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DETECTION OF THE PREVALENCE AND PATHOGENICITY OF CLOSTRIDIUM PERFRINGENS AND CLOSTRIDIUM SPIROFORME ASSOCIATED DIARRHOEA IN RABBITS

(With 5 Tables)

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تقدير مدى تواجد وضراوة ميكروبي كلوستريديم بيرفرنجينز وكلوستريديم اسبيروفورم المصاحبة للأسهالات فى الأرانب

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نظرا للدور الهام للبكتريا اللأهوئية في حالات النزلات المعوية في الأرانب فقد شملت الدراسة الفحص البكتريولوجى لعدد١٤٠ أرنبا منهم٨٠ ارنبا مصابا بالأسهالات والنزالات المعوية و٤٠ حديث النفوق ومذبوحا اضطراريا ومصاب بنزالات معوية و٢٠ أرنب سليما ظاهريا من محافظتي المنيا وأسيوط. وقد تبين بالفحص الميكروسيكوبى والخواص المورفولوجية والتفاعلات البيوكميائية من عزل ٦٥ عترة من ميكروب الكلوستريديم بنسبة (٤٦،٤٠%) منها ٥٥ عترة لميكروب كلوستريديم بيرفرنيجينز بنسبة (٣٩،٣٠%) و١٠ عترات من ميكروب كلوستريديم سبيروفورمى بنسبة (٧،١٤%) من اجمالى العينات المفحوصة٠ وكانت نسبة كلوستريديم بيرفرنيجينز في العينات السليمة ظاهريا والمريضة والنافقة كالأتي (١٠%) و(٣٣،٧٥%) و(٦٥%) على التوالي بينما كانت نسبة ميكروب كلوستريديم سبيروفورمى (٥%) و(٦،٢٥%) و(١٠%) على التوالى٠ أوضحت الاختبارات البيولوجية عزل ١٠ عترات بنسبة (١٨،١٨%) غيرمفرزة للسموم و٤٥ عترة ضارية مفرزة للسموم لميكروب كلوستريديم بيرفرنيجينز بنسبة (٨١،٨٢%) منها ٧ عترات للنوع (ا) بنسبة (١٥،٥٦%) و٢عترة للنوع (ب) بنسبة (٤،٤٥%) و٤ عترة للنوع (د) بنسبة (٨،٨٩%) و٣٢ عترة للنوع (ه) بنسبة (٧١،١١%) وبفحص ضراوة كل من كلوستريديم بيرفرنيجينز وكلوستريديم سبيروفورمى على أرانب تجريبها عن طريق الحقن تحت الجلد كانت نسبة النفوق(٧٥%) في المجموعة المحقونة بكلوستريديم بيرفرنيجينز عترة (ه) و(٣٧،٥%) فى عترة (ا) بينما كانت(٦٢،٥%) للمجموعة المحقونة بكلوستريديم سبيروفورمى وتم وصف الأعراض الإكلينيكية والآفات التشريحية للأرانب المحقونة وتم

عزل الميكروب مرة أخرى من أمعاء وكبد هذه الأرانب. ومن خلال اختبار الحساسية للعترات المعزولة ضد بعض المضادات الحيوية تبين حساسيتها لكل من الامبسللين والنروفلوكساسين والكلورامنيفنيكول بينما كانت مقاومه لكل من ستربتومايس والجنتاميسين. وقد تبين من خلال الدراسة ان الأجهادات واختلال توازن العليقة والأصابة بالكوكسيديا والأستخدام الخاطئ للمضادات الحيوية من أهم العوامل التى تساعد على شدة وضراوة الأصابة بالكستريديم فى الأرانب.

