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ISOLATION AND PATHOGENICITY OF INTESTINAL PATHOGENS ASSOCIATED WITH THE ENTERITIS COMPLEX IN RABBITS WITH SPECIAL REFERENCE TO E.COLI AND SALMONELLA SPP

(With 7 Tables)

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عزل وضراوة البكتريا المعوية الممرضة المصاحبة للنزلات المعوية في الأرانب مع الإشارة الخاصة لميكروبي السالمونيلا والأيشرشيا كولاى

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لدراسة البكتريا المعوية المصاحبة للنزلات المعوية فى الأرانب تم تجميع ٢٠٠ عينة مسحات بكتريولوجية من فتحة المستقيم ومحتويات الأمعاء من أرانب مصابة بالأسهالات وبعضها حديث النفوق ومذبوح (٨٠ عينة من أرانب حديثة الفطام و١٢٠ من أرانب بالغة مصابة بالأسهالات) بالإضافة الى٢٠ عينة من فتحة المستقيم لأرانب سليمة ظاهريا لدراسة المسببات البكتريولوجية للإسهال في الأرانب مع التركيز على ميكروبيي الأيشريشيا كولاى والسالمونيلا مع دراسة شدة ضراوتهما وعمل اختبار الحساسية للعترات المعزولة. وقد أظهرت نتائج الدراسة عن عزل ميكروب الأيشريشيا كولاى والسالمونيلا مصحوبة بأنواع مختلفة من الميكروبات المعوية الأخرى بنسب مختلفة وكانت نسبة ميكروب الأيشريشيا كولاى في الأرانب المصابة ٨٠% بينما كانت ٣٠% في الأرانب السليمة ظاهريا من إجمالي العينات المختبرة وكانت نسبته في الأرانب حديثة الفطام ٨٧،٥% وفى الأرانب البالغة ٨٣،٣% والنسبة المئوية في الصيف ٦٧،٥% والشتاء ٢٣،٥% وتم تصنيف عترات الميكروب القولوني سيرولوجيا إلى 10 (B_{12}) , K_{67} : O_{128} ; 12(7,1%) O_{119}/B_{14} ; 15(8.8%) (B) O_{103} ; 15(8.8%) $O_{55}/K59$ (B_5) ; 7(4.1%) O_{78}/K_{80} (B_1) ; 8(4.7%) (B_{16}) , K_{71} :O_{126} ; (5.9%) و٩٠ غير مصنف. وتم عزل ميكروب السالمونيلا من الأرانب المريضة والنافقة بنسبة ٥،٥% لسالمونيلا تيفميريم ٨،٣% لسالموبيلا انتيريدز بينما لم يتم عزلها من الأرانب السليمة ظاهريا. وبأجراء اختبارات الضراوة للعترات المعزولة من ميكروبيي الأيشريشيا كولاى والسالمونيلا على الأرانب في المعمل بواسطة الحقن عن طريق الفم وجدت أنها ضارية للأرانب حيث بلغت نسبة النفوق

من ٦٠% الى ١٠٠% وقد سجلت الأعراض الإكلينيكية والآفات التشريحية ووجدت أنها تشبه إلى حد كبير تلك التى سجلت في العدوى الطبيعية وتم عزل الميكروب مرة أخرى منها . كما تم عمل اختبار الحساسية للميكروبات المعزولة حيث كان ميكروب الأيشريشيا كولاى حساس لكل من الجنتاميسين والأنروفلاكساسين والكولوستين سلفات بينما كان مقاوم لكل من الأميسللين والأيرثرومايسين والأستربتومايسين والأموكسسللين بينما كان ميكروب السالمونيلا حساس لكل من الجنتاميسين والأنروفلاكساسين والكولوستين سلفات والأمبسللين والنيومايسين سلفات بينما كان مقاوم لكل من الأيرثرومايسين والأستربتومايسين والأموكسسللين. وتم مناقشة الأهمية الصحية من تواجد ميكروبيي الأيشريشيا كولاى والسالمونيلا في الحالات المصاحبة للأسهالات في الأرانب

