Escherichia coli Neonatal Calf Diarrhea in Middle Egypt: Prevalence, Phenotypes, Genotypes and Pathotypes

A.H. Abed[1] and A.M.S. Menshawy[2,*]

[1]*Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt*

[2]*Veterinary Medicine (Infectious Diseases) Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt*

Abstract: Neonatal calf diarrhea is one of the most important problems facing livestock causing great economic losses. *E. coli* is one of the most dangerous pathogens induced neonatal calves diarrhea from birth until about 3 months of age. This study investigated the prevalence of *E. coli* infection in diarrheic calves in middle Egypt Governorates (Giza, El Fayoum and Beni-Suef) to determine the virulence gene markers for *E. coli* isolates; using multiplex-PCR, detecting their pathotypes especially ETEC. Fecal samples were collected from 58 cross breed diarrheic calves up to 3 months and 38 *E. coli* isolates were recovered with prevalence of 65.5%. The highest prevalence was recorded in El-Fayoum (71.4%), followed by Giza (66.7%) while the lowest prevalence was Beni-Suef (57.1%). Serogrouping of *E. coli* isolates showed that 10 O-serogroups were identified. The serogroup O_{26} was the most prevalent (21.1%) followed by O_{103} (18.4%) then, serogroups O_{86} and O_{111} (13.2% and 10.5%, respectively). Moreover, other serogroups were recorded; O_{119}, O_{127} and O_{157} (5.3% for each) and O_{18}, O_{44} and O_{158} (2.6% for each) while, 13.2% of *E. coli* isolates were untyped. Analysis of multiplex PCR results for different *E. coli* serotypes showed that ETEC, AEEC, EHEC genes were possessed in 70%, 70% and 30% of isolates, respectively while none of the isolates possessed STEC gene. The in *in-vitro* susceptibility testing of *E. coli* isolates was applied. Mostly, *E. coli* isolates showed high resistances against the majority of antimicrobials used while; high sensitivity was observed to neomycin only.

Keywords: Neonatal calf diarrhea (NCD), *E. coli* pathotypes, enterotoxigenic *E. coli* (ETEC), *E. coli* serogroups, prevalence, antimicrobial susceptibility.

1. INTRODUCTION

Neonatal calf diarrhea (NCD); also known as calf scouring, is a commonly reported disease in young animals, and still a major cause of productivity and economic losses to the bovine industry worldwide [1]. According to the 2007 National Animal Health Monitoring System (NAHMS) for U.S. dairy [2], 57% of unweaned calf mortality was due to diarrhea especially in calves less than one month old. In Egypt, NCD continues to be the first cause of calf mortality ranging between 27.4 to 55% of the total deaths in young calves [3]. The economic losses occur not only from mortality but also from other costs including treatment, diagnostics, labor, veterinary intervention and decreased number of herd replacements [4] as well as subsequent chronic ill thrift and impaired growth performance [5].

NCD is multifactorial syndrome resulting from the interaction of a number of variables including pathogen (infectious NCD) as well as non-infectious factors related to the animal (immunological and nutritional status), the environment or the management [6]. The multifactorial nature of calf diarrhea makes this disease difficult to be controlled effectively in modern cow-calf operations [1].

Infectious diarrhea is the most significant cause of morbidity and mortality in neonatal dairy calves throughout the world [7]. It can be caused by a variety of pathogens including, viruses (rotavirus and coronavirus), protozoa (*Cryptosporidium parvum*) and bacteria [6]. Among bacteria, enterotoxigenic *E. coli* (ETEC) and *Salmonella* species are known to be the most common and economically important agents [8], but other bacteria have also been identified as cause of enteric disease and NCD, e.g. *Clostridium* species [9] and *Campylobacter* species [10]. Co-infection is frequently observed in diarrheic calves although a single infection can be recorded in some cases. The prevalence of each pathogen and disease incidence can vary by geographical location of the farms, farm management practices, and herd size [1]. Identification of the possible causative agent in outbreaks of diarrhea is important to allow targeted preventative measures, such as vaccination, and identification of possible risk factors or sources of infection [6].

