseudomonas aeruginosa	Staph aureus	Clostridium perfringens
Nitrofuration 300µg	S	HS	S	HS	IM	S	R	R	IM
Gentamycin 10 µg	S	HS	HS	HS	S	IM	S	IM	R
Amoxicillin 10 µg	IM	IM	IM	IM	R	R	R	IM	S
Cefotaxime 30 µg	R	HS	R	HS	IM	IM	R	IM	S
Amoxicillin clavulanic A. 30 µg	IM	IM	IM	S	R	IM	IM	IM	IM
Ofloxacin 5 µg	HS	HS	HS	HS	R	IM	S	IM	S
Polymxin B.5 µg	IM	S	S	S	R	IM	IM	IM	S
CiproFloxacin 5 µg	HS	S	HS	S	S	S	HS	S	IM
Neomycin 30 µg	IM	S	HS	IM	IM	IM	IM	IM	R
Trymethoprime 5 µg	R	HS	R	S	R	IM	R	IM	R
Nalidixic Acid 30 µg	S	R	HS	R	R	IM	R	R	R
Sulphamexazole20 µg	R	R	R	R	IM	R	R	R	R

High sensitive = HS = ++++

Sensitive = S = +++ Intermediate = IM = +++ Resistant = R = -

DISCUSSION

Enteritis is the major cause of diseases in commercial rabbit industry. Diet, stress and management factors are known to affect the incidence and the spread of enteric disease . The rapid effect of these factors could be related to changes in the treatment and prophylactic protocols used in the farms ***(Boucher and Nouaille 1999)***.

Incidence of isolation:

Isolation and identification of bacteria from examined samples revealed that, most conditions of diarrhea in rabbits are due to the infection by Enterobacteriacea while ***Ramirez et al., (2005)*** found that the most conditions of diarrhea in rabbits is multifactor.

And in this research the most prevalent bacteria was *E.coli* followed by *Proteus*, *Klebsiella*, then *Pseudomonas aeruginosa*, *Clostridium perfringens* and finally, *Staph.aureus* at an incidence 80%, 18%, 12%, 6%, 6%, and 4% respectively (Table , 2).

The high ratio of *E.coli* was similar to that obtained by ***Abdel – Rhman et al., (2005)*** 80%, but higher than that obtained by ***Abdel Gwad (1988)*** 33%, ***Blanco et al., (1994)*** 74%, ***Abdel- Nasser (1998)*** 27.6% , and ***Azhar et al., (2009)*** 45%. The incidence of *Proteus* (18%), *Klebsiella* (12%), *Pseudomonas aeruginosa* (6%) were higher than that obtained by ***Al-Shaimaa (2007)***, who isolated them at 14.8%, 7.4%, 2.1% respectively. *Staph. aureus* was isolated at 4%, this was lower than that obtained by ***Azhar et al., (2009)*** 20%.*Cl.perfringens* was isolated at 6% which is lower than that obtained by ***Abdel- Rhman et al., (2007)*** where they recorded *Cl.perfringens* in diarrhotic rabbits at 33.75%.

Table (3) illustrated the biochemical tests characteristic for different isolates that in agreed with ***Abdel – Rhman et al., (2007) Al-Shaimaa (2007),*** and ***Azhar et al., (2009)***.

Concerning serotyping (Table ,4) illustrated that 25 out of 40 isolate of *E.coli* were pathogenic and represented as 12.5% O127, 12.5%O169, 25%O86, 12.5% O1 while ***Al-Shaimaa (2007)*** typed *E.coli* as O111, O114, O125 But ***Abdel- Rhman et al., (2005)*** typed *E.coli* as O26, O44, O59, O114, O126, O127, O128.

