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STUDIES ON MICROBIAL CAUSES OF DIARRHOEA IN CALVES

(With 9 Tables and 4 Figures)

By

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دراسات على الأسباب الميكروبية للأسهال فى العجول

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إبراهيم الدسوقى

خلال هذه الدراسة تم فحص عدد ١٦٥ عينة برازمن ١٦٥ عجل منهم ١٢٥ عجل مصاب بلأسهال وأربعون عجلا صحيحا وقد شملت الدراسة العزل البكتيرى لمجموعة الأنتيروباكتريسى وعزل الفطريات، وقد تم عزل ٣١٤ عترة بالنسب التالية عترة الأيشريشيا كولاى (الميكروب القولونى العصوى) وكانت نسبة عزله٦٦,٢% وعترة البروتيس ميرابيليس ١٢,١% وعترة السيتوباكتر١٢,١% وعترة الأنتيروباكتر ٣,٢%، والكلبسيلا ٢,٢%، والبروتيس فلجاريس ١,٦% وسالمونيللا كونتاكى ٠,٣% وسالمونيللا فريش ٠,٣% وسالمونيللا سيرو ٠,٣% والأدواردسيلا ٠,٦% ومورجانيلا ٠,٦% والسيراتيا٠,٣% وقد عزلت السلمونيللا من العجول المصابة بلأسهال فقط. وقد تم التفريق بين العترات المرضية والغير مرضية لعترات الأيشريشيا كولاى بإستخدام بيئة الكونغو الأحمر حيث كانت نسبة وجوده فى العجول المصابة بالأسهال ٨٣,٧% مما يدل على إرتباطه بالحالات المرضية للعجول. تم إختبار ٥٣ عترة لميكروب القولون العصوى ضد ١٢ نوع من المضادات الحيوية. فوجدنا أنه كان حساسا للسيبروفلوكساسين، الأنروفلوكساسين، اوفلوكساسين والكلورامفنيكول بينما كانت مقاومة لكل من الأوكسستتراسيكلين، سلفات الكوليستين، الكليندامايسينوالأريثرومايسين. وقد أظهرت الفحوص الفطرية للعينات وجود ١٢ عترة من الخمائر تم عزلها وتصنيفها ظاهريا وبيوكيميائيا إلى ٧ عترات كانديدا جلابراتا و٤ عترات كانديدا جويللير موندى وعترة أخرى غير مصنفة من الكانديدا أيضا.

SUMMARY

In this study, a total of 165 calves (125 diarrhoeic and 40 healthy calves) were examined for bacteriological and mycological isolation to study the role of family Enterobacteriaceae and fungi in calf diarrhoea. A total of 314 bacterial isolates were recovered from examined calves and identified into *E. coli* (66.2%), *P. mirabilis* (12.1%), *Citrobacter* spp.

(12.1%), *Enterobacter* spp. (3.2%), *Klebsiella* spp. (2.2%), *P. vulgaris* (1.6%), *Salmonella* spp. (0.9%), *Edwardsiella* spp. (0.6%), *Morganella* spp. (0.6%) and *Serratia* spp. (0.3%). *Salmonella* spp. had been recovered only from diarrhoeic calves. The recovered isolates were *S. kentucky, S. ferruch* and *S. cerro* (0.3% of each). Congo red binding activity of *E. coli* may be considered as an easy diagnostic tool for rough differentiation between pathogenic and non pathogenic strains. A total of 12 yeast isolates were recovered from examined calves and identified into Candida *glabrata* (58.3%), *Candida guillermondii* (33.3%) and other *Candida* spp. (8.33%). *In vitro* sensitivity of *E. coli* to various antimicrobial agents revealed their sensitivity to ciprofloacin, enrofloaxin, ofloxacin and chloramphenicol, while they were resistant to oxytetracycline, colistine, clindamycine and erythromycine, on the other side, *Salmonella* spp. were varied in their sensitivity to different antimicrobial agents.

Key words:*Diarrhoea, calves, Enterobacteriaceae, fungi, antimicrobial agents.*

INTRODUCTION

Recently, neonatal calf diarrhoea remains one of the most important cause of calf mortality and is one of the major problems facing livestock production not only in Egypt but all over the world.

Each year thousands of neonatal calves are suffering from diarrhoea, resulting in economic losses these losses not only by increasing calf fatality but also by decrease in the calf's ability to gain weight, treatment cost, time spent on care as well as subsequent chronic ill thrift and poor growth (Bazeley, 2003).

It has been estimated that neonatal calf diarrhoea accounts for approximately 75% of the mortality of dairy calves under 3 weeks of age (Radostits *et al.*, 1994).

Diarrhoea is a well known clinical sign in neonatal animals. Its etiology is complex involving managemental, environmental, nutritional, physiological variation and various infectious agents as bacterial, viral and protozoa (Marcio *et al.*, 2000).

Family Enterobacteriaceae constitutes a great hazard to enteritis in newly born calves. *E. coli* and *Salmonella* take the major importance as a cause of diarrhoea (Ashraf, 1996).

The fungi, particularly yeasts and moulds are always neglected although they are well known to cause diseases of all animal species

predisposing by their insensitivity to antibacterial antibiotics, so they usually flourish following prolonged antibiotic therapy.

So the aim of present work was planed as an attempt to study this problem from the following points of view:

1. To study the role of members of *Enterobacteriaceae* in calf diarrhoea.
2. Serological identification of the most predominant isolates to focus the most important serotypes involved in this condition.
3. Trails for isolation and identification of the possible fungal causative agents.
4. Finding the most effective antimicrobial agents to be used for treatment.

