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Risk factors associated with *E. coli* causing neonatal calf diarrhea

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ABSTRACT

Calf diarrhea is one of the major health challenges in cattle herds. The bacteriological examination of fecal samples collected from apparently healthy and diarrheic calves' revealed isolation of 26 *E. coli* isolates out of 56 calves with an incidence of 46.4%. Serogroups O1, O26, O44, O55, O115, O119, O125, O146, and O151 were identified from the collected fecal samples. Using PCR all isolates was positive for *ompA* gene species specific for *E. coli*. While *stx*1 and *eae*A genes detected with incidence of 3.8 and 19.2% respectively from the isolates. The presence of *stx*2 gene was negative in the fecal isolates. Among colostrum samples 4 *E. coli* isolates were detected and serogrouped to O26, O55 and O119. They were negative for *eae*A, *stx*1 and *stx*2 except strain number 4 (O55) was positive for *stx*1. *E. coli* strains were sensitive to norfloxacin (80.7%) and resistant to ampicillin and cefotaxime (100% each). Based on our findings, there was no association between occurrence of *E. coli* and age of calf (2–14 days), while bottle feeding calf colostrum may be a source of *E. coli* contamination.

1. Introduction

The occurrence of risk for calf diarrhea and mortality has been reported by Windeyer et al., 2014. Enterotoxigenic *E. coli* was isolated from diarrheic calves by many authors worldwide (Dereje, 2012; Masud et al., 2012). Calf diarrhea is result from multifactorial: incorrect management of calves; feeding, age, and animal breed were the most important risk factors of death rate (Muluken et al., 2017). Ashenafi and Tesfaye (2016) isolated *Escherichia coli* from diarrheic calves, and determined risk factors associated with its isolation. They concluded that younger age and low colostrum feeding were significantly associated with *E. coli* isolation. The occurrence of *E. coli* was lower in the milk stored and transported in stainless steel containers (Nigatu et al., 2017). Awosile and Smith (2017) recorded that shedding of cephalosporin-resistant *E. coli* may be caused by waste milk feeding in calves. Benavides et al. (2018) detected extended spectrum betalactamase producing *Escherichia coli* in bats and domestic animals. A strategy to control calf mortality must start with a confirmed diagnosis of the causative agents and study the risk factors associated with diarrhea. The present investigation was aimed to study occurrence of *E. coli* and the risk factors associated with diarrhea in dairy farms.

2. Materials and methods

2.1. Animals for sampling

This study was conducted in 3 dairy farms in El-Fayoum governorate from November 2015 to April 2016 to estimate the prevalence of *E. coli* from calf's scours up to 3 months of age and assessment of risk factors associated with calf diarrhea as well as antimicrobial sensitivity testing. A total of 56 fecal samples were collected from 26 calves suffering from diarrhea and 30 apparently healthy calves in contact with diseased animals selected from

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different private farms in El-Fayoum farms. As well as 33 colostrum samples were collected from farm number 3 (had high number of diarrheic calves). Samples were collected under complete aseptic condition and transported in ice box as soon as possible to the lab to detect *E. coli*.

2.2. Identification of E. coli isolates

Isolation and identification of *E. coli* among the collected samples were confirmed on the basis of their morphology, cultural and biochemical tests using standard bacteriological procedures described by Quinn et al. (2002) and Murrary et al. (2003). Serological identification of *E. coli* isolates was conducted at Serology Unit, Animal Health Research Institute, Dokki using polyvalent group specific antisera and Monovalent group specific antisera (Mast assure^{tm} pathogenic *Escherichia coli* "O" antisera).

2.3. Procedure for PCR

Extraction of DNA was carried out according to QIAamp DNA mini kit instructions.

Specific sequence oligonucleotide primers were used to amplify a specific product as shown in Table 1. Agarose gel electrophoreses was prepared according to Sambrook et al. (1989) and the data was analyzed through computer software.

2.4. Management of risk factors among the investigated farms

The risk assessment of the farms was generated through a questionnaire and direct observations. The questionnaire was investigated the following:

Herd size, vaccination, calf separation, usage of antibiotics to treat diarrhea, calf separation, calf mortality, diarrhea color, diarrhea duration, presence of calving stable, cleaning and disinfecting calving stable after calving, use of calving stable for animals, cleaning and disinfecting of obstetric material, cleaning and disinfecting of hands, cleaning and disinfecting rear of cows, immediate separating calf from cow after calving, cleaning and disinfecting of calf box after each calf, contact between weaned and un weaned calves, herd clothes are being used for visitors, use of one bucket per calf, milk type.

