

## **Occurrence of Some Bacterial and Parasitic Causes of Diarrhoea in New Borne Calves in Beni Suef Governorate**

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### **Abstract**

In the present study faecal samples and swabs of 170 new borne calves under one month of age in different villages in Beni Suef Governorate were examined for 7 common bacterial and parasitic enteropathogenic organisms. Bacteriological and parasitological examination of 170 of faecal samples from calves (127 apparently normal and 43 diarrhoeic) showed that *E. coli*, *Salmonella* spp., *Campylobacter jejuni*, *Campylobacter coli*, *Cryptosporidium* oocysts, *Eimeria* oocysts and *Ascaris* eggs were found at percentage of (22.94%), (0%), (11.77%), (5.29%), (77.65), (10.59) and (10%) respectively. Serological identification of 39 isolates of *E. coli* revealed that *E. coli* O168 (23.08%), O111 (20.51%), O127 (15.39%), O29 (12.82%), O119 (7.69%), O6 (7.69%) and 5 untypable isolates (12.82%) were found. The results of antimicrobial susceptibility of *E. coli*, *C. jejuni* and *C. coli* to different chemotherapeutic agents cleared that *E. coli* strains were moderately sensitive to ciprofloxacin, nalidixic acid and doxycycline, *C. jejuni* isolates were sensitive to chloramphenicol, and moderately sensitive to nalidixic acid while *C. coli* isolates showed high resistance to all used chemotherapeutic agents. The prevalence of different parasitic affections was studied in relation to age of calves and the mixed parasitic infection was discussed.

### **Introduction**

Diarrhoea in new borne calves particularly under one month of age is considered as one of the most important diseases due to significant economic losses, these losses not only by increasing calf fatality but also by decrease in the calf's ability to gain weight, treatment cost as well as subsequent chronic illness and poor growth (10). The etiology of the disease is complex as it involves an interaction between enteropathogenic bacteria, viruses and protozoa as well as risk factors (21). *E. coli* and *Salmonella* spp. are two of the most common bacterial enteric pathogens capable of causing diarrhoea in new borne calves (2 and 4). In addition there has been recent interest in *Campylobacter* spp., as potential causes of diarrhoea in new borne calves (1). *Campylobacter jejuni* and *Campylobacter coli* are

responsible factors of diarrhoea in several animal species and human (34), although other studies showed that there were no difference in prevalence of *C. jejuni* and *C. coli* between healthy and diseased calves (38 and 11).

Also internal parasitic affections of new born calves are considered one of the most important problems which affect the animal production and may be play a role as predisposing factor to other diseases of other origins (18), *Cryptosporidium* oocysts were detected in faecal samples of diarrhoeic calves (29 and 16), *Eimeria* oocysts were shed by calves within a month from birth (27) while other investigation showed that calves started to shed *E. coli* oocysts in faeces at 15<sup>th</sup> day of age (6) and the *Toxocara vitulorum* eggs were detected in faeces of Asian buffaloes calves at  $22.3 \pm 1.6$  day of age (33).

The purpose of the present study was to investigate the occurrence of some bacterial and parasitic causes of diarrhoea in calves under one month of age at Beni Suef Governorate, serological identification of the isolated *E. coli* and *Salmonella* spp. and studying the resistance of the isolated bacteria to different chemotherapeutic agents in vitro.

### **Material and Methods**

#### **Examined animals:-**

In this study 170 (127 apparently normal and 43 diarrhoeic) new borne calves under one month of age in different villages in Beni Suef Governorate were subjected to clinical examination according to (31).

#### **Samples:-**

Faecal swabs and faecal samples were individually collected from all examined calves and subjected to bacteriological, physical and parasitological examination.

#### **Isolation and identification of enteric bacteria: -**

Isolation and identification of *E. coli*, *Salmonella* spp. and *Campylobacter* spp. were done according to (13) and (30).

**Serological typing of *E. coli*: - (14)**

Agar slants containing generous growth of the isolates of *E. coli* were submitted to agglutination test using polyvalent and monovalent O, *E. coli* antisera obtained from Denka Seiken Co., LTD. Japan.

**Antimicrobial susceptibility: -**

In vitro antimicrobial susceptibility of the isolated bacteria was determined by using disc diffusion technique according to (17).

**Parasitological examination:-**

**Macroscopical examination of faeces:-**

Faecal samples were examined for physical characters, presence of visible blood and adult worms.