SUMMARY

A total of 200 intestinal content and cloaca swabs were collected from a 80 recently weaned rabbit and 120 adult freshly dead and sacrificed rabbits suffered from diarrhoea, addition 20 cloaca swab were collected from apparently healthy rabbits. These samples were collected from privately owned rabbitaries at EL- Minia and Assiut Province for P.M and bacteriological examination. The clinical examination and postmortem lesions revealed to depression, anorexia, exhaustion, rough coat, profuse watery diarrhoea, catarrhal enteritis, peticheal haemorrhages on the internal organs and enlargement of liver and spleen. The bacteriological examination from all examined samples revealed that the highest percentage of E.coli (80%) followed by P. mirabilis (27.3%), Enterobacter cloacae (22.3%) Akaligenes (16.4%), Klebsiella (13.6%) Citrobacter (8%) while Salmonella typhimarium was (5.5%) and Salmonella enteritidis was (8.2%), while could not isolate from apparent healthy rabbits. The incidence percentage of E.coli in summer was (76.5%) while in winter was (23.5%) and Salmonella typhmarium and Salmonella enteritidis were (75%) and (77.8%) in summer while were (25%) and (22.2%) in winter respectively. The most common serotypes of E.coli in order of frequency were O_{55}/K59 (B_5) 15 (8.8%); O $_{103}$ (B) 15 (8.8%); O_{119}/B_{14} 12 (7.1%) ; O_{128}:K_{67},(B_{12}) 10 (5.9%); O_{126}:K_{71}, (B_{16}) 8(4.7%); O_{78}/K_{80} (B_1) 7 (4.1%) and 90 untypable. For virulence assay of E.coli showed that 82.3% of E.coli isolates were Congo red positive (+ve CR) and 17.6% were Congo red negative (-ve CR), while haemolytic activity revealed that (52.9%) of E.coli isolates were positive for haemolytic activity from diarrhoea rabbits, while was (100%) negative haemolytic activity from apparently healthy rabbits. Results of experimental infection of the susceptible animals it is showed that the isolated strains were pathogenic with

mortality rates ranging from 60% to 100% and the clinical symptoms and post-mortem pictures of inoculated rabbits are similarly that showing in examined diarrhoea rabbits in our work. Sensitivity test for the isolates revealed that E.coli strains were highly resistant to Ampicillin, Erythromycin, Streptomycin, Chloramephenical and Amoxycillin, while it is highly sensitive to Enrofloxacin, Gentamycin and Colistine sulphate. Salmonella isolates were more susceptible to Ampicillin, Neomycin sulphate; Gentamycin and Enerofloxacin while are resistant to Erythromycin, Streptomycin and Amoxycillin.

Key words: *Rabbits, Enteritis, E. coli, Sulmonella.*

INTRODUCTION

During the recent years, interest has been focused on diarrhaeo in rabbits, since it is responsible for high economic losses. Broiler rabbits are extremely sensitive to diseases of digestive tract mainly diarrhoea and enteritis which occur specially in newborn and newly weaned rabbits since this condition is responsible for major losses in commercial rabbits (Blanco *et al.*, 1994). E.coli infection is the primary causative agent in most outbreaks of diarrhoea in newly weaned rabbits (Percy *et al.*, 1993). Walf (1997) reported that the enterooxogenic E.coli (ETEC) is leading to infectious diarrhoea world wide, while all E.coli strains cause diarrhoea in rabbits are the classical enteropathogenic E.coli (AEEC). Newton *et al.* (2004) reported that the E.coli was the predominant microorganism isolated from ligated colon and caceum of diarrhoea rabbits Rosario *et al.* (2004) regarded that the most conditions of diarrhoea diarrhoea in rabbits as an enteric infection by enterobacteriaceae, while other outhers agree that the aetiology of the diarrhoea in rabbits is multifactor (Ramirez *et al.*, 2005). Ibrahim (1985) could isolate E.coli belong of to 5 serotypes (O_{128}:K_{67},(B_{12}), O_{126}:K_{71}, (B_{16}), O_{59}:K_{59} (B_5), O_{124}:K_{72} (B_{17}), and O_{26}:K_{60} (B_6). from dead rabbits (6-8 week-old) suffering from intestinal disorders. Okerman *et al.* (1999) isolated E.coli strain from newly weaned rabbits suffering from profuse watery diarrhoea with 23% mortality. Camguilhem *et al.* (1986) isolated E.coli serotype O_{103} from sever outbreak of diarrhoea with 50 -80 % mortality in rabbits aged 5-8 weeks rabbits with enteritis. Mohamed *et al.* (2002) could isolated E.coli belong to 5 serotypes (O_{119}, O_{103}, O_{55}, O_{153}, O_{128} and untype one) from rabbits dirrhoea at percentage of 77.27%from total examined samples. El-Boushy *et al.* (2005) isolated E.coli sero type O_{103} from internal organs of rabbit's dirrhoea. Saad