MATERIALS and METHODS

1- Materials:

1.1. Calves:

As shown in Table (1) a total of 165 calves were subjected to this investigation, out of which 94 cow-calves and 31 buffalo-calves were suffering from diarrhoea with variable degree.

The rest, 25 cow-calves and 15 buffalo-calves were apparently healthy normal calves from the same farms and were used as controls. All the investigated calves were 1-60 days old. Faecal samples were separately collected from examined calves using sterile disposable plastic gloves, which were inverted after sampling and information about date of sampling, number and age of the calf were written on each glove. Samples were collected from calves before any trial of treatment had been initiated. Faecal materials were transported to the laboratory in a cold chamber container to be cultured on the same day of sampling.

Table 1: Number, breeds and state of examined calves in different farms at Kafr El-Sheikh Governorate.

Farms	Species	No. of examined calves		Total
		Diarrhoeic	Apparently healthy	
Sakha	Friesian	44	13	57
Karada	Friesian	50	12	62
Mehallet Mousa	Buffalo	17	8	25
El-Nataf	Buffalo	14	7	21
Total		125	40	165

1.2. Bacteriological media:

1.2.1. Liquid media:

- Nutrient broth (Oxoid)
- Tryptic Soya broth (Biolife)
- Rappaport Vassiliadis (Oxoid)
- Selenite F broth (Oxoid).

1.2.2. Semisolid media (soft agar):

Semisolid nutrient agar (Cruickshank *et al.,* 1975).

1.2.3. Solid media:

- Nutrient agar (Oxoid).
- Mac Conkey's agar (Lab M).
- Eosine Methylene Blue agar (EMB) (Oxoid)
- Xylose Lysine Desoxycholate agar (XLD) (Oxoid)
- Salmonella-Shigella Agar (S.S) (Oxoid).
- Brilliant Green agar (B.G) (Oxoid)
- Blood agar media *(Cruickshank et al., 1975)* Blood agar base to which 5% defibrinated sheep's blood was added.
- Congo red medium *(Berkhoff and Vinal, 1986).*
- The medium consists of trypticase soya agar (Oxoid) supplemented by 0.03% Congo red dye (Sigma) and 0.15% bile salts (Sigma).
- Mueller Hinton media (Oxoid): for antibiotic sensitivity test

1.2.4. Media used for biochemical identification of the isolates:

All media used were prepared according to Cruickshank *et al.* (1975).

- Peptone water 2% (Oxoid):
- Glucose Phosphate broth:
- Simmon's citrate agar (Oxoid):
- Christensen's urea agar base (Oxoid):
- Sugar fermentation media (Oxoid):
- Triple Sugar Iron Agar (TSI) (Oxoid):
- Lysine Iron Agar (LIA) (Sifin):

Table 2: Interpretation of reaction on TSI medium.

Reaction				Possible Organisms
Slant	Butt	Gas	H_2S	
A	A	+	-	*Escherichia* and *Klebsiella* spp.
K	A	+	+	*Salmonella* spp., *Proteus* spp. and *Citrobacter* spp.
K	A	-	-	*Enterobacter* spp. and *Shigella* spp.
A	A	+	+	*Proteus vulgaris*

A: Acid (yellow) K: Alkaline (red)

H_2S: H_2S production (black colouration)

1.3. Media used for isolation of fungi:

- Sabouraud's Dextrose Agar (SDA) (Oxoid)
- Sabouraud's dextrose broth (Oxoid)

1.4. Media used for morphological identification of fungi:

Rice Agar Medium (Refai M., 1987)

1.5. Reagents and chemicals:

- 3% hydrogen peroxide solution for Catalase test.
- P. dimethyl amino benzaldhyde (Kovac's reagent) for Indole test.
- 1% tetramethyl-P-Phenylene diamine dihydrochloride, solution for Oxidase test.
- 0.04% methyl red solution for Methyl red test.
- Solution I of 5% alpha naphthol in absolute ethanol. and solution II 40% potassium hydroxide for Voges Proskauer test.
- 40% sterile urea solution (Oxoid, SR 20). For urease test.
- 1% Andrad's indicator.
- Potassium nitrate:for nitrate assimilation test.
- Sugars: glucose, lactose, sucrose, mannitol, galactose, mannitol, maltose, dulcitol and inositol.

1.6. Diagnostic antisera:

1.6.1. Antisera used for serotyping of *E. coli* isolates.

"O" sera 51 vials (polyvalent 8 vials and 43 monovalent vials) "SEIKEN" (product Code 312001, Japan).

- Polyvalent "1": $O_{1,}$ $O_{26,}$ $O_{86a,}$ $O_{111,}$ O_{119} $O_{127a,}$ $O_{128.}$
- Polyvalent "2": $O_{44,}$ $O_{55,}$ $O_{125,}$ $O_{126,}$ $O_{146,}$ $O_{166.}$
- Polyvalent "3": $O_{18,}$ $O_{114,}$ $O_{142,}$ $O_{151,}$ $O_{157,}$ $O_{158.}$
- Polyvalent "4": $O_{6,}$ $O_{27,}$ $O_{78,}$ $O_{148,}$ $O_{159,}$ $O_{168.}$
- Polyvalent "5": $O_{20,}$ $O_{25,}$ $O_{63,}$ $O_{153,}$ O_{187}
- Polyvalent "6": $O_{8,}$ $O_{15,}$ $O_{115,}$ O_{169}
- Polyvalent "7": $O_{28ac,}$ $O_{112ac,}$ $O_{124,}$ $O_{136,}$ O_{144}
- Polyvalent "8": $O_{29,}$ $O_{143,}$ $O_{152,}$ $O_{164.}$

1.6.2. Diagnostic *Salmonella* antisera:

Diagnostic, polyvalent 1, II and III and monovalent *Salmonella* O and H (phase 1 and phase 2) antisera were obtained from Denka Seiken, Japan.