E. coli can be classified into six pathogroups based on virulence scheme: enterotoxigenic (ETEC), attaching and effacing (AEEC)/or enteropathogenic

*Address correspondence to this author at the Department of Veterinary Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt; Tel/Fax: (+2) 0822327982;
E-mail: elmenshawy81@yahoo.com

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(EPEC), enteroinvasive (EIEC), enteroaggregative (EAEC), enterohaemorrhagic (EHEC) and shiga toxin-producing *E. coli* (STEC) but the most common cause of NCD is ETEC stains that produce the K99 (F5) adhesion antigen (*E. coli* K99+) and heat-stable (*STa* or *STb*) and/or heat-labile (*LT1* or *LT2*) enterotoxins [11]. *E. coli* causes a watery diarrhea and weakness in 1-4 day old newborn calves. Death usually occurred within less than 24 hours due to severe dehydration [9]. The fimbrial adhesion F5 (K99) promotes the attachment of bacterial cells to glycoproteins on the surface of epithelial cells of the jejunum and/or ileum, and bacterial enterotoxin also causes damage to the epithelial cells, resulting in fluid secretion and diarrhea [12]. AEEC and STEC have also been identified as causes of diarrhea and dysentery in calves [13].

The current study was planned to investigate the prevalence of *E. coli* in diarrheic calves in Middle Egypt Governorates as well as investigation of virulence gene markers for *E. coli* isolates; using multiplex-PCR, to detect their pathotypes especially ETEC.

2. MATERIAL AND METHODS

2.1. Animals

A total of 58 cross breed diarrheic calves up to 3 months of age (exhibiting signs of systemic disease; poor appetite, dehydration, decreased mentation and reduced suckle reflex, and had pasty watery feces) reared in different Middle Egypt Governorates (Giza, EL-Fayoum and Beni-Suef) were examined during the period from April to December 2017 (Table **1**).

Table 1: Number of Samples Collected from Diarrheic Calves from Different Governorates

Governorates	Collected samples	
	Number	%
Giza	9	15.5
El-Fayoum	28	48.3
Beni-Suef	21	36.2
Total	58	100

%: Percentages were calculated according to the total No. of samples.

2.2. Samples

Fecal samples were collected directly every 4 weeks for a period of 5 months from the rectum of diarrheic calves individually using sterile rectal swabs. All samples were transferred in an ice box to the laboratory of Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt, with minimal delay for bacteriological examination.

2.3. Bacteriological Examination

Isolation of *E. coli* strains was done according to El-Seedy *et al*. [14]. Briefly, 1 gram of each fecal sample was diluted in 3 mL sterile saline. Then, a loopful from the diluted specimens was inoculated into MacConkey's agar and incubated at 37C for 18-24 hrs. Lactose fermenter (pink) colonies were streaked onto and eosin methylene blue agar and confirmed as *E. col* using the standard biochemical tests [15, 16].

2.3. Serogrouping of *E. coli* Isolates

E. coli serogroups were identified serologically by slide agglutination test using standard polyvalent and monovalent *E. coli* antisera [17].

2.4. Multiplex PCR for Detection of Virulence Genes in *E. coli* Isolates

Multiplex-PCR was applied according to Pourtaghi *et al*. [18] on all *E. coli* isolates to investigate 5 virulence gene markers of *E. coli* to identify their pathotypes by amplifying genes encoding fimbrial gene *K99* (*F5*) and heat-stable enterotoxin a (*STa*) to identify ETEC beside genes encoding the gene of adherence factor intimin (*eaeA*), shiga toxin 1 (*Stx1*) as well as *HlyF* gene to identify AEEC, STEC and EHEC, respectively (Table **2**).

2.5. Antimicrobial Susceptibility Testing of *E. coli* Isolates

All *E. coli* isolates recovered from diarrheic calves were tested for their antimicrobial susceptibility to 10 different antimicrobial discs including; penicillin (10µg), amoxicillin (10µg), enrofloxacin (10µg), gentamicin (10µg), erythromycin (15µg), cefotaxime sodium (30µg), tetracycline (30µg), streptomycin (10µg), sulphamethoxazol-trimethoprim (25µg) and neomycin (20µg). (Oxoid, Basing Stoke, UK). Antimicrobial susceptibility testing was performed using disc diffusion method on Muller Hinton agar according to Clinical and Laboratory Standards Institute [22]. The antibiotic susceptibility was based on the induced inhibition zones according to the guidelines of the **CLSI** [22].