SUMMARY

A total of 140 rectal swabs, 20 of apparently healthy, 80 diarrhoeic rabbits and 40 freshly dead and sacrificed diarrhoeic rabbits 6-8 weeks-old collected from El-Minia and Assiut provinces. Collected samples were examined bacteriologically for prevalence and pathogenicity of clostridia. According to morphological characters and biochemical reactions. The incidence of C.perfringens and C.spiroforme were 55 (39.30%) and 10 (7.14%) respectively. There was variation between the prevalence rate of clostridia according to their general healthy condition, where was 3(15%) in apparently healthy, 32(40) in diarrhoeic rabbits while was 30 (75%) in dead and slaughtered diarrhoeic rabbits. For toxogenic and non-toxogenic type of C-perfringens, the incidence of toxigenic type was 45 (81.82%) while was 10 (18.18%) for non-toxigenic type. ToxIgenic type revealed that type "E" was the most predominant (71.11%), followed by type "A", "D" and "B" were (15.56%), (8.89%) and (4.45%) respectively. The pathogencity test of the isolates revealed high mortality of infected rabbits with C.perfringens type "E" reached to (75%) and (37.5%) for type "A", while reached to (62.5%) for C.spiroforme. All dead infected rabbits showed profuse watery diarrhoea and die within few first days after onset. Postmortem examination showed a varying degree of inflammation and ulcerative lesions on mucosal surface of caecum, colon and ileum while internal organs were congested and sometimes necrotic foci in liver. Sensitivity test of Clostridial strains against some antibiotics in vitro showed that, Ampcillin, Norfloxacin and Chloramphenicol were highly effective, while the strains were resistance to Streptomycin and Gentamycin.

Key words: *Rabbits, clostridium, diarrhoea.*

INTRODUCTION

Recently clostridium Spp appeared to be of high significance among rabbits and constitute one of the most important veterinary problems that face rabbit industry in our country due to the high-income losses. The species implicated in the enteritis complex include Clostridium perfringens, C. difficile, and C. spiroforme. All are Gram positive, anaerobic bacilli. Several reports on the presence of Clostridium perfringens Type E iota in the caeca of diarrheic rabbits have been published, although workers failed to isolate C. perfringens Type "E" from the ceca. (Patton *et al.*, 1978 and Rehg and Pakes. 1982), while Clostridium perfringens Type "A" isolated from the liver and caecum of diseased rabbits by Kunstyr *et al.*, 1975; McDonel and Duncan.1975 and Hughes *et al.*, 1976). Also Patton *et al* (1978) observed that the presence of Clostridium perfringens type E enterotoxin was confirmed in the caecal content of 23 out of 46 rabbits, which had died from enteritis. Bernal *et al.* (1981) revealed that severe diarrhoea and death in 40% of inoculated rabbits with Clostridium perfringens Type "A", while Peeters *et al.* (1986) detected C.spiroforme in commercial rabbits at percentage of 52.4% and causing diarrhea. Nagi *et al* (1988) isolated Clostridium perfringens from caeca of diarrhoeic rabbits. They found that toxigenic strains of type E more prevalent and a few were of type"A", following prolonged therapy with penicillin and ampicillin. Nowakowska *et al* (1991) isolated seven strains of C.perfringens from outbreak of diarroeic rabbits with mortality reached 20%. C. spiroforme is the most common clostridial pathogen associated with the enteritis complex in juvenile rabbits and produces a type E iota toxin. Ellis *et al* (1991) found that enterotoxaemia caused by C.spiroforme was responsible for significant losses in commercial rabbits. Abd-EL-Gwad (1993) recovered one isolate of C.spiroforme from rabbits with an incidence of 2% in Assiut Province. Enterotoxaemia in rabbits is caused by Clostridium spiroforme as a result of stress produced by handling, an imbalanced diet (Carman and Wilkins 1991), and after the administration of some antibiotics (Hara *et al.*, 1991).

So this study was planned for estimate prevalence and pathogenicity of C- perfringence and C-spiroforme associated with enteritis in rabbits and sensitivity test of the isolated bacteria against different members of antibiotics were also achieved.

MATERIALS and METHODS

Materials:

1- Samples:

A total of 140 samples (intestinal content, liver, spleen and cloacal swabs) were collected from 40 freshly dead & slaughtered diarrhoeic rabbits, 80 rabbits suffered from diarrhoea and 20 cloacal swabs were collected from apparently healthy rabbits of various age These samples were collected from privately owned rabbitaries at EL-Minia and Assiut Province to detected the prevalence and pathogenicity of Clostridium Spp in diarrhoeic rabbits.