1.7. Antimicrobial sensitivity disks:

- A total of 12 antimicrobial disks of Oxoid laboratory were used in the present investigation. they included, Chloramphenicol (C30 µg),, Erythromycin (E 15µg), Oxytetracycline (OT 30 µg), Ciprofloxacin (CIP 5 µg), Ofloxacin (OFX 5 µg), Enrofloxacin (ENR 5 µg), Cefotaxime (CTX 30 µg), Clindamycine (DA 2 µg), Cephalexine (CL

30 µg), Ampicillin sulbactam (SAM 20 µg), Amoxycillin Clavulenic acid (AMC 30 µg), and Colistin sulphate (CT 25 µg).

2- Methods:

2.1. Isolation of Enterobacteriaceae:

Faecal samples were subjected for two methods of bacterial examination (Cruickshank *et al.*, 1975).

2.1.1. Direct plating method:

A small quantity of faecal samples was plated onto the surface of MacConkey´s agar plates. The inoculated plates were incubated aerobically at 37 ^{o}C for 24 -48 hours.

2.1.2. Selective enrichment method:

About one gram of faeces was added to both 9 ml of Rappaport Vassiliadis and Selenite F broths. After 18 hours of aerobic incubation at 37^{o}C, aloopful from cultivated broths was plated out on XLD, BG, S-S agar media, then incubated aerobically at 37^{o}C for 24-72 hours.

Pure colonies were picked up and preserved on slope agar for further morphological, biochemical and serological identification according Krieg and Holt (1984).

2.2. Identification of the isolated bacteria.

2.2.1. Morphological and culture examination.

Pure cultures were prepared from all suspected colonies, the shape, size, type of colonies either lactose or non lactose fermenting colonies onto MacConkey's agar or S-S agar and type of haemolysis on blood agar were recorded.

Films were prepared from the purified isolates, stained with Gram´s stain and examined microscopically for detecting their staining reaction and morphological characters. Colonies showing the morphological characters of members of family Enterobacteriaceae were preserved on semisolid agar for further studies.

2.2.2 Biochemical identification.

The obtained bacterial isolates were examined for Catalase and Oxidase tests to be sure that they were belonged to family Enterobacteriaceae, then biochemical identification was achieved as described by Quinn *et al.* (2002). It included sugar fermentation (glucose, lactose, galactose, sucrose, xylose, mannitol, dulcitol, sorbitol and salicin), Indol production, Methyl red, Voges proskauer, Citrate utilization, Urea hydrolysis and H_2S Production.

2.2.3. Detection of bacterial motility.

Motility of the isolated bacteria was studied in soft agar (0.5 % agar) as described by Cruickshank *et al.* (1975).

2.2.4. Serological typing of isolated bacteria:-

a. Serological identification of *E. coli:*

Isolates that were preliminary identified biochemically as *E. coli* were subjected to serological identification according to Ewing (1986).

Each isolate was first tested for its agglutinability of the diagnostic polyvalent "O" antisera, which are intended for use by slide agglutination technique. Once the pathogenic type has been indicated by the use of the polyvalent sera, further serogrouping was made with the appropriate"O" monovalent antisera.

b. Serological identification of *Salmonella*:

Isolates that were preliminary identified biochemically as *Salmonella* were subjected to serological identification according to Kauffmann-White scheme (Kauffmann, 1973) as follow:

2.2.5. *In vitro* differentiation between pathogenic and non-pathogenic *E. coli*:

Identified *E. coli* isolates were cultured on Congo red medium and incubated aerobically at 37ºC for 24 hours and then left at room temperature for additional 2 days (not to exceed 4 days).

2.3. Sensitivity of bacterial isolates to different chemotherapeutic agents:

Bacterial isolates were tested for their susceptibility to 12 different antimicrobial disks according to test diffusion technique as described by Koneman *et al.* (1995).

2.4. Isolation of fungi:

Fecal samples were immersed in Sabouraud's dextrose broth with chloramphenicol and left for 18 hours before culturing.

A loopful from cultivated broth was streaked on SDA containing chloramphenicol at 37ºC and 25ºC for 4-6 days. Isolates were kept on SDA slopes for further identification.

2.4.1. Morphological identification of yeast.

Yeast isolates were streaked on Rice agar plates and after 24-48 hours incubation at 30ºC, the plates were examined under the high power of ordinary microscope (Refai, 1987).

2.4.2. Biochemical identification of the isolated yeasts *(Refai, 1987)*:

a. Sugar fermentation:

Glucose, galactose, sucrose, maltose and lactose sugar media were inoculated with the suspected isolates and incubated for 3-6 days at 37ºC.

b. Nitrate assimilation test:

Nitrate assimilation medium was cooled to 45°C then poured into sterile Petri dishes containing 2 ml saline suspension of the suspected isolates and finally left to dry at room temperature. Sterile filter discs previously soaked in 5% peptone or potassium nitrate solution were put on the surface of the medium, then the plates were incubated for 2-4 days at 37°C.

c. Urease test:

Christensen's urea agar slopes were inoculated with the suspected yeasts and then incubated at 25°C for 2-4 days.

d. Germ tube test *(Finegold and Baron, 1986)*:

- A loopful of suspected yeasts inoculum was suspended in 0.5ml sterile bovine serum in test tube. The tubes were incubated for 3 hours at 37°C, then one drop of yeasts serum mixture was examined microscopically for the presence of germ tube.