Table 1
Oligonucleotide primers sequences.

Reference	Amplified product	Primer sequence 5′–3′	Genes
Ewers et al. (2007)	919 bp	AGCTATCGCGATTGCAGTG GGTGTTGCCAGTAACCGG	*ompA*
Bisi-Johnson et al. (2011)	248 bp	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	*eaeA*
Dipineto et al. (2006)	614 bp	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	*stx1*
	779 bp	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	*stx2*

2.5. Antibiogram sensitivity test of E. coli

Antibacterial susceptibility testing of the isolates was carried out by Kirby-Bauer disk diffusion assay using standard procedures of National committee for clinical laboratory Standard (1998). Ampicillin (AM 10 μg), cefotaxime (CTX 30 μg), clindamycin (DA 2 μg), gentamicin (CN 10 μg), kanamycin (K 30 μg), neomycin (N 30 μg), norfloxacin (NOR 10 μg) and trimethoprim-sulfamethoxazole (STX 25 μg) discs from Oxoid were used. Results were recorded and compared with the interpretation of zone of inhibition in agar diffusion method according to CLSI (2017).

3. Results

3.1. Occurrence of E. coli among the examined farms

Fecal samples were obtained from 30 clinically healthy calves and 26 calves had signs of diarrhea at the time of sampling. The animal population comprised 65.2% males and 34.7 females. 14.3% of calves were between 1 and 14 days of age, 28.6% between 15 and 28 days of age, and 57.1% older than 28 days of age. The occurrence of diarrhea varied markedly between herds, age of occurrence and month of birth.

Among the examined fecal samples 26 *E. coli* isolates were identified and serogrouped to O1, O26, O44, O55, O115, O119, O125, O146 and O151 Table 2.

3.2. Antimicrobial sensitivity test among E. coli isolates recovered from calves

Table 3 recorded that all isolates (100%) were resistant to ampicillin and cefotaxime while 76.9% were sensitive to norfloxacin & gentamicin (each).

3.3. Virulence factors among E. coli isolated:

3.3.1. Among fecal samples

The isolates were examined using multiplex PCR to detect *omp*A, *stx*1 and *stx*2 genes. All 26 isolates were positive for *omp*A gene species specific for *E. coli* (100%). Only one isolate had *stx1* gene 1 (3.8%). No isolate had *stx*2 gene. Among *eae*A, attaching and effacing gene, 5 *E. coli* isolates (19.2%) had *eae*A gene as shown in Figs. 1 and 2.

3.3.2. Among colostrum samples

Four colostrum samples were positive for *E. coli* and serogroups O26, O55 and O119. All were positive for *omp*A gene

Table 2
Occurrence of *E. coli* in different farms samples.

Farm No	Status of animals	No of the examined samples	*E. coli* positive samples		Serogroups
		No	NO	%	
Farm 1	App. healthy	10	3	30	O125 and O55
(20 calves)	Diseased	10	1	10	
Farm 2	App. healthy	8	4	50	O26, O115, and
(12 calves)	Diseased	4	2	50	O146
Farm 3	App. healthy	12	7	58	O119, O151, O1
(24 calves)	Diseased	12	9	75	and O44
Total (56 calves)		56	26	466	

Table 3
Results of antimicrobial sensitivity test among *E. coli* isolates recovered from calves.