2- Culture media:

a - Cooked meat medium "Mast DM 120"

b- Neomycin blood agar medium (neomycin sulphate soultion was added to the media just before the additions of blood to make final concentration of 150ug/ml.

c- Thioglycollate broth medium "Oxoid, GM10)

d- Fermentable media

Sterile solutions 20% of various fermentable substances "glucose, lactose, maltose, sucrose and mannitol" were spread over the surface of blood agar plates, and then the plates allowed drying at 37^0C according to (Levett, 1991).

e- Media used for biochemical tests: -

Sugar fermentation (glucose, lactose, maltose, sucrose and manitol), gelatin medium, glucose phosphate broth medium, pepton water, triple sugar iron agar (T.S.I.), urea agar base, and semi-solid agar media.

f- Medium for toxin production of CL-perfringens:

The medium was recommended by Roberts *et al* (1970)

c-Antiserum"Burrough,s Wellcome, Beckenham, London,England"

RPO4	Cl-perfringens	type A	K	476810
RPO5	Cl-perfringens	type B	K	454010
RPO6	Cl-perfringens	type C	K	354610
RPO7	Cl-perfringens	type D	K	447710
RPO8	Cl-perfringens	type E	K	449710
RPO9	Cl-perfringens	type control	K	447910

h- Experimental animals:

1-Swiss mice with an average weight (20-25gm) were used for the detection of toxin of CL.perfringens in the intestine of infected rabbits.

They were kept under observation for 2weeks before they were inoculated.

2- Experimental rabbits:

Thirty-five rabbits (8-12 week-old) obtained from private rabbit farms in EL-Minia Province were used in the pathogenicity and experimental studies.

i- Antimicrobial sensitivity discs (Oxoid Laboratories):

Antimicrobial sensitivity discs produced by Oxoid LTD, London, England, including Ampcillin (10ug), Neomycin (30ug), Norfloxacin (5ug), Oxytetracycline (30ug), Streptomycin (10ug), Chloramphenicol (30ug), Nalidixic acid (30ug), and Gentamycin (10ug) were used in this experiment.

j- Gas-pack anaerobic jar "BBL-814-12":

It was used for production of anaerobiosis by using disposable hydrogen-carbon dioxide bags with socket. (Baker platinum LTD, London).

Methods

1-Isolation and identification of C. perfringens and C.spiroforme:

Cloacal swab, liver, spleen and small pieces of the intestines with their contents from each sample were inoculated into sterile cooked meat media tubes. Both inoculated media were incubated anaerobically at 37^0C for 48 hours. Only one of the inoculated mediums was heated in water at 60^0C for 30 minutes. Subcultures were made on duplicated neomycin blood agar plates. One set of the inoculated solid media was incubated anaerobically and the other aerobically at 37^0C for 24 hr. only strick anaerobic isolates were examined and identified for microscopic appearance, culture character, motility, then transferred to cooked meat medium for other biochemical tests as described by Konemann *et al* (1988) and Levett (1991).

2- Determination of typing and toxigenic isolates of CL. Perfringens:

Preparation of culture suspension:

A 48 hr. culture in cooked meat medium was prepared from the isolated clostridial organisms. The cultures suspensions were centrifuged for one hour at 4000 r.p.m. Gram stained smears made from the sediment were examined microscopic to insure purity. The sediment was washed three times in saline, and then resuspended in thioglycollate medium. The plate count technique (Crucickshank *et al.*, 1975) was used for determination of the viable count of cell per ml of suspension.

a) - Determination of toxigenic isolates of C. Perfringens: Nagler's reaction test was applied as described by (Smith and Holdeman, 1968

and Levett, (1991). Pathogenicity to Albino guinea-pigs was done according to Willis (1964).