**Microscopical examination of faeces:-**

Each faecal samples were examined microscopically by direct smear and sedimentation technique (37). The examination of faeces for *Cryptosporidium* oocysts was carried out by staining technique according to (32), also modified sheather's sugar flotation method was used (22).

**Modified sheather's sugar flotation method:-**

Place 1-2 ml faecal suspension in 12 ml conical centrifugal tube, added sheather's sugar flotation until the tube is three quarter full, stir vigorously with applicator stirrer, fill the tube with sugar solution, centrifuge at 500 rpm for 10 mn, transfer surface material to glass slide by means of wire loop, cover with cover slip and examined microscopically.

## Results

**Clinical examination:-**

The Clinical examination of 170 calves revealed the presence of some clinical abnormalities in 43(25.29%) which included signs of enteritis, tenesmus and recumbency in some cases, the mucous membranes of all diarrhoeic cases were pale.

**Bacteriological and parasitological examination**

The results of bacteriological and parasitological examination of 170 faecal swabs and faecal samples for isolation of *E. coli*, *Salmonella* spp., *Campylobacter* spp., protozoa and helminthes were cleared in table (1) and fig (1 and 2).

**Table (1) Prevalence of bacterial and parasitic infections in new borne calves**

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Enteropathogenes	Apparently normal calves (n=127)		Diarrhoeic calves (n=43)		Total (n=170)	
	No	%	No	%	No	%
<i>E.coli</i>	27	21.26	12	27.91	39	22.94
<i>Salmonella</i> spp	0	0	0	0	0	0
<i>C.jejuni</i>	14	11.02	6	13.95	20	11.77
<i>C. coli</i>	7	5.51	2	4.65	9	5.29
<i>Cryptosporidium</i>	97	76.38	35	81.40	132	77.65
<i>Eimeria</i>	12	27.91	6	13.95	18	10.59
<i>Ascaris</i>	15	11.81	2	4.65	17	10

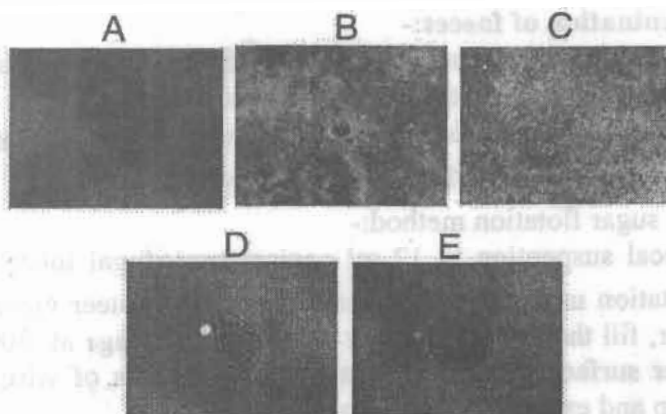
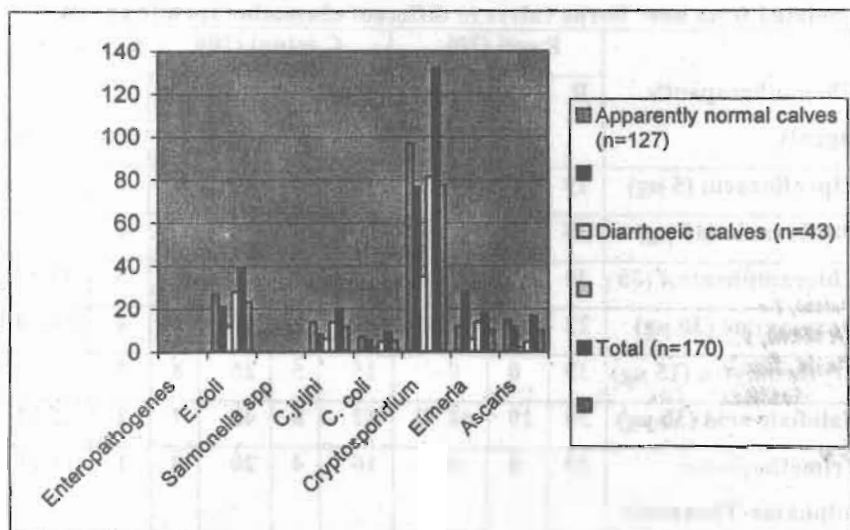


Fig (1) *Cryptosporidium* oocyst (A) by flotation technique, B, and C *Cryptosporidium* oocyst stained by modified Zeil Nelson (Brilliant green)x1000. *Eimeria* oocyst x40 (D). *Ascaris* egg x40 (E)

