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EPIDEMIOLOGY AND DIAGNOSIS OF *ESCHERICHIA COLI* CALF SCOUR

By

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ABSTRACT

One of the most important problems in young calves, Neonatal calf diarrhea or what is called undifferentiated diarrhea beside calf pneumonia are considered the most dangerous life threatening, economic problems of the young growing calves. In the current study, a total number of 160 fecal samples collected from cattle and buffalo calves under one month old were examined bacteriologically for isolation of *E. coli*. All 44 (27.5%) *E. coli* isolates recovered from fecal samples collected from diarrheic calves were confirmed by PCR targeting 16SrRNA gene. All *E. coli* isolates were typed by serogrouping into O146 (50%), O1 & O119 (13.5%), O78 (9.25%) and O26 & O127 (6.8%). Epidemiology of *E. coli* was studied where; a higher prevalence rate was recorded in cattle calves (32.46 %) than buffalo calves (22.89%). A higher prevalence was found in female calves (34.6%) than male calves (21.1%). In different seasons, the higher incidence was in summer (38.7%) followed by winter (31.5%), spring (22.5%) and autumn (21.5%). The antibiotic susceptibility was also evaluated, where the isolates exhibited a high resistance to Enrofloxacin (91%), in the same time the isolates were sensitive to Sulphmethoxazole / Trimethoprim (54.5%), followed by amoxicillin / Calvulanic acid (29.5%).

Keywords:

Diarrhea, Calves, *E. coli*, PCR, Serotypes, Serogroups, Antibiotic Sensitivity.

INTRODUCTION

Neonatal calf diarrhea (NCD) defined as diarrhea of calves from one week up to 12 weeks old. The disease considered as one of the major health problem among newly born calves **(Bazeley, 2003),** causing economic losses include high morbidity and mortality rates, reduced growth rate, treatment costs and time spent caring for the affected calves **(Anderson *et al.*, 2003 and Ok *et al*., 2009)**.

The infectious causes of diarrhea include bacteria such as *Escherichia coli* (*E.coli*), *Salmonella* spp. and *Clostridium perfringens* **(Brown *et al.*,2007)**, viruses such as bovine rotavirus, bovine coronavirus and bovine viral diarrhea virus **(Cho and Yoon,2014)** and parasites such as *Cryptosporidium parvum*, *Eimeria* spp. and *Giardia* **(Schulze, 1992)**.

E. coli has been incriminated as a major cause of diarrhea, which characterized by progressive dehydration and death may occur depends on the age of the calf when scour started and on the particular pathotypes of *E. coli* **(Tan *et al.*, 2011)**.

Polymerase chain reaction (PCR) is used for the diagnosis of *E. coli* with high accuracy, and considered as an easy tool for amplifying genes of interest specifically present in a target pathotype or serogroup **(Begum *et al*., 1993).**

According to mechanism of pathogenesis and virulence factors the diarrheagenic *E.coli* divided into groups or categories **(Anis *et al.*, 2013)** First intestinal *E. coli* which comprises Enteropathogenic *E. coli* (EPEC); Enterotoxigenic *E. coli* (ETEC); Enteroinvasie *E. coli* (EIEC); Enterohemorrhagic, verotoxogenic or shiga toxin-producing *E. coli* (EHEC-VTEC-STEC); Enteroaggregative *E. coli* (EAEC) and Diffusely Adhering *E. coli* (DAEC).

The second ones are extra intestinal *E. coli* which comprises Associated *E.coli* Meningitis (MAEC); Uropathogenic *E. coli* (UPEC).

The present study was undertaken to study the prevalence of *E. coli* in diarrheic calves. Biotyping, serotyping and antibiotic sensitivity of the isolated strains of *E. coli* was also carried out.

MATERIAL AND METHODS

Animals and samples:

A total number of 160 fecal swabs were directly collected from the rectum of the examined diarrheic cattle and buffalo calves using sterile cotton swabs which inserted into the upper third of amies transport media and transferred to the laboratory on ice box.

Bacteriological Identification of *E. coli*:

Fecal swabs were inoculated into trypticase soya broth (TSB) and incubated at 37 °C for 24 hrs for propagation of *E. coli*. Subcultures from trypticase soya broth were streaked on MacConkey agar and EMB agar media and incubated at 37 °C for 24 hrs. **(Quinn *et al.*, 2002)**. Identification of the isolated bacteria was done based on colonial morphology, staining characters and biochemical reaction. After complete identification, the bacterial isolates were stored at-20°C into brain heart infusion broth containing 16% glycerol for long term preservation.