Nagler, s reaction test:-In this test the plate egg yolk medium was soaked with few drop of antiserum of type "A", the second with antiserum of type "B" and the third acted as control and the same work was done on the other plate to type C, D, and E. After the dryness of antiserum, then added the centrifuged supernatant (3000 r.p.m.) cooked meat culture in ever part. The plates were incubated anaerboically at 37^0C for 24hr and the results were recorded. An opalescence area appeared considered as positive cases.

b) Determination typing of toxigenic C. Perfringens isolates: Neutralization test in mice was performed according to (Smith and Holdeman, 1968).

Toxin neutralization tests: It was performed by adding 0.1ml. of specific antisera (A, B, C, D, and E of C.perfringes) (Burrough, s wellcome, Beckenham, London, England) to 3ml. of the centrifuged supernatant (3000 r.p.m.) cooked meat culture. Supernatant culture of only type "D" was treated with 0.1 trypsin for 45 minutes at 37^0C. The mixture was left for 30 minutes at 37^0C before its injection in mice.

3- Pathogenicity tests:

Experimental infection design: For studies on the pathogenicity of the isolated organisms. The experiment was performed to study the pathogenicity of isolated microorganisms including C. Perfringens and C. spiroforme

a) Pathogenicity to Swiss mice:

Swiss mice inoculated in tail vein with 0.3 ml. of centrifuged suspected C. Perfringens and C- spiroforme cases. The animals were kept under observation for 72 hr.

b) Albino guinea-pigs:

Albino guinea-pigs with an average weight of 250-450gm were used in dermo-necrotic reaction for typing of isolated C. Perfringens. The animals were kept under observation for 2 weeks before the beginning of the experiment.

c) Pathogenicity to rabbits: Thirty-five, 6-8 week-old rabbits were used for studying of pathogenicity of isolated Clostridial microorganisms. The animals were kept in cages and observed for a period a week. A random samples of 3 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 into 3 groups Each group contain 8 rabbits:

Group 1: 8 rabbits were inoculated subcutaneously by 0.1 ($1x10^8$) of 24hr cooked meat broth culture of the identified toxigenic C.perfringens isolates type "E"
Group 2: 8 rabbits were inoculated subcutaneously by 0.1 ($1x10^8$) of 24hr cooked meat broth culture of the identified toxigenic C.perringens isolates type "A"
Group 3: 8 rabbits were inoculated subcutaneously by 0.1 ($1x10^8$) of 24hr cooked meat broth culture of the identified C-spiroforme isolates
Group 4: 8 rabbits were kept without inoculation as control.
All groups were kept for 30 days (period of observation) with daily examination for clinical signs. Dead and sacrified rabbits. Survived rabbits till the end of the observation period were subjected to P.M as well as bacteriological examinations for lesions and trials reisolation were conducted.

Sensitivity test:

The isolates were tested for sensitivity to different chemotherapeutic agents. One ml of 24hr. broth cultures was spread on the surface of blood agar. Antibiotic sensitivity discs were placed on the surface seeded agar. Plates were incubated anaerobically at 37^0C for 24hr. The sensitivity was judged according to the diameter of clearance zone around the discs according to (Perelman *et al.*, 1991).

RESULTS

Table 1: The percentage of C.perfringens and C.spiroforme isolated from 140 rabbit samples.

Types of samples rabbits	Total No. Of examined samples	Total No. Of +ve samples		C-perfringens		C-spiroforme	
		No.	%	No.	%	No.	%
Apparently healthy	20	3	15.0	2	10.00	1	5.00
Diarrhoeic rabbits	80	32	40.0	27	33.75	5	6.25
Dead & slaughtered diarrhotic rabbits	40	30	75.0	26	65.00	4	10.0
Total	140	65	46.4	55	39.30	10	7.10

Table 2: The percentage of toxigenic and non- toxigenic types of C. perfringens isolated from 140 rabbits samples.