(1970) isolated Salmonella from 13 dead rabbits suffering from enteritis with frequency of 0.97%, he found S.typhimarium in 10 cases and S.pullorum in three cases. Ghoniem *et al.* (1971) could isolate of S. typhmarium, S. heidelbrug, S. hidalgo and S. pullorum from rabbit diarrhoea. Joshi and Sardeshpende (1980) isolated 37 cases of Salmonella from 82 rabbits, which died during an outbreak of salmonellosis. Abbassi *et al.* (1966) isolated Proteus and Klebsiella from normal and disease rabbits suffering from diarrhoea. Mcleod and Katz (1986) and Abdel-Gwad (1988) isolate E.coli, Pr. Mirabilis, Citrobacter and Klebsiella from caecum of rabbit with mucoid enteritis.

The objective of this study was the investigation of bacterial causes of diarrhoea in rabbits and detection of virulence of isolated E.coli and Salmonella by different methods and antibiogram sensitivity test.

MATERIALS and METHODS

A- Samples:

A total of 200 intestinal contents and cloaca swabs were collected from a 80 recently weaned rabbit and 120 adult freshly dead and sacrificed rabbits suffering from diarrhoea, additional 20 cloaca swab were collected from apparently healthy rabbits of various age These samples were collected from privately owned rabbitaries at EL-Minia and Assiut Province for P.M and bacteriological examination

B- Isolation and Identification

Loopfuls of feacal samples and intestinal contents were collected a aseptically and directly transferred to modified tetrathionate broth as well as selenite F. broth, and incubated at 37^0C for 18-24 hr., then streaked onto MacConkay[s], brillant green phenol red agar and S.S agar as well as Eosine-methylene blue agar (Oxoid Manual, 1982). The inoculated plates were incubated for 24-48 hr. at 37^0C. Suspected colonies from the different media were screened morphologically, biochemically according to (Edward and Ewing, 1972, Buchanan and Gibons, 1974 Crucickshank, *et al.* 1982 and Speck, 1984) and those suggestive of Salmonella and E.coli were confirmed serologically

C- Serological tests: -

1- Serological identification of E.coli:- Identified strains of E.coli suspected isolates were serologically identified after their purification by determination of the "O" and "K" group antigen were serologically investigated by the slid agglutination technique for the determination of the enteropathogenic strains according to (Edward and Ewing, 1972).

The elimination of nonspecific-shared antigen was carried out by heating the bacterial suspensions in a water bath at 100^0C for 60 min. The sera used were purchased from Behring werk, AG Marburg, Labn, Germany. O_{26}:K_{60} (B_6) ; O_{55}:K_{59}(B_5); O_{59}:K_{59} (B_5); O_{78}:$K_{80\ (B1)}$; O $_{103}$ B; O_{114}/K_{90}; O_{119}/B_{14}; O_{124}:K_{72} (B_{17}); O_{125}/B_{15}; O_{126}: K_{71}, (B_{16}), O_{127}/B_8; and. O_{128}:K_{67},(B_{12}).

The test sera were diluted in their vials with saline solution. The polyvalent sera were diluted 1:2 and the monovalent 1:4. A loop of bacterial culture was placed on clean dry slide in addition to one drop of diluted test sera and carefully mixed with bacterial mass without formation of lumps. The slide was then carefully tilled for thorough mixing and agglutination results were read within 2 minutes. Each isolate was tested with 2 polyvalent sera, 1 and sera, 11. The appurtenance of any evidence of clumping or marked smooth agglutination, indicated a positive agglutination test, delayed or partial agglutination was considered as negative or false agglutination results. In case of positive agglutination with the polyvalent sera 1and 11, the isolate was similarly tested with the monovalent sera. A control suspension was similarly prepared at the other end of glass slid, using sterile saline solution instead of E.coli antiserum.