RESULTS

1. Results of bacteriological examination of calves:

Bacteriological examination of 165 faecal samples collected from diarrhoeic and apparently healthy calves revealed that a total of 314 bacterial isolates were recovered. The identification of such isolates revealed that 208 isolates of them belonged to *E. coli* with an incidence of 66.2%, 38 isolates belonged to each *P. mirabilis* and *Citrobacter* spp. (12.1% of each), 10 isolates belonged to *Enterobacter* spp. (3.2%), 7 isolates belonged to *Klebsiella* spp. (2.2%), 5 isolates belonged to *P. vulgaris* (1.6%), two isolates belonged to each *Morganella* spp. and *Edwardsiella* spp. (0.6% of each) and one isolate belonged to each *S. kentucky, S. ferruch S. cerro and Serratia* spp. (0.3% of each).

1.1. Recovered bacteria from diarrhoeic calves:

As shown in Table (3) and Figure (1). A total of 259 bacterial isolates were identified. They include 166 isolates of *E. coli* (64.1%), 35 isolates of *P. mirabilis* (13.5%), 31 isolates of *Citrobacter* spp. (12%), 10 isolates of *Enterobacter* spp. (3.9%), 6 isolates of *Klebsiella* spp. (2.3%), 5 isolates *P. vulgaris* of 2 isolates of *Edwardsiella* spp. (0.8%) and one isolate belonged to each *S. kentucky, S. ferruch, S. cerro* and *Morganella* spp. (0.4% of each).

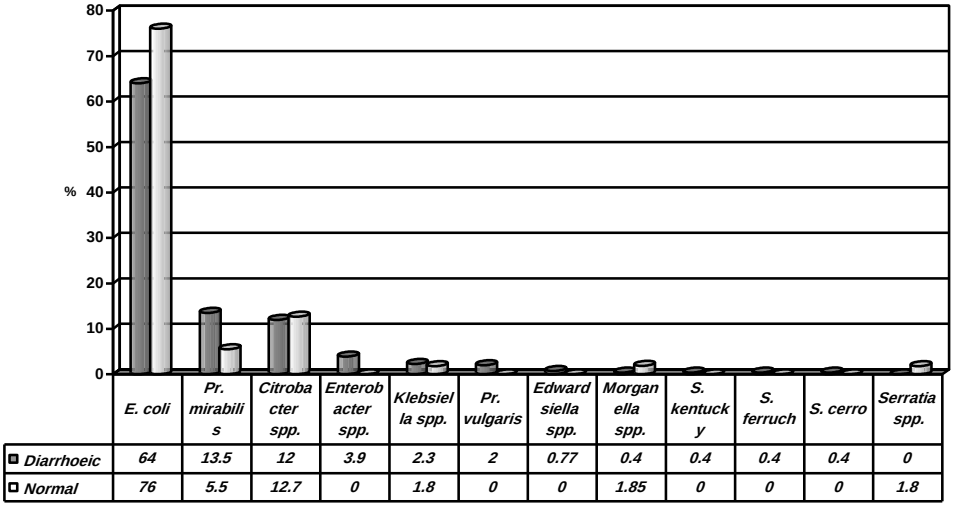

Fig. 1: incidence of recovered bacteria among the examined calves.

Table 3: Types of recovered bacteria from the examined calves.

Recovered bacteria	Diarrhoeic (125)		Normal (40)		Total (165)	
	No.	%	No.	%	No.	%
E. coli	166	64.1	42	76.4	208	66.2
Pr. mirabilis	35	13.5	3	5.5	38	12.1
Citrobacter spp.	31	12	7	12.7	38	12.1
Enterobacter spp.	10	3.9	0	0	10	3.2
Klebsiella spp.	6	2.3	1	1.8	7	2.2
Pr. vulgaris	5	2	0	0	5	1.6
Edwardsiella spp.	2	0.77	0	0	2	0.6
Morganella spp.	1	0.4	1	1.8	2	0.6
S. kentucky	1	0.4	0	0	1	0.3
S. ferruch	1	0.4	0	0	1	0.3
S. cerro	1	0.4	0	0	1	0.3
Serratia spp.	0	0	1	1.8	1	0.3
Total	259	100	55	100	314	100

1.2. Recovered bacteria from apparently healthy calves:

As shown in Table (4) a total of 55 bacterial isolates were recovered. They include 42 isolates of *E. coli* (76.4%), 7 isolates of *Citrobacter* spp. (12.7%), 3 isolates of *P. mirabilis* (5.5%) and one isolate belonged to each *Klebsiella* spp., *Serratia* spp. and *Morganella* spp. (1.8% for each).

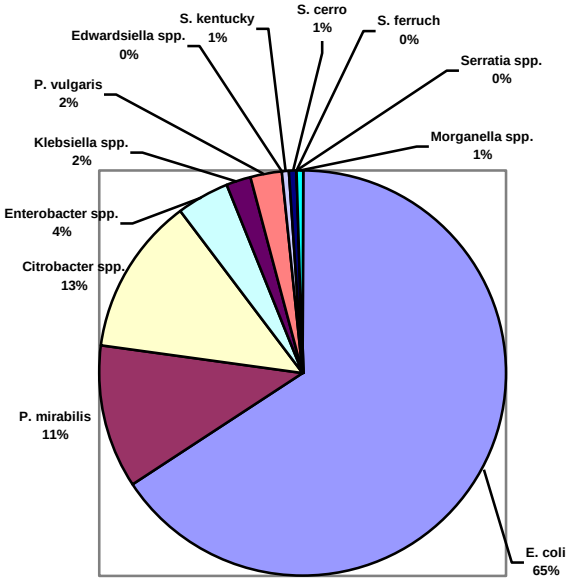

Fig. 2: Incidence of the recovered bacteria among examined cow calves.