Samples	No. +ve C.perfringens		Total No. of non-toxigenic		Total No.of toxigenic		Type of toxigenic organism							
							Type "A"		Type "B"		Type "D"		Type "E"	
	No	%	No	%	No	%	No	%*	No	%*	No	%*	No	%*
Apparently healthy	2	10.00	2	100	-	-	-	-	-	-	-	-	-	-
Diarrhotic rabbits	27	33.75	7	25.93	20	74.07	3	15.00	1	5.00	2	10.00	14	70.00
Dead&slaughtered diarrhoeic rabbits	26	65.00	1	3.85	25	96.15	4	16.00	1	4.00	2	8.00	18	72.00
Total	55	39.30	10	18.18	45	81.82	7	15.56	2	4.45	4	8.89	32	71.11

%* Calculated according to the total No. of toxigenic isolates

Table 3: Showing of results of pathogenicity of CL-perfringens type "A", "E" and CL-Spiroforme isolated from examined 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	5-15	16-20	20	25	25-30			
Group 1	8	C-perfringens type "E"	S/C	0.1 ml ($1X10^8$) cfu	3	2	1	0.0	0.0	0-0	6	2	75%
Group 2	8	C-perfringens type "A"	S/C	0.1 ml ($1X10^8$) cfu	1	1	0.0	1	0.0	0.0	3	5	37 5
Group 3	8	C-spiroforme	S/C	0.1 ml ($1X10^8$) cfu	2	1	2	0.0	0.0	0-0	5	3	62.5%
Group 4	8	Non	Control	0	0	0	0	0	0	0	0	8	0.0%

Table 4: Biochemical reaction of the suspected Clostridium isolates

Type of isolates	Biochemical reactions									
	Indol	H2s	Urease	Hydrolysis of gelatin	Haemolysis	Manitol	Glucose	Lactose	Maltose	Sucrose
C.perfringens	-	+	V	+	+	-	+	+	-	+
C.spiroforme	-	-	V	-	-	-	+	+	-	+

Table 5: Sensitivity of the recovered isolates types of C-perfringens and C-spiroforme to chemotherapeutic agents.

Antibacterial agent	C.perfringens isolates								C.spiroforme isolates	
	"A"		B		"D"		"E"			
	No	%	No	%	No	%	No	%	No	%
Ampcillin(10ug)	4	80	2	40	3	60	4	80	4	80
Streptomycin(10ug)	1	20	1	20	0.0	0.0	1	20	0.0	0.0
Neomycin(30ug)	0.0	0.0	0.0	0.0	1	20	1	20	1	20
Norfloxacin (10ug)	4	80	3	60	4	80	4	80	4	80
Chloramphenicol (30ug)	4	80	3	60	3	60	4	80	3	60
Oxytetracycline(30ug)	3	60	2	40	1	20	3	60	2	40
Gentamycin (10ug)	0.0	0.0	0.0	0.0	0.0	0.0	1	20	0.0	0.0
Nalidixic acid(30ug)	2	40	1	20	2	40	3	60	1	20

5 Strains clostridia species were tested from each

DISCUSSION

Intestinal clostridial infections are a common problem among health of rabbits causing severe losses specially when complicated with stress produced by handling, an imbalanced diet (Carman and Wilkins 1991), and after the administration of some antibiotics (Hara *et al.*, 1991).

In our study attentions were carried to investigate the incidence and pathogenicty of Clostridium Perfringens and Clostridium spiroforme associated with diarrhoeic rabbits in Assiut and El-Minia provinces.

1- Identification of the isolates:

Out of 140 examined rabbits with average age (6-8 weeks). Of these 20 apparent healthy, 40 freshly dead & slaughtered diarrhoeic rabbits, and 80 cases suffering from enteritis varied from catarrhal to haemorrhagic and ballooning of the intestine. Morphological characters, direct microscopic examination and biochemical reaction of the suspected C.perfringens colonies on neomycin blood agar plates were circular, smooth, glistening and gave double zone of haemolysis around the colony, an inner clear zone (complete haemolysis), and outer hazy zone (incomplete haemolysis). In cooked meat medium produced gases and the meat particles turned pink without digestion. The organisms were gram-positive, large bacilli, stained smears often showed coccobacillus with blunt end. They were non-motile, ferment glucose, lactose, maltose, and sucrose and did not fermented mannitol, liquefied