2- Serological identification of salmonella "O" and "H" antigens as well as the phase of the organism were detected by using Agglutination sera test was carried by the glass-slid technique according to the modified Kauffmanns and White scheme described by (Mcwhorter *et al* 1977). Suspected culture was mixed thoroughly with a drop of saline on clean slide. A small drop of polyvalent Salmonella antisera was mixed thoroughly with the bacteria suspension by tilting the slide for one minute. Positive agglutination was recognized by formation of fine granules or large aggregate, delayed or partial agglutination was considered as negative or false agglutination. Cultures which gave positive results were similarly tested using monovalent group- for determination of specific "O" antigen and within group of "H" antigen both phase,1 and phase 11

The sera used were purchased from Wellcome Research Laboratories Beckengham, England. The serological tests were carried out in Microbiology Dep. Fac. of Med. Assiut Univ.

D- Virulence Assay of E.coli: -

a) Haemolysis assay: -

E. coli isolates were propagated on blood agar base supplemented with 5% washed sheep erythrocytes. Blood agar plates

then incubated at 37 ^{O}C for 24 hrs and colonies producing clear zones of haemolysis were then recorded as hemolysin positive (Heller and Drabkin, 1977, Vidotto *et al.*, 1990).

b) Congo red Binding Assay: -

The medium used for determination of Congo red binding of the isolates was prepared according to (Berkhoff and Vinal, 1986). Trypticase Soy Agar was supplemented with 0.003% Congo red dye (Sigma) and 0.15% bile salts. Each isolate was cultured on a separate plate and incubated at 37^{O}C for 24hrs. After 24hrs incubation, the cultures were left at room temperature for 48hrs Invasive E. coli were identified by their ability to take up Congo red dye. The positive isolates produced red colonies. The negative isolates appeared as colorless

E-Experimental infection design:

Studies on the pathogenicity of the isolated organisms in rabbits:

The experimental was performed to study the pathogenicity of the isolated microorganisms including E.coli O_{55}:$K_{59}(B_5)$ and S.typhmarium. A total of 35 rabbits (6-8 week-old) apparently healthy obtained from commercial rabbit farms in EL-Minia Province were used in the pathogenicity and experimental studies. The animals were kept in cages and observed for a period a week. A random samples of 5 rabbits were slaughtered and exposed to post-mortem, parasitology and bacteriological examination, which proved their healthy status and free from diseases and the other rabbits were classified in to 3 groups Each group contain 10 rabbits. Ten isolates cultures of each E.coli and S.typhmarium were collected by centrifugation and resuspended in a saturated solution of $NaHCO_3$ in water. The first group was inoculated by oro-gastric with ($4x10^{10}$/CFU/ml.) of E.coli. and the second group was the same inoculated with S.typhmarium ($1x10^{8}$/ CFU/ml), while the last group was inoculated with sterile normal saline (Cantey and Black, 1977). During the observation period (one month) clinical signs, P.M lesions were recorded and trials for reisolations of inoculated strains were conducted.

E-Antimicrobial sensitivity discs (Oxoid Laboratories):

Disc diffusion method was done according to Finegold and Martin (1982) to E.coli and Salmonella isolates on Muller Hinton agar using antibiotic discs produced by Oxoid LTD, London, England, (Oxoid Manual, 1982) including Ampcillin (10ug), Amoxicillin (25ug) Gentamycin (10ug) Neomycin (30ug), Enerofloxacin (5ug), Amoxycillin (25ug), Colistine sulphate (25ug), Erythromycin (15ug), Oxytetracycline (30ug), Streptomycin (10ug), Chloramephenical (30ug)

and Nalidixic acid (30ug). The results were interpreted according to Quinn *et al.* (1994).