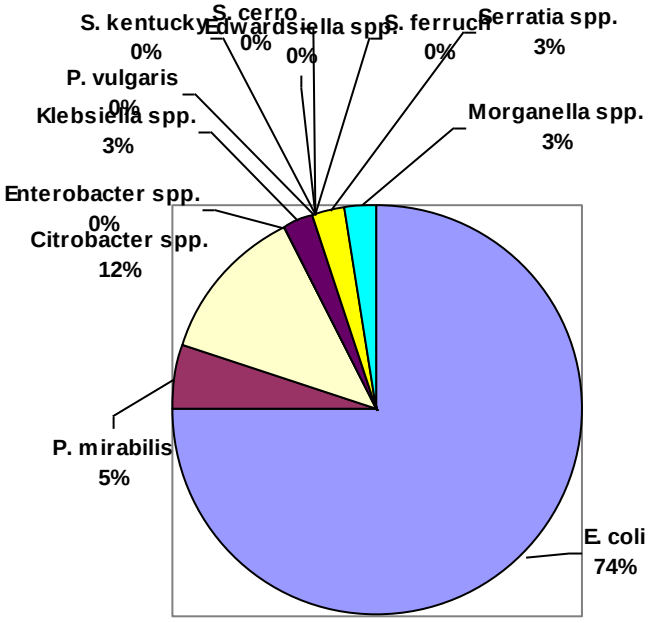

Fig. 3: Incidence of the recovered bacteria among examined apparently normal calves.

As shown in Table (5) a total of 32 cases from examined calves were contained more than one type of bacteria with a percentage of 19.3%. They include 12 of *E. coli* with *Citrobacter* spp. (7.3%), 8 of *E. coli* with *P. mirabilis* (4.8%), 4 of *E. coli* with *P. vulgaris* (2.4%), 3 of *E. coli* with *Klebsiella* spp. (1.8), 2 of *E. coli* with *Morganella* spp. (1.2%) and the last three cases were *E. coli* with *S. kentucky, E. coli* with *S. cerro* and *P. mirabilis* with *S. ferruch* (0.6% of each).

3. Results of serological identification of *E. coli:*

The result of the serotyping of 10 *E. coli* isolates recovered from examined diarrhoeic calves revealed that O_{165} represent the most common serogroups (3 isolates) followed by O_{25}, O_{115}, O_{111}, O_{167} and O_1 (one isolate for each) and the last two serotypes were untypable.

4. Results of serotyping of the recovered *Salmonellae*:

A total of 3 isolates of *Salmonellae* were recovered from examined diarrhoeic calves and serologically typed as *S.kentucky, S. ferruch* and *S. cerro* (one for each) with an incidence of 0.3% (of each), as shown in Table (6).

Table 6: Serotyping of the recovered Salmonellae.

Salmonella	No. of	Calf	Antigenic formula	
Serovars	isolates	species	Group	
S. kentucky	1	Cow	C	O_8-H_{1i}-H_2Z_4
S. ferruch	1	Buffalo	C	O_8-H_{1eh}-H_2 1.5
S. cerro	1	Cow		$O_{6.14.1}$-$H_1Z_4.Z_{23}$-H_2 (1.5

5. Congo red binding activity of the recovered *E. coli*:

As shown in Table (7) and Figure (4) a total of 208 isolates of *E. coli* (166 from diarrhoeic calves and 42 form apparently normal calves) were tested by their phenotype on Congo red medium.

Table 7: Results of in vitro differentiation between pathogenic and non pathogenic *E. coli*.

Calf state	No. of examined E. coli	Congo red reaction			
		+ve		-ve	
		No.	%	No.	%
Diarrhoeic	166	139	83.7	27	16.3
Normal	42	13	31	29	69
Total	208	152	73	56	27

As shown in Table (7), 152 isolates (73%) produced red colonies, 139 from diarrhoeic calves (83.7%) and 13 from apparently normal

calves (31%) while 56 isolates (26.9%) produced white colonies (27 from diarrhoeic calves (16.3%) and 29 from apparently normal calves (69%).

6. Antibiotic susceptibility of *E. coli* isolated from diarrhoeic calves:

As shown in Table (8) a total of 53 isolates of *E. coli* were tested for their sensitivity to 12 antimicrobial agents. Most of the tested isolates were highly sensitive to ciprofloxacin, enrofloxacin and ofloxacin, but they were sensitive to chloramphenicol, ampicillin and cefotaxime, on the other hand, the tested isolates were resistant to oxytetracycline, colistin sulphate, clindamycin, erythromycin and amoxycillin.

7. Antibiotic susceptibility of *Salmonella* species isolated from diarrhoeic calves:

As shown in Table (8), 3 isolates of Salmonella recovered from diarrhoeic calves were tested for their sensitivity to antimicrobial agents.

S. kentucky was highly sensitive to chloramphenicol, cefotaxime, amoxycillin and ampicillin, while it was completely resistant to ciprofloxacin, erythromycin, ofloxacin, clindamycine and oxytetracycline.

Table 8: Results of antibiogram of the isolated bacteria.

Antimicrobial agents	*E. coli* (53)			*S. kentucky* (1)			*S. ferruch* (1)			*S. cerro* (1)		
	No	%	Result	No	%	Result	No	%	Result	No	%	Result
Ciprofloxacin	51	96.23	S	1	0	R	1	100	S	1	0	R
Enrofloxacin	47	88.68	S	1	100	S	1	100	S	1	0	R
Ampicillin	29	54.72	S	1	100	S	1	0	R	1	100	S
Amoxycillin	19	35.84	R	1	100	S	1	0	R	1	100	S
Erythromycine	21	39.62	R	1	0	R	1	0	R	1	0	R
Chloramphenicol	41	77.3	S	1	100	S	1	100	S	1	100	S
Ofloxacin	45	85	S	1	0	R	1	100	S	1	0	R
Clindamycine	8	15.1	R	1	0	R	1	0	R	1	0	R
Oxytetracycline	9	17	R	1	0	R	1	0	R	1	0	R
Colistin	16	30.2	R	1	0	R	1	0	R	1	100	S
Cephalexine	13	24.5	R	1	100	S	1	100	S	1	100	S
Cefotaxime	29	54.7	S	1	100	S	1	100	S	1	100	S

S. ferruch was highly sensitive ciprofloxacin, cefotaxime and enrofloxacin, while it was resistant to erythromycin, oxytetracycline and clindamycine.