3. RESULTS

3.1. Prevalence of *E. coli* in Neonatal Diarrheic Calves in Different Governorates

The overall prevalence of *E. coli* was 65.5% (38/58). On the other hand, 20 samples showed negative *E. coli*

Table 2: Primers used in Multiplex-PCR for Detection of Virulence Genes in *E. coli* Isolates

Primer		Primer Sequences	Size of product	Reference
Stx1	F	*5′-TTCGCTCTGCAATAGGTA-3′*	555 bp	[19]
	R	*5′-TTCCCCAGTTCAATGTAAGAT-3′*		
HlyF	F	*5′-GGCCACAGTCGTTTAGGGTGCTTACC-3′*	450 bp	[20]
	R	*5′-GGCGGTTTAGGCATTCCGATACTCAG-3′*		
eaeA	F	*5′-ATATCCGTTTTAATGGCTATCT-3′*	425 bp	[21]
	R	*5′-AATCTTCTGCGTACTGTGTTCA-3′*		
K99 (F5)	F	*5′-TATTATCTTAGGTGGTATGG-3′*	314 bp	[19]
	R	*5′-GGTATCCTTTAGCAGCAGTATTTC-3′*		
STa	F	*5′-GCTAATGTTGGCAATTTTTATTTCTGTA-3′*	190 bp	
	R	*5′-AGGATTACAACAAAGTTCACAGCAGTAA-3′*		

Table 3: Prevalences of *E. coli* in Neonatal Diarrheic Calves in Different Governorates

Governorates	No. of samples	*E. coli* isolates		Negative isolation	
		No.	%	No.	%
Giza	9	6	66.7	3	33.3
El-Fayoum	28	20	71.4	8	28.6
Beni-Suef	21	12	57.1	9	42.9
Total	58	38	65.5	20	34.5

%: Percentages were calculated according to the corresponding No. of samples.

isolation (34.5%). The highest prevalence was recorded in El-Fayoum Governorate; as 71.4% (20/28), followed by Giza; as 66.7% (6/9). Meanwhile the lowest prevalence was recorded in Beni-Suef Governorate as 57.1% (12/21%) (Table **3**).

3.2. Serogrouping of *E. coli* Isolates

Out of 38 *E. coli* isolates, 10 O-serogroups were identified. The serogroup O_{26} was the most prevalent represented 21.1% (8 isolates) followed by serogroup O_{103} as 18.4% (7 isolates) then, serogroups O_{86} (13.2%; 5 isolates) and O_{111} (10.5%; 4 isolates). Afterthat, serogroups O_{119}, O_{127} and O_{157} were represented as 5.3% (2 isolates) for each. And finally, serogroups O_{18}, O_{44} and O_{158} were represented as 2.6% (1 isolate) for each. Moreover, there were 5 isolates (13.2%) were untyped with the available antisera (Table **4**).

3.3. Multiplex PCR for *E. coli* Isolates

Multiplex-PCR was applied on 33 isolates of *E. coli* representing all the identified serogroups to investigate 5 virulence gene markers of *E. coli* to identify their pathotypes by amplifying genes encoding fimbrial gene *K99* (*F5*) and heat-stable enterotoxin a (*STa*) to identify ETEC beside genes encoding the gene of adherence factor intimin (*eaeA*), shiga toxin 1 (*Stx1*) as well as

Table 4: Serogroups of *E. coli* Recovered from Diarrheic Calves

E. coli Serogroup	No.	%
O_{26}	8	21.1
O_{103}	7	18.4
O_{86}	5	13.2
O_{111}	4	10.5
O_{119}	2	5.3
O_{127}	2	5.3
O_{157}	2	5.3
O_{18}	1	2.6
O_{44}	1	2.6
O_{158}	1	2.6
Untyped	5	13.2
Total No. of isolates	38	100

%: was calculated according to the total No. of isolates.

Table 5: Prevalence of Virulence-Associated Genes among the Examined *E. coli* Isolates

Serogroups	No. of tested isolates	Virulence Genes									
		Stx1		*HlyF*		*eaeA*		*K99(F5)*		*STa*	
		No	%	No	%	No	%	No	%	No	%
O_{18}	1	0	0	0	0	1	100	1	100	1	100
O_{26}	8	0	0	0	0	6	75	5	62.5	5	62.5
O_{44}	1	0	0	0	0	0	0	1	100	1	100
O_{86}	5	0	0	2	40	4	80	3	60	0	0
O_{103}	7	0	0	5	71.4	5	71.4	0	0	0	0
O_{111}	4	0	0	0	0	0	0	4	100	4	100
O_{119}	2	0	0	0	0	2	100	2	100	2	100
O_{127}	2	0	0	0	0	0	0	2	100	2	100
O_{157}	2	0	0	2	100	2	100	0	0	0	0
O_{158}	1	0	0	0	0	1	100	0	0	0	0

%: was calculated according to the corresponding No. of testeds isolates.