Identification of *E. coli* isolates by PCR:

DNA was extracted from the bacterial colonies by boiling method **(Wani *et al.,* 2003)**. The samples were tested using primer set (16SrRNA) for identification of *E. coli*, where PCR was targeting **(Wang *et al.,* 2002)**.

The sequence of primer used for amplification of 16SrRNA gene was forward: 5' CCCCCTGGACGAAGACTGAC 3' and Reverse: 5' ACCGCTGGCAACAAAGGATA 3' The PCR cycling protocol was applied as following: initial denaturation at 94°C for 8 min., followed by 30 cycles of denaturation at 95 °C for 30 sec., annealing at 58°C for 30 sec. and extension at 72 °C for 30 sec., followed by a final extension at 72 °C for 7 min.

Agarose Gel Electrophoresis and Visualization of PCR Products:

Amplified PCR products with product size of 401 bp were analyzed by electrophoresis in 1.5% agarose gel containing ethidium bromide (0.5 µg/ml). The products were visualized under UV illumination and analyzed with Gel pro analyzer® version 4 **(Sambrook and Russell, 2001)**.

Serotyping of isolated strains of *E. coli*.

Isolates that were preliminary identified biochemically and confirmed by PCR as *E. coli* were subjected to serological identification using slide agglutination test for identification of the somatic "O" antigen and determination of adherence of capsular "K99" antigen **(Ewing, 1986)**.

Antibiotic sensitivity of *E. coli*.

The disc diffusion technique was applied according to **Finegold and Martin (1982)** as well as the types of antibiotic sensitivity discs were selected according to **CarterandCollee (1990)**, the procedure for the inoculation of antibiotic sensitivity test plates as recommended by **NCCLS (1998).**

RESULTS

A total number of 160 fecal swabs collected from diarrheic cattle and buffalo calves were screened for detection of *E. coli* with a prevalence rate of 27.5% Fig.(1). *E. coli* colonies were bright pink and green metallic sheen on MacConkey and EMB agar media, respectively (Photo 1) with gram negative coccobacilli. The prevalence of pathogenic *E. coli* among all collected fecal samples from the buffalo and cattle calves suffered from diarrhea were 27.5% categorized as 32.46% (25 out of 77) and 22.89% (19 out of 83) in cattle and buffalo, respectively (Table 1). The result of the amplification of 16SrRNA gene using PCR revealed that, all *E. coli* strains were positive for 16SrRNA gene (100%) (Photo 2). All the 44 *E. coli*

strains isolated from the diarrheic calves were serologically screened for identification the different serotypes of pathogenic *E. coli*, where all the 44 *E. coli* strains isolated from the diarrheic calves were examined for detection of 6 non-O157 serogroups (O1, O146, O78, O127, O126, O119) using Dryspot *E. coli* seroscreen agglutination kit, with the certificate of Animal Health Research Institute "AHRI". The Kit detected 6-non O157 serogroups in *E. coli* strains at a percentage of 74% (N=37), while 26% (N=7) of *E. coli* strains were negative.

Also, the serological identification of *E. coli* isolates revealed that O146 serotype was the most predominant isolate (22 out of 44) with a percentage of 50%, followed by serotypes O1 and O119 (6 each out of 44) with a percentage of 13.6%, serotype O78 (4 out of 44) with a percentage of 9.1%, serotype O26 and O127 (3 each out of 44) with a percentage of 6.8% each. Also, serological detection of K99 Ag was studied for clarifying the linkage between serotypes in case of diarrheic calves proven to be caused by *E. coli* where K99 Ag was used, and we found that all O146 strains gave a positive result for k99.The Epidemiological aspects of *E. coli* as a causative agent of diarrhea (calf scour) in buffalo and cattle calves were also studied, where different factors were studied to make a relationship between *E. coli* and these factors which are very important for the field point of view as diagnosis and dealing with such a case (Tables 2 - 11)**,** Fig. (2, 3).The antibiotic susceptibility was also evaluated, where the isolates exhibited a high resistance to Enrofloxacin (91%), in the same time the isolates were sensitive to Sulphmethoxazole / Trimethoprim (54.5%), followed by amoxicillin / Calvulanic acid (29.5%).