S. cerro was highly sensitive to chloramphenicol, cefotaxime and amoxycillin, but it was resistant to enrofloxacin, erythromycin, oxytetracycline and clindamycine.

Collectively, all the tested *Salmonellae* varied in their sensitivity to ofloxacin, colistin, cephalexine, but they were completely resistant to erythromycin, oxytetracycline and clindamycine.

8. Results of fungal examination:

8.1. Recovered fungi from diarrhoeic calves:

As shown in Table (9) and Fig. (5) it is evident that 9 yeast isolates were obtained from 125 faecal samples of dirrhoeic calves (7.2%). The identification of the isolates is demonstrated in Table (9) where 6 isolates were identified as *Candida glabrata*, 2 isolates as *Candida guillermondii* and one isolate of other *Candida* species.

8.2. Recovered fungi from apparently healthy calves:

Three yeast isolates were obtained from 40 faecal samples of apparently healthy calves (1.5%) as indicated in Table (9) and Fig. (5) identified as *C. glabrata* (one isolate) and *C. guillermondii* (two isolates).

Table 9: Number of yeast isolates and their prevalence in faecal samples of examined calves.

Recovered Yeasts	Diarrhoeic		Apparently healthy		Total	
	No.	%	No.	%	No.	%
C. glabrata	6	66.6	1	33.3	7	58.3
C. guillermondii	2	22.2	2	66.6	4	33.3
Other *Candida* spp.	1	11.1	0	0	1	8.33
Total	9	100	3	100	12	100

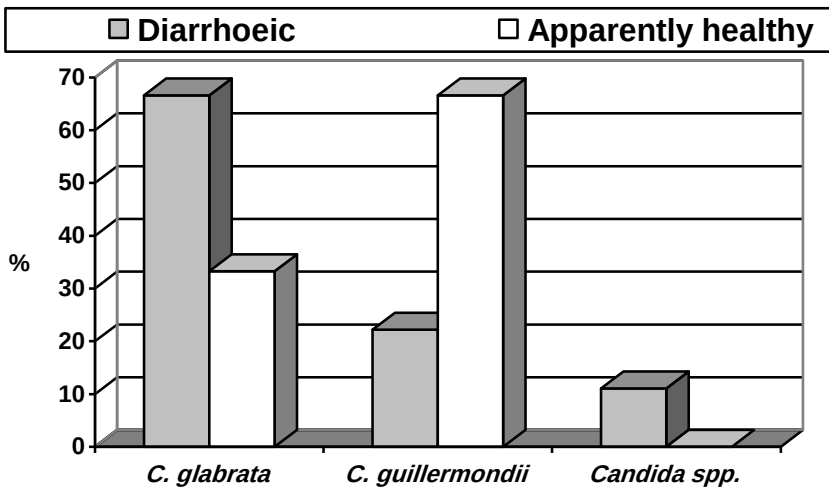

Fig. 4: incidence of yeast isolated from examined calves.

DISCUSSION

During the last decade, a great attention was paid towards calf breeding projects to diminish the gap between the increased population and their demands from such animal proteins. Such projects were objected with many obstacles represented in a financial and general health condition that may lead to decrease in their productivity. Diarrhoea is one of main problems that face such projects. The disease remain largely unchecked because of its complex aetiology (Tzipori, 1981).

The recovered bacterial isolates were *E. coli* (208 isolates), *P. mirabilis* (38), *Citrobacter* spp. (38), *Enterobacter* spp. (10), *Klebsiella* spp. (7), *P. vulgaris* (5), *Edwardsiella* spp. (2), *Morganella* spp (2), *Salmonella* spp. (3) and *Serratia* spp. (1) with an incidence of 66.2%, 12.1%. 12.1%, 3.2%. 2.25%, 1.6%, 0.6%, 0.6% 0.9% and 0.3% respectively

The result in the present thesis showed that *E. coli* was the predominant spp. among members of family Enterobacteriaceae. This result coincides with that mentioned by Ashraf (1996).

In the present study, *E. coli* was isolated with an incidence of 64% and 76% from diarrhoeic and healthy calves respectively. These results agree with the result obtained by Navade *et al.* (2000) who recorded that *E. coli* was the primary cause of diarrhroea. The second, third, fourth and fifth authors reported that its incidence were 53%, 66%, 68.6% and 68%, respectively. This high incidence of *E. coli* in the diarrhoeic calves let us to conclude that *E. coli* is the most common cause of diarrhoea, although various types of *E. coli* are normal inhabitants in the intestine.

Meanwhile, other researcher isolated *E. coli* from diarrhoeic calves with relatively low incidence as described by Tanios *et al.* (2000) who isolated *E. coli* with an incidence of 10.8%, 34%, 28%, 32.8%, 14.5%, 37%, 33.3%, 33%, 32% and 34.9% in there order, on contrary higher incidence of *E. coli* was recorded by Oliveir *et al.* (1989) and Ashraf (1996). Their results were 74.45%, 95.4% and 79.7%, respectively. These variation may be attributed to the pathogenicity of *E. coli* for calves which had been correlated with numerous extrinsic and intrinsic factors. These extrinsic factors include environmental condition, exposure to other infectious agent, improper feeding, unsanitary condition for drinking, bad hygienic surrounding and deprivation of colostrum one of the most important factors. Some

viruses and bacteria beside the bad sanitary environmental condition assist the pathogenecity of *E. coli*, such facts was previously mentioned by Woode and Bridger (1975).