Antimicrobial group	Antimicrobial agent	S		I		R	
		n	%	n	%	n	%
Penicillin	Ampicillin	–	–	–	–	26	100
Aminoglycosides	Gentamicin	20,121	76.9	1	3.8	51,312	19.2
	Kanamycin		46.2	1	3.8		50
	Neomycin		3.8	13	50		46.2
Cephalosporins	Cefotaxime	–	–	–	–	26	100
Lincosamide	Clindamycin	1	3.85	1	3.85	24	92.3
Quinolone	Norfloxacin	20	76.9	1	3.8	5	19.2
Sulfonamides	Trimethoprim-Sulfamethoxazole	8	30.8	2	7.7	16	61.5

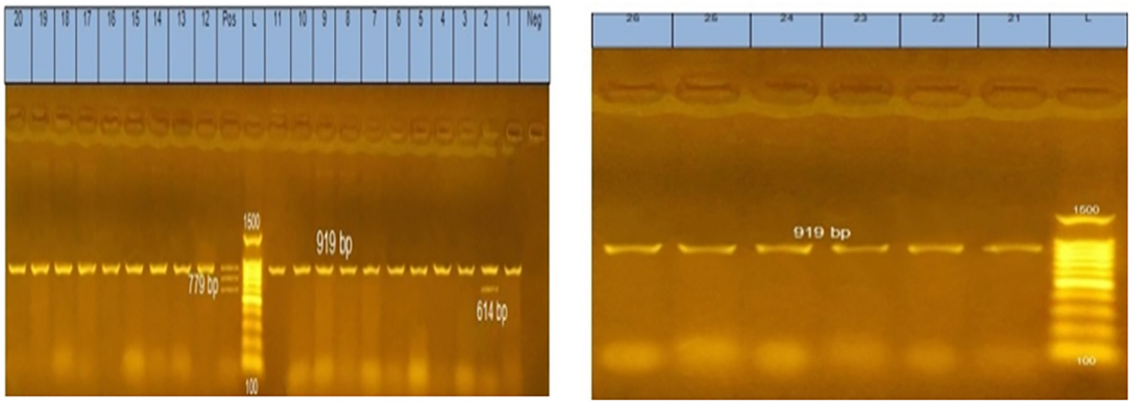

Fig. 1. Agarose gel electrophoresis showing amplification of 919 bp fragments for *ompA* gene from all *E.coli* isolates and amplification of 614 bp fragments for *stx1* gene among strain number 2 isolated from apparently healthy calf from farm number 1 in comparison with DNA marker (QIAGEN).

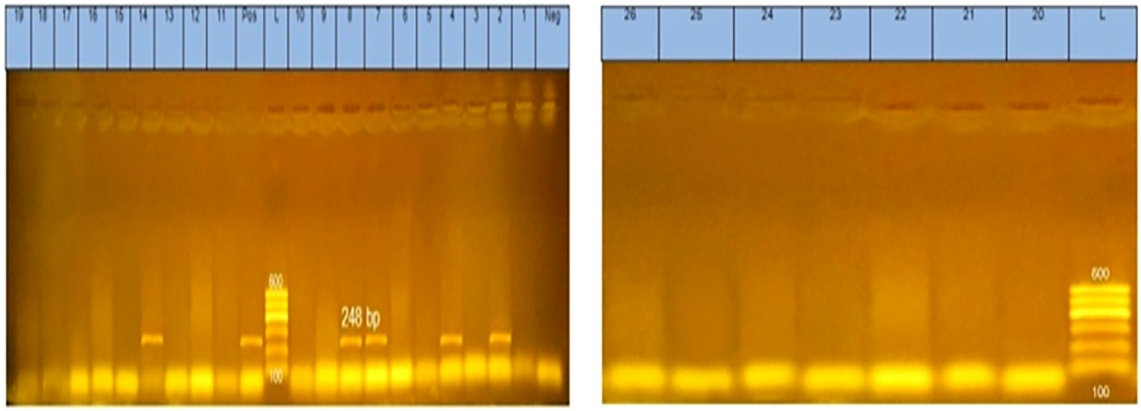

Fig. 2. Agarose gel electrophoresis showing amplification of 248 bp fragments for *eaeA* gene among strain number 2 isolated from apparently healthy calf from farm number 1 and strains number 4 and 7 isolated from diarrheic calves from number 2 and strains number, 8 and 14 isolated from diarrheic calves from frame number 3 in comparison with DNA marker (QIAGEN).

species specific for *E. coli* and negative for *eaeA, stx1* and *stx2* except strain number 4 (O55) was positive for *stx1* as shown in Fig. 3.

3.4. Investigate risk factors

Risk factors associated with calf diarrhea Table 4 include age, management, larger herd size was associated with an increased incidence of *E. coli* causing calf diarrhea.