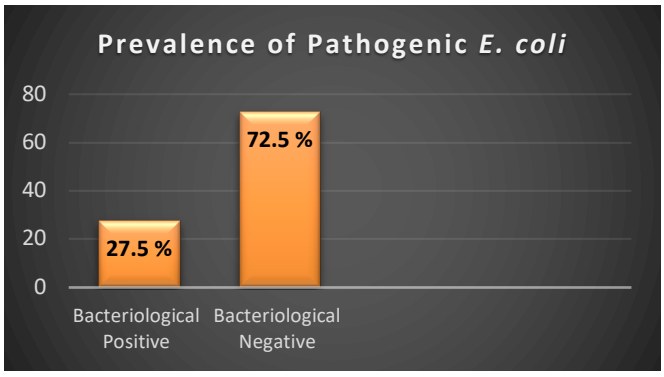


Fig. (1): The prevalence of pathogenic *E. coli.*

Table (1): The prevalence of pathogenic *E. coli* among cattle and buffalo calves.

Animal (Calves)	Number of samples	Bacteriological positive samples	
		No.	%
Cattle	77	25	32.46
Buffalo	83	19	22.89
Total	160	44	27.5

Table (2): Prevalence of *E. coli* among cattle and Buffalo calves according to their localities.

Governorate	Total number of samples	Bacteriologically positive samples	
		No.	%
Giza	45	12	26.7
Gharbia	35	9	25.7
Ismailia	40	8	20
Beni-suef	40	15	37.5
Total	160	44	27.5

Table (3): The incidence of *E. coli* among Cattle and buffalo calves.

Animal species	No. of samples	Bacteriologically positive samples	
		No.	%
Cattle calves	77	25	32.5
Buffalo calves	83	19	22.9
Total	160	44	27.5

Table (4): Prevalence of *E. coli* among cattle calve according to their localities and sex.

Governorates	Cattle Calves Fecal Samples			Bacteriologically positive Cattle Calves Fecal Samples					
	Male	Female	Total	Male		Female		Total	
				NO.	%	NO.	%	NO.	%
Giza	11	9	20	2	18.2	5	55.6	7	35
Gharbia	9	11	20	2	22.2	3	27.3	5	25
Ismailia	10	10	20	2	20	2	20	4	20
Beni-suef	10	7	17	4	40	5	71.4	9	53
Total	40	37	77	10	25	15	40.5	25	32.5

Table (5): Prevalence of *E. coli* among buffalo calves according to their localities and sex.

Governorates	Buffalo Calves Fecal Samples			Bacteriologically positive Buffalo Calves Fecal Samples					
	Male	Female	Total	Male		Female		Total	
				NO.	%	NO.	%	NO.	%
Giza	10	15	25	2	20	3	20	5	20
Gharbia	10	5	15	2	20	2	40	4	26.6
Ismailia	10	10	20	2	20	2	20	4	20
Beni-suef	15	8	23	2	13.3	4	50	6	26.1
Total	45	36	83	8	17.8	11	30.5	19	22.9

Table (6): The correlation between *E. coli* and sex among Cattle and Buffalo Calves.

Sex	Cattle Calves		Buffalo Calves	
	No.	%	No.	%
Male	10	25	8	17.8
Female	15	40.5	11	30.5
Total	25	32.5	19	22.9

Table (7): The Correlation of *E. coli* to age.

Age	No. of samples	Bacteriologically positive samples	
		No.	%
1st week	31	9	29
2nd week	25	9	36
3rd week	37	10	27
4th week	67	16	23.9

Table (8): The Correlation of *E. coli* to season.

Seasons	No. of samples	Bacteriologically positive samples	
		No.	%
Sumer	31	12	38.7
Autumn	42	9	21.4
Winter	38	12	31.6
Spring	49	11	22.5

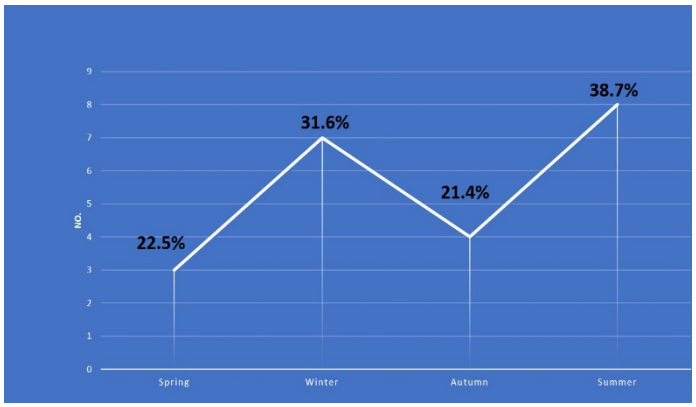


Fig. (2): The correlation of *E. coli* serotype O_{146} with the season.

Table (9): The Correlation of *E. coli* to the feeding system.