RESULTS and DISCUSSIONS

Identification of the isolates:

A total of 200 intestinal content and cloacae swabs were collected from a 80 recently weaned rabbits and 120 adult freshly dead and sacrificed rabbits suffered from diarrhoea, addition of 20 cloacae swab were collected from apparently healthy rabbits of various age These samples were collected from privately owned rabbitaries at EL-Minia and Assiut Province for P.M and bacteriological examination

During recent years was directed to study diarrhoea in rabbits since this condition is responsible for major losses in commercial rabbis, where the E.coli infection was the most serious problem among the enteric diseases in rabbits (Newton, 2004).

Clinical signs: The main clinical sings of the affected rabbits were depression, anorexia, exhaustion, rough coat, a perineal area covered with greenish brown faecal material and profuse watery and other mucous diarrhoea, dehydration and often a bloated abdomen.

P.M lisons: The postmortem examination revealed that the most examined cases showed catarrhal enteritis, peticheal haemorrhages on the internal organs and congested of liver and spleen, The faeces is often covered with mucus and mucus fluid-filled caecoum. The intestine was congested, edematous and in some cases distended with gas and enlarged mesenteric lymph nodes. The clinical sings and postmortem lesions in the present work are completely in agreement with those previously described by (Awaad, 1972; Peeters, 1994 and Okerman, 1999).

Bacteriological examination: -

Concerning the results of bacteriological examination of infected rabbits resulted indicated isolated of different pathogenic strains of bacteria instullated in Table (1) It is clear that the highest percentage of E.coli (80%) followed by P. mirabilis (27.3%), Enterobacter cloacae (22.3%) Akaligenes (16.4%), Klebsiella (13.6%) while Salmonella typhimarium was (5.5%) and Salmonella enteritidis was (8.2%), and Citrobacter (8%) from all examined samples. Also show in Table (1) it is indicated that the incidence of E.coli isolated from the recent weaned rabbits was (87.5%) is higher than isolated from adult rabbits (83.3%) while Salmonella typhmarium and Salmonella enteritidis isolated from

adult rabbits were (8.3%) and (10.0%) is higher than obtained from recent weaned rabbits (2.5%) and (7.5%) respectively. One or more species of the above-mentioned organisms that isolated at different percentage, a partial similarly that were previously isolated from rabbits diarrhoea by several authors (Abbassi *et al.* (1966); Saad, 1970; Ghoniem *et al.*, 1971, Ibrahim, 1985. Mcleod and Katz (1986); Abdel-Gwad, 1988 and Aisha and Yousief, 1999). From Table (2) It is revealed to the incidence of the isolates from diarrhoea rabbits during seasons of summer and winter. It is indicated that the total isolate in summer was 274 isolates while in winter was 105 isolates. The incidence percentage of E.coli in summer was (76.5%) while in winter was (23.5%) and Salmonella typhmarium and Salmonella enteritidis were (75%) and (77.8%) in summer while were (25%) and (22.2%) in winter respectivily. The higher incidence of isolates in summer than winter related to many factors contribute to heat stress, lack of drinking water and reduce feed intake resulted in decrease caecal volatile fatty acid and increase pH of intestine leading to immunosupperssion which favour of E.coli and other microorganisms proliferation causing diarrhoea (Peeters *et al.*, 1984b).

Serological identification of E.coli and Salmonella isolated: -

The aim of this study was identified of the serotypes of suspected E.coli and Salmonella isolates from examined rabbits. Serotype of E.coli isolates was carried out by slid agglutination test using coli test –sera anti O-K group antigen while Salmonella suspected isolates were serologically typed agglutination technique using both Polyvalent and Monovalent antisera against O and H Salmonella antigen. E.coli infection is the primary of diarrhoea causative agent in most outbreaks of diarrhoea in rabbits (Peetera *et al.*, 1984 and Percy *et al.* 1993). From our results in Table (1) the E.coli was the most frequently isolates (85%) which considered the main cause of diarrhoea in newly weaned rabbits and adults, the obtained results are higher than that recorded by Asdrubali *et al.*, 1977 (70%); Ibrahim, 1985 (16.9%); Abdel-Gwad, 1988 (33%); Banco *et al.*, 1997 (74%); Aisha and Yousief, 1999, (42%) while nearly agreement with the results obtained by Mohamed *et al.*, 2002 (80%) who recovered E.coli from diarrhoea rabbits. From Table (3) It is clear that 80 out of 170 suspected E.coli strains could be identified serologically into 13 serotypes and 90 untypes. The most common serotypes in order of frequency were O_{55}/K59 (B_5) 15 (8.8%); O $_{103}$ (B) 15 (8.8%); O_{119}/B_{14} 12 (7.1%); $O_{128}:K_{67}$,(B_{12}) 10 (5.9%); $O_{126}:K_{71}$, (B_{16}) 8(4.7%); O_{78}/K_{80} (B_1) 7 (4.1%).