gelatin), while the suspected isolates of C.spiroforme show circular, convex shiny, white to gery and non-haemolytic colonies on neomycin blood agar. The organisms were gram-positive, non-motile, spiral shape with terminal and sub terminal round spores. Fermented glucose, lactose, sucrose and did not ferment maltose and mannitol. Gelatin was not liquefied, urease was variable did not produce indol and H_2S as illustrated in Table (4). These findings agree with those reported by Kaneuchi *et al* (1979), Bernal *et al.* (1981), and Nagi *et al.* (1988). They recovered coild spore- forming organisms (C.spiroforme) from faeces of healthy chickens and rabbits causing mediated enterotoxaemia. The organism was non-proteolytic and non-gelatinolytic, fermented glucose, and produced terminal to subterminal round spores. From Table (1) it was found that there are great variations between the incidence of Clostridial isolates and general status of examined rabbits, where it was found that higher incidence was (75%) among dead & slaughtered diarrhoeic rabbits and (40%) among diarrhoeic rabbits, while it was (15%) among apparently healthy. Moreover, the overall incidence of clostridial isolates was 65(46.4%) from all examined samples. Nearly similar results to our findings obtained by McDonel and Duncan (1975) who recorded that the incidence of clostridial infection among rabbits was (37.6%), Szemerdi *et al* (1983) recorded more or less similar results (39.9%) and Abdel-Gawad (1993) who isolated Clostridium microorganisms among all examined diarrhoeic rabbits with incidence of (39.4%). For the C-perfringens isolates, Table (1) revealed that 55 isolates of C.perferingens from all examined samples at percentage of (39.30%). It is clear that incidence of C.perfringens was lower among apparently healthy 2(10.00%), and 27(33.75%) for diarrhoeic rabbits, while was 26(65.00%) among dead and slaughtered diarrhoeic rabbits. These results of isolation are lower than that obtained by McDonel and Duncan (1975) who isolated C.perfringnes in high incidence reached (97.3%) from diarrhoeic rabbits and patton *et al* (1981) who isolated C.perfringnes in an incidence of (95%) and Abde-EL-Gwad (1993) who isolated C.perfringnes in an incidence of (82.3%) from dead rabbits and results obtained by Peeters and Charlier (1985) who reported that the incidence of C.perfringnes isolates was (70%) from digestive disorders of rabbits, while higher than the percentage obtained by Haffar *et al.* (1988) who detected C.perfringnes in 13% of 600 adults diarrhoeic rabbits. For of C. spiroforme isolates, Table (1) indicated that 10 isolates of C spiroforme with incidence of (7.1%) from all examined samples, one isolate from apparent healthy with incidence of (5.0%). and 5

isolates from diarrhoeic rabbits with incidence of (6.25%) while 4 isolates from dead and slaughtered diarrhoeic rabbits with incidence of (10.0%). These results are lower than that reported by. Peeters *et al.* (1986) who detected C.spiroforme by gram stain in (34. 4%) of 149 caecal samples of rabbits with enteritis complex while higher than that obtained by Abde-EL-Gwad (1993) who recovered one isolate of C.spiroforme from dead diarrhoeic rabbits with an incidence of 2% in Assiut Province.

2- Results of typing toxigenic strains of C.perfringens isolated from examined rabbits:

Table (2) revealed both toxigenic and non-toxigenic isolates of C-perfringens. The toxigenic isolates of C-perfringens were recovered with incidence of (81.8.2%), while non-toxigenic was (18.18%) for all examined samples. These results nearly similar to those obtainted by Abde-EL-Gwad (1993) who recorded that the incidence of toxigenic isolates of C.perfringens was (73.6%) while non-toxigenic type was (26.4%) from all examined rabbits. The overall incidence of toxigenic types in diarrhoeic rabbits was (74.07%) while in dead & slaughtered diarrhoea rabbits was (96.15%). These results are higher than that obtained by Abde-EL-Gwad (1993) who isolated toxigenic types of C. Perfringens from dead diarrhoea rabbits at incidenc of (76.2%), while seemed to agree with results obtained by patton *et al* (1981), Szemerdi *et al.* (1983) and Wang (1985) they found that, toxigenic type of C.Perfringens was closely higher among dead diarrhoeic rabbits than apparently healthy. The incidence of toxigenic types of C.perfringens for typing," A", "B", "D" and "E" were. (15.0 %), (5.0%), (10.0%), and (70.0%) in diarrhoeic rabbits, while were (16.0%), (4.0%), (8.0%) and (72.0%) for typing "A", "B", "D and "E" respectively in dead & slaughtered diarrhoea rabbits. This indicated that type "E" is widely distributed in both of diarrhoeic and dead diarrhoeic rabbits. The overall incidence of toxigenic type "E" was (71.11%). These results are higher than that reported by Abd-EL-Gwad (1993) who recorded that the incidence of type "E" was (33.3%) from all examined rabbits. Also the overall incidence of type "A" was (15.6%). These results are lower than that obtained by Abd-EL-Gwad (1993) who reported that the incidence of type "A" was (48.7%), while the overall incidence of type "D" (8.9%) and type "B" (4.5%) are nearly similarto that obtained by Abd-EL-Gwad (1993) who reported that incidence of type "D" and "B" were (7.7%) and (2.6%) respectively.

3- Results of pathogenicity test of the isolated C. Perfringens type "E & A" and C. spiroforme in rabbits:

Results of experimental infection of the susceptible animals illustrated in Table (3) it is showed that the isolated strains were pathogenic with mortality rates reached to 75% within first few days in rabbits infected s/c for C.perfringens type "E". These results are lower than that obtained by Abd-EL-Gwad (1993) who reported that mortality rate 100% in rabbits infected with C.perfringens type "E", while the rate of mortality of infected rabbits with C-perfringens type "A" was (37.5%). These results are lower than obtained by Abd-EL-Gwad (1993) who recorded that (75%) mortality of infected rabbits with C.perfringens type "A", while nearly similar findings have been reported by McDonel and Duncan (1977) and Matthes (1981) who recorded that mortality rate reached 25%-36% and 30%-50% respectively in experimentally infected rabbits with C.perfringens type "A" according to the route of injection and the does of viable bacterial cells. For the pathogenicity of C. spiroforme, the results indicate that mortality rate reached to 62.5% in infected rabbits. These results are higher than obtained by Abd-EL-Gwad (1993) who reported that 25% mortality rate of infected rabbits with C. spiroforme while these finding nearly from data obtained by Halen *et al.* (1986) and Yonushonis *et al.* (1987). The main clinical signs were loss of appetite, ruffled fur, depression and increased thirty, anorexia, severe diarrhea, tympany, dehydration then death. Similar symptoms are showed by Patton *et al.* (1981), Peeters *et al* (1986) and Abd-EL-Gwad (1993). P.M of dead rabbits showed profuse watery diarrhea and death within a few hours after onsetas well as varying degree of inflammation of caecum, severe haemorrhages and ulceration on the mucosal surface of colon and caecum, necrotic foci in liver showed in some cases. Reisolation of the organisms from experimental dead rabbits was done.

4- Results of sensitivity tests:

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.

In-vitro tests the proper antimicrobial agents against clostridium isolates, illustrated in Table (5) revealed that, Ampcillin, Norfloxacin and Chloramphenicol were highly effective against clostridium isolates (60% to 80%) while Oxytetracycline was of moderate effect (40%) but both Neomycin and Nalidixic acid have the lowest effect (20%) on other

hand clostridium isolates were resistant to Streptomycin, and Gentamycin at rate of (0.0%) These findings in general agreement with those of (Long and Truscott, 1976; Ibrahim, 1979; Perelman *et al,* 1991 and Das *et al,* 1997).

Conclusion: From the abovementioned results, it can be concluded that, intestinal clostridial infections are a common problem among rabbits causing severe losses specially when complicated with stress produced by handling, an imbalanced diet and after the administration of some antibiotics and infestation with coccidia so, the samples of diseased rabbits must be subjected to the examination for anaerobic microorganisms in the routine work in the research laboratories.

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