Feeding	No. of samples	Bacteriologically positive samples	
		No.	%
Natural suckling	67	17	25.4
Artificial (milk replacer)	95	27	28.4

Table (10): The Correlation of *E. coli* to the locality, age, season and feeding system.

Factors \ Serotypes		O146	O1	O119	O78	O26	O127
Localities	Giza	7	1	1	1	1	1
	Gharbia	3	2	1	1	1	1
	Ismailia	4	1	1	1	-	1
	Beni-suef	8	2	3	1	1	-
Age	1st week	4	2	1	-	1	1
	2nd week	5	1	-	1	2	-
	3rd week	6	1	1	1	-	1
	4th week	7	2	4	2	-	1
Seasons	Summer	8	1	2	-	-	1
	Autumn	4	2	-	1	1	1
	Winter	7	1	1	2	1	-
	Spring	3	2	3	1	1	1
Feeding	Natural suckling	8	2	3	3	1	-
	Artificial	14	4	3	3	2	3

Table (11): The correlation of *E. coli* serotypes with the age.

Serogroup	Total	1st week		2nd week		3rd week		4th week	
		NO.	%	NO.	%	NO.	%	NO.	%
O146	22	4	18.1	5	22.7	6	27.3	7	31.8
O1	6	3	50	2	33.3	1	16.7	0	0
O119	6	2	33.3	2	33.3	1	16.7	1	16.7
O78	4	2	50	1	25	0	0	1	25
O26	3	2	66.7	0	0	1	33.3	0	0
O127	3	1	33.3	2	66.7	0	0	0	0

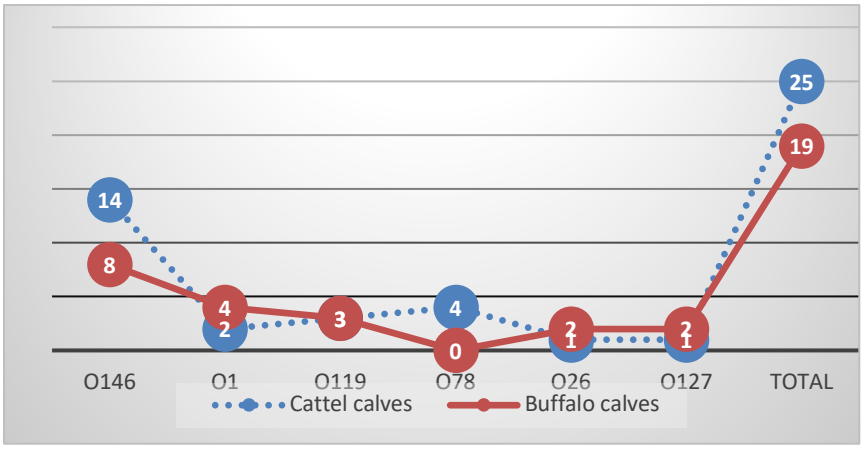


Fig. (3): The correlation of *E. coli* serotypes with the animal species.

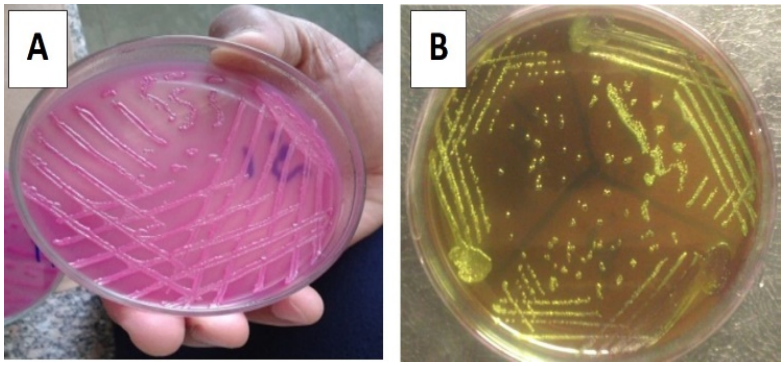
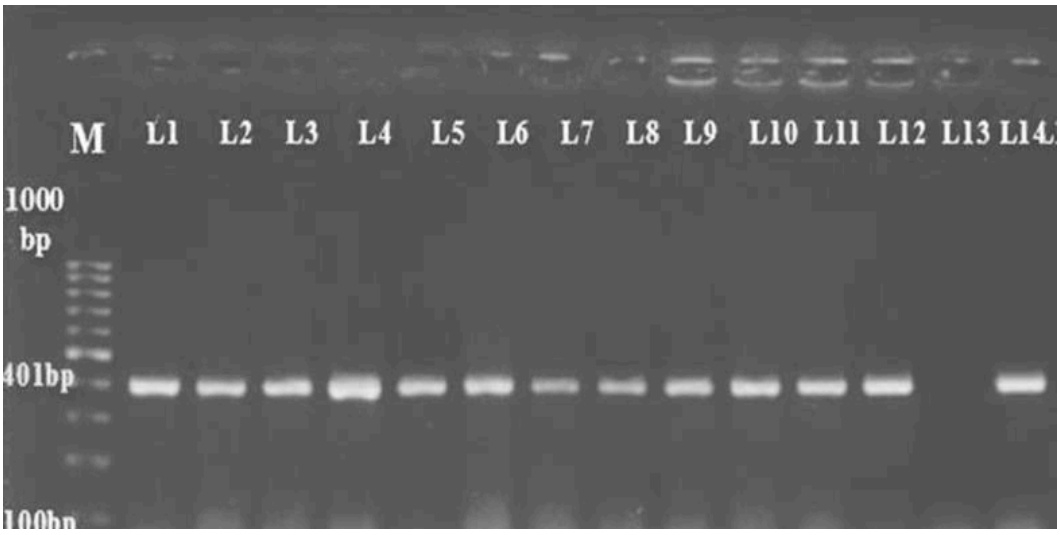