It is enterest that in pathogenicity tests (Table ,5) revealed that *E.Coli* O127, *E.Coli* O169, and *Pseudomonas aeruginosa* were found to be highly pathogenic to rabbits, while isolated *E.Coli* O1, *Proteus*, *Klebsiella* , and *CL.perfringens* caused mortality rate 66.6%, *E.Coli* O86 and *Staph* . *aureus* were the less pathogenic in this study, they cause mortality rate at 33.3%. ***Compos et al.,(1994)*** reported that *E.coli* serotypes may classified as EPEC causing out breaks of diarrhea, while ***Roa and Char (1986)*** studied 232 strains of *E.Coli* from rabbits and identified different O groups of *E.Coli* and some of which were of zoonotic importance.

In table (6), It was found that E.coli serotypes were sensitive to Nitrofuratoin,Gentamycin, Ciprofloxacine but were resistant to Sulphamexazole and that agree with ***Al-Shaimaa (2007)*** who found O114, O125 were highly resistant to Sulphamexazole.

It was noticed that *Proteus* isolates were sensitive only to Gentamycin and Ciprofloxacin but resistant to many antibiotics. *klebsiella* were sensitive to Ciprofloxacin and NitroFuratoin but resistant to Amoxicillin this result is nearly similar to that reported by ***Dhand et al.,(2001)***.

Pseudomona s aeruginosa reported high resistance to many antibiotics, while it was highly sensitive to Ciprofloxacin, Sensitive to Gentamycin and Ofloxacin.

Ciprofloxacin was the drug of choice for *Staph .aureus* which reported high resistance to other types of antibiotics.

Cl.Perfringens was sensitive to Amoxicillin, Polymxin B, and Ciprofloxacin but resistant to Nalidixic acid, Gentamycin and Neomycin and these agreed with that reported by ***Abdel-Rhman et al., (2007).***

Finally, it was recommended that, efforts must be continued to detecte causes of diarrhea in rabbits with improving the diagnosis and hygienic measures to help prevention of the disease in weaned rabbits.

Paying attention to the use of antibiotics is recommended to be of value in treatments.

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استقصاء الأسباب البكتيرية للأمراض المعوية فى الأرانب الصغيرة فى محافظة الإسكندرية

أجريت هذه الدراسة على عدد 50 من الأرانب التى تتراوح أعمارها من 1-4 شهور وكانت تعانى من الإسهال والنزلات المعوية و قد تم تجميع عينات البراز للفحص البكتريولوجى فكانت أما مسحات أو عينات براز من أرانب تم ذبحها اضطراريا او نفقت حديثا. وتم أخذ العينات من مناطق مختلفة بمحافظة الاسكندرية. أنتج الفحص البكتريولوجى عزل ميكروبات الايشيرشيا كولاى، البروتيس، الكلبسيلا، السودوموناس ارجينوزا، الكلوستروديم بيرفرنجنز، والميكروب الذهبى العنقودى بنسب :80-18-12-6-6-4 % على التوالى ولم يتم عزل ميكروب السلمونيلا فى هذه الدراسة. تم إجراء الاختبارات السيرولوجية لميكروبات الايشيرشيا كولاى حيث تواجدها كان الاعلى نسبة فى المعزولات فصنفت سيرولوجيا. وتم فحص ضراوة العترات المصنفة من الميكروب القولونى O127 – O169 – O86 – O1.

والعترات الاخرى المعزولة على أرانب عمر 4-6 اسابيع فكانت نسبة الوفيات 100% فى حالة الميكروبات O127 – O169 و السودوموناس ايروجينوزا بينما وصلت الى6. 66% فى حالة ميكروباتO1 – البروتيس – الكلبسيلا – والكلوستروديم بيرفرنجنز, على الجانب الاخر وصلت نسبة الوفيات الى 3 .33% فى حالة الميكروب O86 والميكروب العنقودى الذهبى. هذا و قد تم تسجيل الاعراض الاكلينيكية و الفحص التشريحى بعد الوفاة و بإجراء اختبارات الحساسية على العترات المعزولة تبين ان معظم الميكروبات لديها حساسية للسيبروفلوكساسين لكن لوحظ ان المعزولات لديها مقاومة واضحة لكثير من المضادات الحيوية المستخدمة لذا يجب انتقاء علاجات فعالة من اجل الحصول على النتيجة المطلوبة لمقاومة المرض.