Most of these serotypes of E.coli isolated from diarrhoea rabbits in our results were agreement which recovered by several authors Matthes (1969) detected O_{55} and O_{44}, Saad (1970) isolated O_{119}:K_{64} (B_{14}) and O_{59}: k:$_{59}$ (B_5), Ibraim (1985) revealed that the isolates belonged to "5" serotypes :O_{55}:k_{69} (B_5), O_{26}:k_{60} (B_6), O_{28}:K_{67} (B_{12}), $O_{124}K_{72}$(B_{17}) and $O_{126}K_{71}$ (B_{16}) were associated with Colibacillosis in rabbits. Recently Peeters *et al.* (1988) have characterized over 500 strains of E. coli isolated from healthy and diarrheic rabbits. Abdel-Gwad (1988) isolated "7" serotypes of E.coli in diarrhoea rabbits belonged to O_{78}:K_{80}(B), O_{26}:K_{60}(B_6), O_{111}:K_{58}(B_4), O_{44}:K_{71}(L), O_{119}:K_{69}(B_{14}) and O_{55}:K_{59}(B_5) and 12 untypes. Percy *et al.* (1993), Blanco *et al.* (1994), Leroy *et al.* (1994) and Zienab (2000) isolated O_{103}, O_{128}, O_{119}, O_{55} and untyped from newly weaned diarrhoea rabbits. Azza (1999) recovered O_{119}, O_{128}, Aish and Youseif (1999) isolated O_{128} and untyped, while Blance *et al.* (1991), Saad (1994) and Jakeen *et al.* (1999) reported thatO_{55} and O_{153} were associated with newly weaned rabbit diarrhoea. Okerman (1999) declared that several E.coli strains of varying virulence cause diarrhoea in weaned rabbits belong to different serotypes (O_{15}, O_{103}, O_{119}, O_{26} and O_{132}).

Virulence Assay of E.coli:-

The result of detection of virulence of E.coli by using Congo red binding assay and haemolytic activity are shown in Table (4) The result of Congo red assay showed that 82.3% of E.coli isolates were Congo red positive (+ve CR), and 17.6% were Congo red negative (-ve CR), while E.coli strains isolated from apparently healthy were 100% of negative Congo red assay (-ve CR). The haemolytic activity studies revealed that 90 (52.9%) out of 170 E.coli isolates were positive for haemolytic activity E.coli Berkhoff and Vinal (1986), Vidotto *et al.* (1990) and Style and Flamer (1991) suggested that there is a positive association between the (+ve CR) E.coli and diarrhoea rabbits. Walmrch et al. (1994) and Okerman (1999) reported that performing haemolysin of E.coli considered an important virulence factor in E.coli infection in rabbits while Wooly *et al.* (1992a) found that there is no connection between virulence of E.coli and their production of haemolysin.

Seriological identification of Salmonella isolated:-

Salmonellosis in rabbits is characterized by septicemia, acute enteritis and rapid death, while pregnant does commonly abort Sadek and Mostufa (1970), Ghoniem *et al.* (1971) and Casaro *et al.* (1979) isolated S.typhmerium from diarrhoea rabbits. In Table (5) it is showed that the total number of salmonella isolates was 30 (15.0%) recovered

from 200 samples of dead and diarrhoea rabbits out of these 12 (6.0%) Salmonella typhimarium and 18 (9.0%) Salmonella enteritidis, while could not isolate from apparent healthy rabbits. The incidence (15.0%) is higher than reported by Saad (1970) 1.9% and Awaad *et al* (1972) 0.79% while lower than obtained by Joshi and Sardeshpende (1980) who isolated 37 cases of Salmonellosis from 82 samples of outbreak of diarrhoea rabbits at incidence of 45.1% and Abdel-Gwad (1988) isolated salmonella from diarrhoea rabbits at incidence of (18.96%)