HlyF gene to identify AEEC, STEC and EHEC, respectively.

The results shown in Table **5** revealed that all the tested serogroups (*n=10*) carried at least one virulence gene marker. Seven serogroups (70%) possessed ETEC virulent genes; 6 serogroups included O_{18}, O_{26}, O_{44}, O_{111}, O_{119} and O_{127} carried both *K99* and *STa* genes while O_{86} carried *K99* gene only. Moreover, 7 serogroups (70%) included O_{18}, O_{26}, O_{86}, O_{103}, O_{119}, O_{157} and O_{158}, possessed AEEC virulent gene (*eaeA*) while 3 serogroups only (30%); O_{86}, O_{103} and O_{157}, possessed EHEC gene (*HlyF*). On the other hand, none of the isolates possessed STEC gene (*Stx1*).

3.4. Antimicrobial Susceptibility Testing of *E. coli* Isolates

Results of *in-vitro* Antimicrobial sensitivity tests of *E. coli* isolates against 10 antimicrobial agents revealed that the different serogroups as well as the untyped group showed different degrees of sensitivity against the tested antibiotics. Mostly, *E. coli* isolates showed high resistances against the majority of antimicrobials used while; high sensitivity was observed against neomycin only (Table **6**).

4. DISCUSSION

Neonatal calf diarrhea remains one of the most important problems facing livestock and causing great economic losses not only from calf mortality and treatment costs, but also from losses in future growth and production [23]. Calves are at greatest risk of developing diarrhea within the first month of life and the incidence of diarrhea decreases with age [24].

NCD is still problematic due to the multifactorial nature of the disease. Along with infections with multiple enteric pathogens, many additional parameters such as nutritional factors, management practices, hygiene conditions and environmental factors, also contribute to the final outcome of the disease [25, 26]. Overfeeding, overpopulation, cold temperature, bad hygiene, stress and colostrum deprivation, are all non-infectious factor which can be important in the complex etiology of NCD [9]. The multifactorial nature of NCD makes this disease hard to control effectively. Therefore, prevention and control of such disease must be based on a good understanding of those disease complexities during the calving period before disease outbreaks [1]. Identification of the possible causative agent in outbreaks of diarrhea is important to allow targeted preventative measures, such as vaccination, and identification of possible risk factors or sources of infection [6].

Among bacteria, *E. coli* and *Salmonella* are the most common identified pathogens in scouring calves less than 2 months of age [8]. Their prevalences vary by geographical location of the farms, farm management practices, and herd size [1].

The current study aimed to investigate the prevalence of *E. coli* in diarrheic calves in Middle Egypt Governorates as well as investigation of virulence gene markers for *E. coli* isolates; using multiplex-PCR, to detect their pathotypes especially ETEC.

Table 6: Antibacterial Sensitivity Testing of *E. coli* Isolates

Antimicrobial disc	Disc content (µg)	O_{18} (n=1)		O_{26} (n=8)		O_{44} (n=1)		O_{86} (n=5)		O_{103} (n=7)		O_{111} (n=4)		O_{119} (n=2)		O_{127} (n=2)		O_{157} (n=2)		O_{158} (n=1)		Untyped (n=5)	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Enrofloxacin	10	100	0	12	88	0	100	0	100	14	86	0	100	0	100	50	50	100	0	0	100	20	80
Gentamicin	10	0	100	25	75	0	100	0	100	28	72	25	75	0	100	0	100	0	100	0	100	0	100
Erythromycin	15	0	100	12	88	0	100	0	100	28	72	0	100	0	100	0	100	0	100	0	100	0	100
Cefotaxime sodium	30	0	100	25	75	25	75	0	100	28	72	0	100	50	50	0	100	0	100	0	100	0	100
Amoxicillin	10	0	100	0	100	0	100	0	100	0	100	0	100	0	100	50	50	0	100	100	0	60	40
Penicillin	10	100	0	0	100	0	100	0	100	14	86	0	100	0	100	0	100	0	100	100	0	80	20
Tetracycline	30	0	100	88	12	0	100	100	0	14	86	0	100	0	100	0	100	0	100	0	100	0	100
Streptomycin	10	0	100	25	75	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100	0	100
Sulphamethoxazol-trimethoprim	25	0	100	25	75	25	75	0	100	28	72	0	100	0	100	0	100	0	100	0	100	20	80
Neomycin	20	100	0	88	12	100	0	100	0	86	14	100	0	0	100	50	50	0	100	0	100	80	20