Serotyping of the recovered *E. coli* is not only useful procedure for epidemiological purpose, but also as a tool for delimiting different pathophysiological syndrome. The serological identification of the recovered *E. coli* were O_{111}, O_{115}, O_{165}, O_{167}, O_{167}, O_1, O_{25} and untypable strains. These results agree with Tanios *et al.* (2000) who isolated two isolates of *E. coli* belonged to O_{111} from diarrhoeic calves and Farid *et al.* (1976) who proved that O_{111} and O_{115} serotypes were present as a causative agents of diarrhoea in calves.

The obtained result also simulated with the result recorded by Sirvastava and Arya (1979) who isolated E. coli from calves suffering from gastroenteritis and typed them as O_{25}. Similar results also described by Verma and Aldakha (1970) who stated that the most strains of E. coli that associated with diarrhoea in calves belonged to O_1 and O_{115}.

Through this study, a total of three *Salmonella* serotypes (two from diarrhoeic cow calves and one from diarrhoeic buffalo calves) were recovered with an incidence of 0.9% and identified serologically as *S. ferruch*, *S. cerro* and *S. kentucky*. Our result showed that *Salmonella* spp. were isolated totally with an incidence of 0.95% which differs from that obtained by Tanios (2000) who recovered Salmonella spp. with an incidence of 18.5%, 4.7%, 7.7%, 23.6%, 8.6%, 8.9% and 13.6%, respectively from diarrhoeic calves. The obtained result were simulated with the result recorded by Abou Zid (1976) who isolated two strains of S. typhimurium from 150 diarrhoeic calves and Zrelli *et al.* (1990). Who detected S. typhimurium in diarrhoeic calves with an incidence of 1.8%.

Serotyping of recovered *Salmonella* spp. revealed that *S. ferruch* was isolated from diarrhoeic buffalo calves with a percentage of 0.3%.

The incidence of S. ferruch recorded in the present study was nearly similar to that obtained by Abou Zid (1979). Single isolate of S. cerro was recovered in this study with a total percentage of 0.3% from diarrhoeic cow calves. These results are near to the results obtained by Labib (1998) who mentioned that the incidence of S. cerro recovered from calves with diarrhoea were 1.4%, 1.6% and 0.9% in their order.

Regarding to *S. kentucky*, it was isolated from diarrhoeic cow calves with an incidence of 0.4%. According to the available literatures, it is the first record to be isolated from cow calves in Egypt.

From the above mentioned results, it could be concluded that all the recovered *Salmonella* serotypes were isolated from diarrhoeic calves,

while, no *Salmonella* spp. were recovered from apparently normal calves. These results agree with that mentioned by Wani *et al.* (2003) that indicated their relation to the disease.

Wells *et al.* (2001) reported that Salmonella faecal shedding in milk cow was detected in 21% of dairies and 66% of cull dairy cow market and the most common serotypes of *Salmonella* shed from dairy cow were *S. kentucky* (8.05%), *S. cerro* (13.3%) and other type of *Salmonellae,* so in our study, isolation of these serotypes from diarrhoeic calves may be attributed to bad hygienic measures in the examined farms that lead to faecal contamination of udder of infected or carrier cow, subsequently infection of newly born calves during milk suckling.

Concerning the incidence of *Proteus* spp. as a causative bacterial agent of enteritis in calves in this study. The obtained finding revealed that it was present in the examined cases with an incidence of 13.7% out of them, *P. mirabilis* was the predominant spp. (12.1%). *P. mirabilis* was recovered from diarrhoeic and healthy calves with an incidence of 13.5% and 5.5%, respectively. *P. vulgaris* was isolated only from diarrhoeic calves with an incidence of 1.6%. The obtained results are to certain extent near to the results obtained by Abd El-Galil *et al.* (1983) who isolated Proteus spp. from diseased buffalo calves with an incidence of 5%, but our results disagree with the results recorded by Mona (1995a) who recovered P. mirabilis and P. vulgaris from calves with enteritis in a percentage of 1.33% and 13.32%, respectively and Olivier *et al.* (1989) who stated that incidence of P. vulgaris recovered from diarrhoeic calves was 8.2%. Also, the present results were lower than obtained by Al-Khayyat *et al.* (1977) who recovered Proteus spp. from diarrhoeic calves with an incidence of 25.33% and 18%, respectively.

With regard to *Citrobacter* spp., they were recovered from diarrhoeic calves with a percentage of 12%. This result agrees with that obtained by Vertanyan *et al.* (1990) who stated that Citrobacter spp. was the most prevalent bacteria among diarrhoeic calves after E. coli and Proteus spp. Also, the obtained results go hand in hand with those recorded by Marcio *et al.* (2000) who isolated Citrobacter spp. with an incidence of 12.5% from buffalo calves suffering from diarrhoea. The present results are higher than that obtained by Mona (1995a), Hussain and Saikia (2000) who recovered Citrobacter spp. with an incidence of 8% and 6.55%, respectively. The obtained result showed that it was isolated with higher percentage form apparently healthy calves (12.7%). This result agrees with that obtained by Corsalini (1969) who mentioned

that Citrobacter spp. was the most frequent enterobacteria isolated from healthy calves.