Results of pathogenicity test:

Results of experimental infection of the susceptible animals instullated in Table (6) it is showed that the isolated strains were pathogenic with mortality rates ranging from 60% to 100%. The clinical symptoms and post-mortem pictures of inoculated rabbits are similarly that showing in examined diarrhoea rabbits in our work and agree with reported by the author Casararo *et al.* (1979) who inoculation five rabbits by mouth with S.typhimarium isolated from rabbits, three of them developed the disease and died between 7^{th} to 10^{th} day with symptoms of acute diarrhoea and lesion of enteritis. Kuman and Singh (1980) produce diarrhoea in young rabbits by injection with O_{15} and O_{22} pathogenic serotypes through the orogastric route. Abdel-Gwad (1988) reported the nearly the same results which we obtained from inoculated the rabbits with E.coli (O_{26}/K_{60} (B_6) and Salmonella typhmarium.

Antimicrobial sensitivity test:-

The extensive use of antibiotics as growth promoters and prophylactic agents for disease control in veterinary medicine has undoubtedly been responsible for large numbers of bacteria that have become resistant to different antibiotics. Antibigram is necessary because many strains of E.coli are resistant to antibiotic commonly used in rabbits (Okerman, 1999). Results of the antibiotic susceptibility pattern of E.coli are clearly shown in Table (7). E.coli strains were highly resistant to Ampicillin, Erythromycin, Sterptomycin, Chloramephenical and Amoxycillin, while they was highly sensitive to Enrofloxacin, Gentamycin and Colistine sulphate, these finding are in agreement with observation by Nicolas *et al.,* (1984) Erganis *et al.* (1989), Moharana *et al.* (1993) and Jakeen *et al* (1999), they found that most E.coli strains were resistant to Ampicillin, Erythromycin, Chloramephenical and Tetracycline while they sensitivity to Enrofloxacin, Gentamycin, Colistine sulphate. Antimicrobial resistance of Salmonella isolates was common and the plasmid may play a role in this resistance. As illustrated in Table (7) Salmonella isolates were more

susceptible to Gentamycin, Enerofloxacin Ampicillin and Neomycin sulphate, while are resistant to Erythromycin, Streptomycin, Oxytetracycline and Amoxycillin, these finding are in agreement with obtained by Hoda (1994) and Abou-zaid *et al.* (2002). They recorded that Salmonella typhimurium were sensitivity to Gentamycin, Ampicillin, Nalidixic acid and Neomycin.

It was concluded that the high mortality among diarrhoea rabbits were mainly attributed to virulence serotypes of E.coli. Therefore it was recommended that: to employ the biochemical parameters, pathogenicty and serological tests for the diagnosis of diarrhoea rabbits. Disease

Table 1: Incidence of bacteria isolates from 220 of intestine content and cloacal swabs of Diarrhoeic rabbits and Apparently healthy

Bacteria isolates	Apparently healthy (n=20)		Diarrhoeic rabbits (n=200)				Total	
			Recent weaned rabbits (No. = 80)		Adult rabbits (No.= 120)			
	No.	%	No.	%	No.	%	No.	%
E.coli	6	30	70	87.5	100	83.3	176	80.0
S. typhimarium	0	0.0	2	2.5	10	8.3	12	5.5
S. enteritidis	0	0.0	6	7.5	12	10.0	18	8.2
P.mirabilis	2	10.0	17	21.25	41	34.2	60	27.3
Enterobacter cloacae	2	10.0	18	22.5	29	24.2	49	22.3
Citrobacter	3	15	1	1.25	15	12.5	19	8.6
Akaligenes	1	5.0	7	8.75	28	23.3	36	16.4
Klebsiella	2	10	10	12.5	18	15.0	30	13.6
Total	16		131		253		400	

Table 2: The incidence of the bacteria isolates from the 200 intestinal contents and cloacal swabs of rabbit's diarrhoea (80 recent weaned rabbits and 120 adult rabbits) during summer and winter seasons:

Bacteria isolates	No. of +ve	%	Seasons			
			Summer		Winter	
			No.	%	No.	%
E.coli	170	85	130	76.5	40	23.5
S. typhimarium	12	6	9	75	3	25
S. enteritidis	18	9	14	77.8	4	22.2
P.mirabilis	58	28	45	80.4	13	19.6
Enterobacter cloacae	47	22	32	68.1	15	31.9
Citrobacter	16	8	8	50.0	8	50.0
Akaligenes	35	16	28	87.5	7	12.5
Klebsiella	28	14	8	28.6	20	71.4
Total	384		274		110	

Table 3: Incidence of serotyping of E.coli isolated from Diarrhoeic and Apparently healthy rabbits

O serotyp of E.coli	Diarrhoeic rabbits		Apparently healthy	
	No.	%	No.	%
O_{26}:K_{60} (B_6)	4	2.4	1	16.6
O44/K71 (L)	5	2.9	0	0.0
O_{55}/K59 (B_5)	15	8.8	0	0.0
O_{78}/$K_{80\ (B1)}$	7	4.1	0	0.0
O_{59}:K_{59} (B_5)	0	0.0	1	16.6
O_{103} B	15	8.8	1	16.6
O_{114}/$K_{69\ (B14)}$	2	1.2	0	0.0
O_{119}/B_{14}	12	7.1	0	0.0
O_{124}:K_{72} (B_{17})	2	1.2	0	0.0
O_{125}/B_{15}	0	0.0	0	0.0
O_{126}:K_{71}, (B_{16})	8	4.7	0	0.0
O_{127}/B_8	0	0.0	0	0.0
O_{128}:K_{67},(B_{12})	10	5.9	2	33.3
Untypable	90	52.9	1	16.6
Total	170		6	

Table 4: Detection of virulence factors using Congo red medium and haemolytic activity of E.coli strain isolated from apparently healthy and diarrhoea rabbits

Source of isolation	No. of tested strain	Red Colony CR (+ve)		Colorless Colony CR (-ve)		Positive haemolysis		Negative haemolysis	
		No.	%	No.	%	No.	%	No.	%
Diarrhoea rabbits	170	140	82.4	30	17.6	120	70.6	50	29.4
Apparently healthy	6	0	0.0	6	100	0	0.0	6	100

Table 5: Incidence of serotypes strain of Salmonella isolated from 80 recent weaned rabbits and 120 adult diarrhoeic rabbits

Salmonella serotypes	Serotype	No. of isolates		Antigenic formula		
				O antigen	H antigen	
		No.	%		Phase1	Phase2
S. typhimarium	B	12	6.0	1,4, (5).12:1:	1	1,2
S. enteritidis	D1	18	9.0	1,9, 12{vi}:	g , m	{1,7}
Total		30	15.0			

Table 6: Showing of results of pathogenicity of E.coli and S. typhimarium isolates from diarrhoea rabbits

Groups	No of infected rabbit	Type of inoculation	Rout of infection	Does of inoculums	Daily deaths post infection						Total No of death	No. of survivors	Mortality rate
					1-4	14	15	16	17	18-30			
Group 1	10	E.coli O_{55}:$K_{59}(B_5)$	Orally	($4x10^{10}$/CFU /ml	0	1	0	1	1	0-0	3	2	60%
Group 2	10	S.typhmarium		($1x10^{8}$/ CFU/ml)	2	1	1	1	0	0-0	5	0	100%
Group 3	10	Normal saline		0	0	0	0	0	0	0	0	5	0.0%

Table 7: Antibiotic sensitivity test for E.coli and Samonella isolates from diarrhoea rabbits

Antibacterial agent	Isolates	
	E.coli	Salmonella
Ampcillin (10ug),	R	++
Neomycin (30ug)	++	++
Enerofloxacin (5ug)	+++ (S)	+++ (S)
Amoxycillin (25ug)	R	R
Colistine sulphate (25ug),	+++ (S)	++
Erythromycin (15ug)	R	R
Oxytetracycline (30ug)	+ (L)	R
Streptomycin (10ug),	R	R
Nalidixic acid (30ug)	R	+ (L)
Chloramephenical (30ug),	R	++
Gentamycin (10ug)	+++ (S)	+++ (S)

S = +++ Highly sensitivity ++ = Intermediate sensitivity + = Low sensitivity R = - Resistance

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