Fig (2) Prevalence of bacterial and parasitic infections in new borne calves



**Table (2) Incidence of different E.coli serotypes in new borne calves**

E.coli serotypes (n=39)	No	%
O168	9	23.08
O111	8	20.51
O127	6	15.39
O29	5	12.82
O119	3	7.69
O6	3	7.69
Untypable	5	12.82
<b>Total</b>	<b>39</b>	<b>100.00</b>

**Table (3): The in vitro antimicrobial susceptibility of *E.coli*, *C.jejuni* and *C. coli* isolated from new borne calves to different chemotherapeutic agents.**

Chemotherapeutic agents	E.coli (39)			C.jejuni (20)			C. coli (9)		
	R	S		R	S		R	S	
		No	%		No	%		No	%
Ciprofloxacin (5 µg)	19	20	51.28	20	0	0	9	0	0
Norfloxacin (10 µg)	25	14	35.9	20	0	0	9	0	0
Chloramphenicol (30 µg)	30	9	23.08	2	18	90	6	3	33.33
Doxycycline (30 µg)	22	17	43.59	17	3	15	5	4	44.44
Erythromycin (15 µg)	39	0	0	15	5	25	8	1	11.11
Nalidixic acid (30 µg)	20	19	48.72	12	8	40	7	2	22.22
Trimethoprim+ sulphame-Thoxazole (1.25+23.75 µg)	39	0	0	16	4	20	8	1	11.11

**Table (4) Prevalence of different parasitic affections in relation to age in new borne calves**

Age	No of examined calves	Cryptosporidium		Eimeria		Ascaris	
		No	%	No	%	No	%
One week	46	41	89.13	-	0	-	0
1-2 week	38	36	94.74	-	0	-	0
2-3 week	41	28	68.29	7	17.07	4	9.67
3-4 week	45	27	60	11	24.44	13	28.89

**Table (5) Single and mixed parasitic affections in new borne calves**

Exam. calves (n=170)	Single parasitic affection			Mixed parasitic affection		
	Crypt.	Eimeria	Ascaris	Crypt. + Ascaris	Crypt. + Eimeria	Ascaris + Eimeria
No	104	1	2	13	15	2
%	61.18	0.59	1.18	7.65	8.82	1.17

## **Discussion**

In the present study faecal samples and swabs of 170 new borne calves under one month of age in different villages in Beni Suef Governorate were examined for 7 common bacterial and parasitic enteropathogenic organisms. Bacteriological examination of 170 ( 127 apparently normal and 43 diarrhoeic) new borne calves under one month of age (Table-1 and Fig.-2) revealed isolation of 39 strains of E.coli 27(21,26%) and 12(27.91%) from apparently normal and diarrhoeic calves respectively, the incidence of E.coli was high in diarrhoeic calves this restriction of E.coli to young calves has been noted by many authors, (15) isolated E.coli from 14.8% of diarrhoeic calves up to 28 days of age and (20) isolated E.coli from 68.9% of apparently normal and 94.2% of diarrhoeic calves. Salmonella spp. were not detected in faecal swabs of all examined calves, similar result was recorded by (16) while (20) isolated Salmonella spp. from 26.2% of diarrhoeic calves and (24) isolated Salmonella spp. from 1.6% of diarrhoeic calves and failed to isolate Salmonella spp. from apparently normal calves, this may be attributed to the difference in localities.

C. jejuni and C.coli were isolated from new borne calves at rates of (11.77%) and (5.29% ) respectively, C. jejuni was isolated in high rate from diarrhoeic calves (13.95%) than from apparently normal calves (11.02%) while C.coli nearly isolated from both at the same rate,( 5.51% apparently normal calves and 4.65% diarrhoeic calves ) this result agreed with (20) who isolated C. jejuni from 9.5% apparently normal calves and 16.5% diarrhoeic calves and C. coli from 4.1% apparently normal calves and 3.9% diarrhoeic calves and (26) who isolated C. jejuni and C. coli from 20.7% and 6.4% of cattle farms respectively. Serological identification of 39 isolates of E.coli (Table 2) cleared that E.coli O168 (23.08%), O111(20.51%), O127(15.39%), O29 (12.82%), O119 (7.69%), O6 (7.69%) and 5 untypable isolates (12.82%) were found, such E.coli serogroups most frequently isolated from faecal samples of diarrhoeic calves (8, 35 and 20) .