In the present study, the prevalences of *E. coli* in neonatal diarrheic calves in different Governorates were illustrated in Table **3**. Out of 58 faecal samples collected from diarrheic calves, 38 *E. coli* isolates were recovered with prevalence rate of 65.5%. The negative isolation of some fecal samples (20 samples; 34.5%) may be attributed to presence of other bacteria especially *Salmonella* which need pre-enrichment selective media such as selenite F broth or tetrathionate broth [15] and it wasn't aimed to be studied in the current study. Also, some microorganisms can't grow on the used culture media either bacteria as *Clostridium* species [9] and *Campylobacter* species [10] that requiring specific or enriched culture media, viruses that requiring tissue culture or protozoa. Additionally, the presence of antibiotic residues may explain falsely negative bacteriological results because the withdrawal time is not regarded in our herds.

The prevalence of *E. coli* in the current study was nearly coincided to other findings in Egypt; 63.6%, [27] while higher than others; 5.4% [28], 35.83% [29] and 50% [30]. On the other hand, it was lower than other studies; 100% [31], 82% [32], and 75.6% [14].

Regarding other countries, it was similar to that recorded in Mexico; 63.7%, [33]. Meanwhile it was higher than those recorded in India; 23% [34], Sweden; 11.5% [35], Pakistan; 54%, [36], Germany; 42% [37], France; 20.3% [38], Northern Spain; 35.9% [39] and Australia; 17.4% [6]. On contrary, this result was lower than those reported in India; 75% [40] and Iran (86.7%) [18]. The differences of the prevalence rates of *E. coli* in diarrheic calves may be attributed also to the geographical locations and management practice as well as hygienic measures where ETEC infection occurs mainly through ingestion of contaminated food or water [1].

Regarding the prevalence of *E. coli* isolation in different Middle Egypt Governorates, the highest prevalence was recorded in El-Fayoum (71.4%), followed by Giza (66.7%) while the lowest prevalence was recorded in Beni-Suef Governorate (57.1%). The highest prevalence was in El-Fayoum because this governorate has highly intensive calves rearing systems, they collect young suckling calves from different sources or localities to be reared in closed farms and that system usually put the young calves under stressful conditions leading to decreased immunity and later on increases susceptibility for infectious diseases specially *E. coli* infection. Giza and later on Beni-Suef had lower prevalence rates due to the lower intensive calf rearing and production systems

In the current study, serogrouping of *E. coli* isolates represented in Table **4** revealed that 10 O-serogroups were identified. The serogroups O_{26} was the most prevalent (21.1%) followed by serogroup O_{103} (18.4%) then, serogroups O_{86} and O_{111} (13.2% and 10.5%, respectively. Moreover, serogroups O_{119}, O_{127} and O_{157} were represented as 5.3% for each while serogroups O_{18}, O_{44} and O_{158} were represented as 2.6% for each. Meanwhile, 13.2% of *E. coli* isolates were untyped with the available antisera. These results run hand to hand with other studies in Egypt where the most common *E. coli* serotypes in isolated diarrheic calves were O_{26} (23.52%), O_{103} (19.6%), and O_{119} (17.64%) followed by O_{86}, O_{111}, and O_{157} (5.88%); O_{44}, O_{125}, and O_{128} (3.92%); O_{78} (1.96%); and untyped *E. coli* (7.84%) [41]. In another study, O_{26} and O_{103} were the most prevalent serogroups (17.7% for each) followed by O_{127} (14.6%) and O_{119} (13.6%) then serogroups O_{86}, O_{111} and O_{157} (5.2% for each), as well as O_{44} and O_{158} (4.2% for each) and finally serogroup O_{78} (3.1%) and untyped *E. coli* isolates (9.4%) [14]. Moreover, Ibrahim [42] recovered *E. coli* serotypes O_{26}, O_{86}, O_{111} and O_{127} from scouring calves. The present findings were also nearly similar to those obtained by other authors in Egypt; where 102 *E. coli* strains were recovered from diarrheic calves, goat and sheep belonging to seven O serogroups; O_{25}, O_{78}, O_{86}, O_{119}, O_{158}, O_{164} and O_{157} [27]. Also, 8 *E. coli* serogroups were recovered including O_{55}, O_{111}, O_{26}, O_{153}, O_8, O_{18}, O_{86}, O_{157}, and “5” isolates were untyped from diarrheic calves at different localities in Qalyoubia Governorate [29]. Concerning the other countries, in Japan the most common *E. coli* serogroups isolated from diarrheic fecal samples were O_{119}, O_{111}, O_{126}, and O_{78} [43] found that. Similarly, Badouei *et al*. [44] recovered $O_{157:}H_7$, O_{111}, and O_{26} serotypes from diarrheic and non-diarrheic calves and the most common serogroup was O_{26} (18.4%).