4. Discussion

Colibacillosis is an important diseased in newborn calf. Our target is to study the risk factors associated with *E. coli* among diarrheic calves. The occurrence of *E. coli* in this study, 26 (46.4%) out of 56 fecal samples is higher than the reports of Masud et al. (2012) 22 (44%), Dereje (2012) 25 (43.1%) and lower than Paul et al. (2010) 76 (76%). This high and low occurrence of *E. coli* may be due to the difference in study area, age of calves, farm size, and sample size, managements, and hygiene measurements.

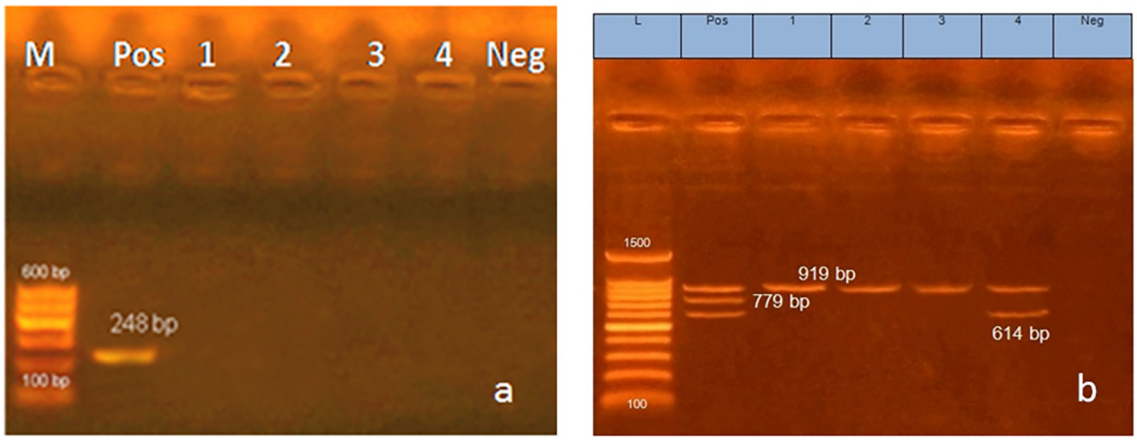

Fig. 3. Result of *eae*A gene (a) and *ompA str1 & 2* genes (b) among the colostrum isolates.

Table 4
The collected data among the study area (Questionnaire).

Independent variable	Farm1	Farm 2	Farm 3
Farm type	Dairy	Dairy	Dairy – meat
Herd size	240	800	240 – 200
No. of calves	40	150	35
Vaccination	FMD-LSD-IBR-Rota – *E. coli* Para Influenza – Corona – *Clostridium* – *Pasteurella*-		FMD – LSD (pox) – *Clostridium*
Usage of antibiotics to treat diarrhea	Use rehydration solution orally + Marbocyl injection.	Marbocyl Gentamicin Clamoxyl	Flagyl – florfenicol Streptomycin Gentamicin
Calf mortality	No	Yes	Yes
Diarrhea form	Soft	Soft – watery	Soft – watery
Diarrhea color	Mostly yellow , a little green	Mostly yellow	Mostly yellow
Age of diarrheic calves	2–14 day	2–7 day	2–12 day
Diarrhea duration	1–2 days	1–2 days	1–2 days
Presence of calving stable	Yes	No (close up)	Yes
Cleaning and disinfecting calving stable after calving	Yes	Yes (close up)	Yes
Use of calving stable for animals	Yes	Yes (close up)	No
Cleaning and disinfecting of obstetric material	Yes	Yes	Yes
Cleaning and disinfecting of hands	Yes	Yes	Yes
Cleaning and disinfecting rear of cows	Yes	Yes	eYes
Immediate separating calf from cow after calving	Yes	Yes	No
Cleaning and disinfecting of calf box after each calf	Yes	Yes	Yes
Contact between weaned and un weaned calves	No	No	yes
Clothes are being used for visitors	Yes	No	No
Use of one bucket per calf	Yes	Yes	No
Milk type	Yes	Yes	Bottle hand colostrum
Incidence of *E. coli* in feces	20%	50%	66%

Serological test showed the identification of serogroups O1, O26, O44, O55, O115, O119, O125, O146 and O151. Mosaad et al. (2008) reported the percentage of *E. coli* in diarrheic Frisian calves was 48.4% non O157.