Concerning *Enterobacer* spp. in the present investigation, it was recovered from diarrhoeic calves with an incidence of 3.9%. These obtained results agree with that mentioned by Ashraf (1996), Hussain and Saikia (2000), who recovered Enterobacter spp. from diarrhoeic calves with an incidence of 3.6% and 2.45%, respectively. The present results are different from that described by Marcio *et al.* (2000) who recorded high recovery rate of Enterobacter spp. with an incidence of 62.5% and 30.1% from diarrhoeic and apparently healthy calves respectively.

Regarding to *Klebsiella* spp. as a causative bacterial agents of enteritis in calves, *Klebsiella* spp. was isolated from diarrhoeic calves with a percentage of 2.3%. This result agrees with that obtained by Ashraf (1996) and Hussain and Saikia (2000). They isolated Klebsiella spp. from diarrhoeic calves with an incidence of 3.8%, 2%, 1.8% and 3.2%, respectively. On the other hand, our result differs from that obtained by Mona (1995) and Marcio *et al.* (2000). They recovered Klebsiella spp. from calves with diarrhoea in a percentage of 13.3% and 35.4%, respectively.

As *E. coli* normal inhabitant in the intestinal tract of all worm blooded animals so its isolation from faeces of calves either diarrhoeic or apparently healthy have no significance unless we determine if it was pathogenic or non pathogenic. For this purpose Congo red binding activity of *E. coli* isolates was determined in this work. The result showed fundamental difference between the percentage of Congo red (CR) positive *E. coli* (Pathogenic) which recovered from diarrhoeic calves (83.7%) and apparently healthy calves (31%), while the Congo red negative *E. coli* (non pathogenic) was higher in apparently healthy calves (69%) than from diarrhoeic (16.3%). These results coincide with that obtained by Hoda (2006). They found that 87.3% (from diseased camels) and 80% (from diarrhoeic calves), respectively were CR positive E. coli. Also El-Bialy and Abd El-Aty (2002) demonstrated that all *E. coli* strains isolated from diarrhoeic foals and kids gave positive CR. Also, our results coincide with the results obtained by Mona (1995b). Accordingly, we can conclude that CR medium can be used as a rough method for *in vitro* differentiation between pathogenic and non pathogenic *E. coli*.

The pattern of antibiotic susceptibility of the most prevalent intestinal pathogens was done *in vitro* and the obtained data revealed

that ciprofloxacin is the most effective antibiotics for *E. coli* with an activity percentage of 96.23% followed by enrofloxacin, ofloxacin and chloramphenicol with activity percentage of 88.68%, 85% and 77%, respectively. The obtained results agree with that reported by Khaled (2004) who recorded higher sensitivity of E. coli to enrofloxacin and ciprofloxacin. On the other hand, these *E. coli* strains were resistant to clindamycin, oxytetracycline, colistin sulphate, amoxycillin and erythromycine (15.1%, 17%, 30%, 35.84% and 39.62%, respectively). These results agree with Khaled (2004) who reported that E. coli isolates were resistant to tetracycline and ampicillin. This may be possibly due to frequent use of these antibiotics where these farms commonly use a wide variety of antibiotics in the feed, water or as injectable drugs so the bacterial strains develop resistance to these antibiotics. Moreover, Abd El-Fattah (1990) reported that the sensitivity pattern of E. coli isolates from newly born calves against antibiotics was as follow: ampicillin (100%), colistin (89.1%), oxytetracycline (59.3%) and neomycine (87.2%).

Regarding to *Salmonella*e, three *Salmonella serotypes* were tested and found to be completely sensitive to chloramphenicol, cephalexine, cefotaxime, ampicillin (except *S. ferruch*), amoxycillin (except *S. ferruch*) and enrofloxacin (except *S. cerro*) but they were completely resistant to oxytetracycline, clindamycin and erythromycin.

Concerning *S. kentucky*, it was highly sensitive to chloramphenicol, cefotaxime, amoxycillin and ampicillin, while it was completely resistant to enrofloxacin, ertythromycin, oxytetracycline and clindamycin.

Regarding to *S. ferruch*, it was highly sensitive to ciprofloxacin, cefotaxime and enrofloaxacin, while it was completely resistant to erythromycine, oxytetracycline and clindamycine.

Concerning to *S. cerro*, it was highly sensitive to chloramphenicol, cefotaxime, and amoxycilinin while it was resistant to enrofloxacin, erythromycine, oxytetracycline and clindamycine. These results agree with that obtained by *Dargatz et al. (2000)* who reported that most of *Salmonella* isolated were resistant to tetracycline, ampicillin, chloramphenicol and streptomycine. So, the multidrug resistance phenomena are clear for salmonellae.

Regarding to possible involvement of fungal agents in calf diarrhoea in this study, the obtained finding revealed hat 12 isolates of yeasts were recovered and identified with an incidence of 7.2%.

Candida *glabrata* was the most prevalent fungal spp. isolated from routine samples (58.3%). This result was near to that obtained by Elad *et al.* (1998) who recorded that *C. glabrata* was the only fungus shed by the calves. Higher incidence of *C. glabrata* was observed among diarrheic calves than healthy (66.6% and 33.3%, respectively) that suggests their role as one of the causes of diarrhoea although some yeast spp. may be considered as commonsals of digestive tract of calves (Elad *et al.*, 1998).

C. guillermondi were isolated from diarrhoeic calves as well as healthy calves with an incidence of 22.2% and 66.6%, respectively. Total Candida isolates were the most common isolates of yeasts recovered from cases of calf diarrhoea, this result is in agreement with Sebryokov *et al.* (1984).

However, it is important to study the role of these yeasts in the aetiology of diarrhoea. The more isolation doesn't indicate this role. However, the above cited authors agree in incriminating these yeasts as potential pathogens and as being capable of causing diarrhoea particularly after prolonged antibiotic therapy.

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