In the current study, Multiplex-PCR was applied on 33 isolates of *E. coli* representing all the identified serogroups to investigate 5 virulence gene markers of *E. coli* to identify their pathotypes by amplifying genes encoding fimbrial gene *K99* (*F5*) and heat-stable enterotoxin a (*STa*) to identify ETEC beside genes encoding the gene of adherence factor intimin (*eaeA*), shiga toxin 1 (*Stx1*) as well as *HlyF* gene to identify AEEC, STEC and EHEC, respectively.

The results illustrated in Table **5** indicated that all the tested serogroups carried at least one virulence

gene marker. Regarding ETEC virulent genes, *K99* gene was detected in 7 serogroups (70%) of them, 6 were mixed with *STa* gene included O_{18}, O_{26}, O_{44}, O_{111}, O_{119} and O_{127} while O_{86} carried *K99* gene only meanwhile O_{103}, O_{157} and O_{158} possessed neither *K99* nor *STa* genes.

Analysis of these results revealed that 70% of the tested *E. coli* isolates were ETEC due to carrying *K99* and *STa* genes. A close correlation between enterotoxigenicity and the presence of the *K99* antigen has been confirmed by some authors [45, 46], but others have reported non-enterotoxigenic *E. coli* possessing the *K99* antigen [47]. Most bovine ETEC produce *STa* enterotoxin and *K99* fimbriae [48]. Also, all ETEC were reported to carry K99 fimbriae and possess *STa* enterotoxin gene [18]. Although, Achá *et al*. [8] not detected enterotoxins; neither *STa* nor *LT*, in any *E. coli* isolates from the diarrheal calves, 40% of these isolates were K99 positive. The present results was supported with that obtained by other authors [11, 49] who reported that the most common cause of NCD was ETEC stains that produce the *K99* (*F5*) adhesion antigen (*E. coli K99*+) and heat-stable (*STa* or *STb*) enterotoxins. *K99* is a fimbrial adhesin distinct from the capsular polysaccharide K antigens [50] promoting the attachment of bacterial cells to glycoproteins on the surface of epithelial cells of the jejunum and/or ileum [51]. After colonization of the gut epithelium, heat-stable toxin production induced by ETEC causes damage to the epithelial cells, resulting in up-regulation of chloride secretion into the gut. This osmotically pulls water into the intestinal lumen and leads to the development of secretory diarrhea in calves [12].

On the other hand, 70% of serotypes also were AEEC (carried AEEC virulent gene; *eaeA*); of which 30% were mixed with ETEC only (O_{18}, O_{26} and O_{119}), 20% were mixed with EHEC (O_{103} and O_{157}), 10% were mixed with both ETEC and EHEC (O_{86}) and 10% was pure AEEC (O_{158}). Unlike ETEC, EPEC strains do not produce toxins [52]. Enteropathogenic *E. coli* (EPEC) Or Attaching and effacing *E. coli* (AEEC) produces an outer membrane protein, *intimin*, which mediates the intimate attachment of bacteria to the enterocyte, causing typical A/E (attaching and effacing) intestinal lesions. Intimin (*eae*) protein is considered one of the most important virulence factors in *E. coli* strains [53]. These results were supported with those recorded in Egypt where *eaeA* gene was detected in 6 *E. coli* isolates from calves and high gene combinations were found between *eaeA*, *hylA*, *K99* and *Stb* genes [27]. On the other hand, other authors investigated the presence of virulence genes in 156 *E. coli* isolates recovered from neonatal diarrheic calves with 2 multiplex PCR protocols and reported that none of the isolates carried *eaeA* gene [18].