The virulence factors produced by *E. coli* strains in the examined farms were investigated. The 26 *E. coli* isolated from the fecal samples *ompA, stx1, stx2* and *eae*A genes were found with incidence of 100, 3.8, 0 and 19.2% respectively. Among 4 *E. coli* isolated from colostrum samples only one strain had *stx1*. It is clear that *stx1* was found in 1 isolate (3.8%); while higher percentage 12.7% and 16.1% were recorded by Leomil et al. (2003) and by Salvadori et al. (2003) respectively.

There are association between *eae*A gene and the capacity of the *E. coli* strains to cause human illness (Boerlin et al., 1999). Leomil et al. (2003) reported a frequency of *eae* carriage of 41.0%, in STEC isolates from calves. Among diarrheic calves shiga toxin producing *E. coli* had *stx* genes along with the *eae* gene (Weiler et al., 1996). Prevalence of STEC in diarrheic claves was 26.3% (41 isolates) and *stx1* gene was the most prevalent variant among the isolates (Taghadosi et al., 2018)

From the questionnaire survey, it was clear that *E. coli* was significantly higher in calves at 2–14 days old. Yeshiwas and Fentahun (2017) concluded that *E. coli* is one of the most common diseases of newborn calves (9–10 days of age) characterized by watery diarrhea and the affected calves die within 2–3 days. Temesgen (2004) and Dereje (2012) reported that calves aged between 0–30 days were at great risk of diarrhea and risk decreases with age. Calf diarrhea was apparently higher in medium and large sized dairy farms than small dairy farms (Yeshiwas and Fentahun, 2017).

The questionnaire survey indicated that the prevalence of *E. coli* was found high in hand (bottle) feeding method colostrum. During

bottle fed the colostrums might be contaminated with many environmental pathogens due to careless management systems. The prevalence of *E. coli* was higher in calf pens having bedding material than without bedding material and in hand feeding source of colostrum than suckling also feeders of colostrum > 24 h than before 6 h (Temesgen, 2004; Amoki, 2001). Milk samples transported under poor hygienic conditions may lead to high health risk to the consumers (Nigatu et al., 2017). The occurrence of *E. coli* is high in muddy or wet livestock floor (Yeshiwas and Fentahun, 2017).

We concluded that although colostrum feeding to calves is economically benefited it had the risk of dissemination of *Escherichia coli* to newborn calves.

Mostly diarrheic calves did not receive appropriate treatments (Aggernesh, 2010; Dereje, 2012). Unfortunately, the usage of antibacterial agents for disease prevention and growth promotion of animals has been a widespread habit on our farms. This could result the increase of STEC strain's multidrug resistance population and, contamination of animal food products (Zhao et al., 2001).

To establish the antimicrobial resistance profile, the susceptibility of the isolates to a panel of eight antibacterial agents was determined. The eight antibacterial agents included in this study are ampicillin, cefotaxime, clindamycin, gentamicin, kanamycin, neomycin, norfloxacin and sulphonamid-trimethoprim. These drugs were chosen because they are extensively used in Egypt. Nigatu et al. (2017) recorded that *E. coli* were resistant to kanamycin streptomycin and tetracycline.

In our study 100% of isolates tested were resistant to more than one of the drugs tested. Ampicillin, cefotaxime and clindamycin showed the highest rates of resistance, 100%, 100% and 92.3% respectively followed by kanamycin (50%), in agreement with Lazaro et al. (1994). The isolates were susceptible to norfloxacin and gentamicin (76.9% each) and less susceptible to kanamycin (46.2%).

The antibiogram study of Yeshiwas and Fentahun (2017) revealed that the *E. coli* isolates were highly sensitive to tetracycline, sulfamethoxazole, chloramphenicol, streptomycin, oxacillin; less sensitive to amoxicillin, ceftazidime, nitrofurantoin, kanamycin and resistance to cefotaxime, vancomycin.

The high prevalence of antimicrobial resistance in *E. coli* is due to uncontrolled human and veterinary use of these antimicrobials in Egypt.

E. coli as a leading health problem in the present study suggests the significance of poor hygiene measurements among farms number 2 and 3. Further study should be carried out on large number of animals to investigate microbial causes of calf diarrhea and control measures.

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