Photo (1): The cultured plates showing suspected *E. coli* isolates **(A)** on MacConkey agar media as bright pink colonies and **(B)** on EMB agar media as metallic sheen colonies.



Photo(2): Agarose gel electrophoresis of 16S rRNA gene in *E. coli* strains. Lane M: Molecular weight marker (100-1000 bp.); Lanes 1-12: positive samples with band of amplicon size at 401bp; Lane 13: Negative Control; Lane14: Positive control.

DISCUSSION

Colibacillosis is an infectious disease of enzootic importance especially in newborn calves **(Kolenda *et al.,* 2015).** Aspects of the etiology and pathogenesis of *E. coli* infections of young calves had been studied. Although colibacillosis can affect buffaloes and cattle at any age from the first month of embryonic life onwards, yet it received little attention among this animal species.

The present study was undertaken to study the prevalence of *E. coli* in diarrheic calves. Biotyping, serotyping and antibiotic sensitivity of the isolated strains of *E. coli* was also carried out.

Concerning the prevalence rate of *E. coli* as a causative agent for calf scour (27.5%), the results nearly agreed with **Bashir *et al*., (2015)** (35%) and **Gebregiorgis *et al.,* (2016)** (36%). On the other hand, the results were disagreed with **Fard *et al*., (2005)** (87.5%); **Hossain *et al*., (2013)** (49%)**; Islam *et al*., (2015)** (57%); **El-Seedy *et al*., (2016)** (75.6%).

All the isolated 44 *E. coli* strains were positive for the presence of 16S rRNA gene. This result is supported by the findings of **Sabat *et al*., (2000), Achtman (2001)**, **Galal *et al.,* (2013)**, **Hossain *et al*., (2013)**, **Bashir *et al*., (2015) and Kolenda *et al*., (2015)**.

Concerning the serotypes of *E. coli*., Forty-four isolates were isolated from diseased calves

identified according to "O" serogroups as O146, O1, O119, O78, O26 and O127 these results show the variant of *E. coli* serotypes isolated from diseased calves which agree with **Blanco *et al.,* (1996)** who recorded 74 isolates O2, O104, O128, O153, O157, O78, O26, O113 and O4. Also, O26, O119 and O114 were reported by **Saridakis *et al.,* (1997)** in addition **Fecteau *et al., (*2001)** determined 25 *E. coli* strains belonging to serotypes O78, O119 to be isolated from diseased calves.

The effect of feeding type on the incidence of enteric colibacillosis in calves was also studied where the incidence with the artificial (milk replacer) feeding was higher than the natural feeding. These results may be due to the contamination of teats from soil may be occurred but in artificial (milk replacer) with the bad hygienic measures and bad handling from workers

CONCLUSION

Colibacillosis is an infectious disease of enzootic importance especially in newborn calves. Aspects of the etiology and pathogenesis of *E. coli* infections can affect buffalo and cattle at any age from the first month of embryonic life onwards, yet it received little attention among those animal species. So, PCR as a tool is a sensitive, specific, and rapid technique for the confirmation of the *E. coli* hand to hand with the serotyping of the isolates for detection of pathogenic *E. coli*. Furthermore, all the pathogenic serotypes must be included in the vaccine provided for the dam in pregnant for supplying calves with readymade antibodies for on-spot protection against the life-threatening infections.

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