Moreover, 30% of serotypes (O_{86}, O_{103} and O_{157}) were EHEC (carried *HlyF* gene). All of them were mixed with EAEC and 10% were mixed with ETEC (O_{86}). These results were supported with other studies in Egypt [27].

On the contrary, none of the isolates possessed STEC gene (*Stx1*). These results run parallel to those obtained by others who fund that *E. coli* strains recovered from diarrheic calves were negative for both *stx1*and *stx2* [27]. On the contrary, the present results were not coincided with many authors who identified shiga toxin-producing *E. coli* (STEC) as a cause of diarrhea and dysentery in calves [13]. Nguyen *et al*. [54] found that 50 *E. coli* isolates recovered from diarrheic carried genes for Shiga toxins while another study reported that *Stx1* represented 82.8 % of the virulence markers identified [49].

Despite the increased availability of vaccines against ETEC and other pathogens associated with NCD and continued emphasis on optimizing colostral transfer of passive immunity, improved treatment protocols for calf diarrhea are required. Although the administration of intravenous fluids and oral electrolyte solutions plays a central role in treatment, the efficacy of antimicrobial agents in treating calf diarrhea is controversial [55].

One of the steps in the treatment of NCD is the use of the appropriate chemotherapeutic agents. Many authors reported that calves with diarrhea are more likely to have failure or partial failure of passive transfer, and this group of calves, in turn, is more likely to be bacteraemic [55-57] and this is an additional cause that antimicrobial agents might be indicated in the treatment of calf diarrhea. On the contrary, other authors mentioned that antibiotics rarely affect the outcome of this disease, while fluid support is critical to survival [58]. Vaccination of dry cows and good colostrum feeding can eliminate this problem. The type of antibiotic drug should better be selected on the basis of its sensitivity which could be detected by laboratory examination and the antimicrobial treatment of diarrheic calves should therefore be focused against bacteria in the twosites of infection; the small intestine and blood [55].

Amoxicillin, chlortetracycline, neomycin, oxytetracycline, streptomycin, sulfachloropyridazine, sulfamethazine, and tetracycline administered orally are currently labeled in the United States and all over the world for the treatment of calf diarrhea [55]. These antimicrobials were nearly similar to those used in the present study to perform the in-vitro sensitivity tests for the recovered *Salmonella* serotypes and *E. coli* serogroups.

In the present study, *in-vitro* antimicrobial sensitivity test of *E. coli* isolates against 10 antimicrobial agents was applied. The results represented in Table **6** revealed that the different *E. coli* serogroups as well as the untyped group showed different degrees of sensitivity against the tested antimicrobials. Mostly, *E. coli* isolates showed high resistances against the majority of antimicrobials used while; high sensitivity was observed against neomycin only. These findings were in agreement with studies in pre-weaned dairy calves obtained by other many authors [59-61].

The high occurrence of antimicrobials resistance can be anticipated since a large proportion of the animals are probably treated with antimicrobials [60]. Other authors discussed the epidemiology of resistant *E. coli* in calves as multifactorial, complex and e.g. influenced by co-selection due to linkage of resistance genes [62]. But widespread resistance is fundamentally a consequence of historical and current use of antimicrobials and associations between use of antimicrobials and resistance in enteric *E. coli* of calves have been documented [61, 63, 64]. One proposed factor is a linkage between resistance genes and genes conferring selective advantage to colonize the intestinal lumen of calves. Walk *et al.* [64] hypothesized that, regardless of use of antimicrobials, antibiotic resistance in *E. coli* is co-selected in calves by an unknown “beneficial mutation”.

5. CONCLUSION

Neonatal calf diarrhea is one of the most important problems facing livestock causing great economic losses despite vaccination programs and management measures, necessitating treatment with antibiotics and fluid therapy. *E. coli* is one of the most dangerous pathogens induced neonatal calves from birth until about 3 months of age. The serogroup O_{26}, O_{103}, O_{86} and O_{111} were the most prevalent serogroup. Analysis of multiplex PCR results for different *E. coli* serotypes showed that ETEC, AEEC, EHEC genes were possessed in 70%, 70% and 30% of isolates, respectively while none of the isolates possessed STEC gene. Mostly, *E. coli* isolates showed high resistances against the majority of antimicrobials used. The in *in-vitro* susceptibility testing of *E. coli* isolates mostly showed high resistances against the majority of antimicrobials used while; high sensitivity was observed to neomycin only.

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