The results of antimicrobial susceptibility of E.coli, C.jejuni and C.coli isolated from new borne calves to different chemotherapeutic agents were cleared in Table (3). E.coli strains isolated from new borne calves were moderately sensitive to ciprofloxacin, nalidixic acid and doxycycline, some strains were sensitive to norfloxacin and chloramphenicol and all strains were

resistant to erythromycin and trimethoprim+ sulphamethoxazole different rates of resistance of *E. coli* to these chemotherapeutic agents were recorded by (20) and (24), the variation in the degree of resistance may be due to the over use of these chemotherapeutic agents in treatment of diseased animals. *C. jejuni* isolates were sensitive to chloramphenicol, and moderately sensitive to nalidixic acid while *C. coli* isolates showed high resistance to all chemotherapeutic agents used, similar results were recorded by (7) who showed that *C. jejuni* isolated from cattle farms was 58.8% susceptible to ciprofloxacin, nalidixic acid, and erythromycin while *C. coli* isolates were 72.2% to 89.3% resistant to these chemotherapeutic agents.

The clinical examination of 170 calves revealed that (25.29%) of calves showed some clinical abnormalities similar findings among calves were reported by many authors and differed from one author to another, such variation in prevalence may be attributed to several factors, one of them is the hygienic condition. The clinical abnormalities were signs of enteritis, tenesmus and recumbency in some cases, the mucous membranes of all diarrhoeic cases were pale, similar pictures were observed by (9), (5). The results of parasitological examination of 170 faecal samples from new borne calves Table (1) and Fig. (1 and 2) revealed that *Cryptosporidium*, *Eimeria* and *Ascaris* were found at percentage of, (77.65 %), (10.59%) and (10%) respectively. Concerning with *Cryptosporidiosis* among clinical infected calves was (81.40%), low percentage was recorded by (36) and (3), the high percentage in this study may be attributed to the difference in the techniques used for detection of the protozoan or due to the spreading nature of the parasite and the high susceptibility of calves to be infected from adult, this observation was studied and confirmed by (25) and (28).

Clinical *Enteriasis* was detected in (13.95) of calves, similar results were recorded by (39), (31) and (12). In case of clinical *Ascariasis* was detected in (10%) of calves such prevalence may be due to higher exposure of young calves to ingestion of larvae in colostrums as reported by (33). In this study the prevalence of different parasitic affections was studied in relation to age of calves that ranged from one day up to one month of age Table (4) the results showed that infestation with *Cryptosporidium* was highest in first days of age and decrease in older calves, this indicated the high susceptibility of calves at this age, this result agreed with that reported by (23). Infestation



with *Eimeria* spp. in the present study was detected in 2-4 week of age, the obtained results agreed with that reported by (19) who reported that coccidiosis was appeared mainly in suckling calves due to the high rate of exposure to bad environmental conditions. In case of Ascariasis began in calves 2-4 weeks age this may be due to prenatal infection and also the ingestion of colostrum or milk containing infective larvae (33). The results recorded in Table (5) indicated that calves may be infested with more than one parasite at the same time such as association of *Cryptosporidium* and *Ascaris*, *Cryptosporidium* and *Eimeria* and *Ascaris* and *Eimeria*, this will expose the infested calves to a lot of harm due to mixed infestation.

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مذوق تواجد بعض مسببات الأسهال البكتيرية والطفيلية في العجول المصغرة في محافظة بنى سويف

ساميه ابراهيم عفيفي و خالد احمد شقير

عند دراسة بعض مسببات الأسهال في العجول الرضيعه في محافظة بنى سويف تم أخذ ١٧٠ عينة بزاز حيث تبين أن ٢٢.٩٤% و ٠% و ١١.٧٧ و ٥.٢٩% و ٧٧.٦٥% و ١٠.٥٩% و ١٠% منها تحتوى على عترات ميكروب الأشيريشيا القولونى وميكروب السالمونيلا وميكروبى الكاميلوباكتر جوجونى و الكاميلوباكتر كولاي و طفيل الكريبتوسبورديوم والكوكسيديا وبويضات الأسكارس بالنسب السابقه على التوالي كما صنفت عترات ميكروب الأشيريشيا القولونى وكانت بالأنواع الآتية O 6 و O 119 و O 29 و O 127 و O ١١١ و O ١٦٨ وكانت بالأعداد الآتية ٣ و ٣ و ٥ و ٦ و ٨ و ٩ واح يتحدد نوع عترات وتم دراسه حساسية البكتيريا المعزولة لبعض المعالجات الدوائية حيث أظهرت معظمها نسب مقاومه متفاوتة كما تم دراسه الأعمار بالنسب المختلفه بالأعمار العجول.