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Research Article

Correlation between Virulence Genes Profile of Currently Circulating *E. coli* Pathotypes Isolated from Diarrheic Calves and Humans

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Abstract

Introduction: Diseases causing diarrhea are one of the major causes of deaths in low and middle income countries and responsible for high mortality rate in young calves resulting in economic losses. Several studies concluded to the high distribution of *Escherichia coli* (*E. coli*) strains in infectious calf diarrhea. STEC causes human gastrointestinal illnesses with diverse clinical spectra. So this study was planned for isolation, identification and molecular characterization of the currently circulating *E. coli* between calves and related workers in Egypt and to determine the role of virulence genes and pathotypes of *E. coli* in diarrhea in both calves and humans.

Material and methods: A total of 161 Holsteins calves with varying ages in four different farms in Egypt were examined clinically for diarrhea as well as related human workers in these farms. 43 fresh fecal samples were collected from diarrheic calves as well as 18 stool swab samples from workers then transferred to microbiological laboratory for bacteriological and molecular examination.

Results: The prevalence rate of *E. coli* was 53% among diarrheic calf samples and the highest isolation rate was 77% among Group I (age <month) and decreased with age. Meanwhile isolation rate in human samples was 50% (9 out of 18). Regarding virulence genes, *VT1*, *VT2* and *eaeA* virulence gens were successfully amplified in 1, 7 and 7 out of 23 calf isolates respectively. On the other hand human isolates showed only positive reaction with *VT1* and *VT2* were recovered from 5 and 4 out of 9 isolates while *eaeA* gave no positive reaction.

Conclusion: Depending on the virulence gene profiling of *E. coli* isolates, there was 8 out 23 animal isolates were Shiga Toxin producing *E. coli* (STEC) representing 35% of total animal isolates and 7 out of 23 animal isolates were Enteropathogenic *E. coli* (EPEC) representing 30% of total animal isolates. Meanwhile, 100% of the human isolates were STEC.

Keywords: Calf diarrhea; E. *coli*; Human diarrhea; Virulence genes

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Introduction

Diarrhea is an important disease of young calves with both infectious and non-infectious factors making it responsible for high mortality rate in young calves and so great economic losses. Diseases causing diarrhea are one of the major cause of death in low and middle income countries [1]. Several studies have been addressed the high distribution of *Escherichia coli* (*E. coli*) strains in infectious calf diarrhea [2]. Pathogenic *E. coli* strains have different virulence factors that allow them to colonize the host's small intestine and stimulating the deleterious inflammatory response to produce diarrhea [3]. Among all strains, Enteropathogenic *E. coli* (EPEC) and Shiga Toxin producing *E. coli* (STEC) affect the young calves between the ages of 2-8 weeks [4]. STEC produce shiga toxin (which called also verotoxin) structurally related to shiga toxin of *Shigella dysenteriae* Type 1 (*VT1*) and / or (*VT2*) and EPEC produce Intimin encoded by the attaching and effacing (*eaeA*) gene [5]. The diarrheagenic STEC exerts their role mainly in calves that destroys intestinal microvilli resulting in hemorrhagic diarrhea 2-5 weeks' old [6].

STEC is an enteric pathogen that has been linked to outbreaks from foodborne, waterborne and contact sources. STEC causes human gastrointestinal illnesses with diverse clinical spectra, ranging from watery and bloody diarrhea to hemorrhagic colitis. It may also cause hemolytic-uremic syndrome and renal failure [7,8]. Rapid detection of Shiga Toxin-producing *Escherichia coli* (STEC) enables appropriate treatment. In the same time the non-O157H7 STEC serotypes have increased significantly in the past decade that not ferment sorbitol making it difficult to be detected by conventional methods [9]. Molecular detection of Virulence genes (*VT1*, *VT2* and *eaeA*)

accelerates more sensitive and accurate detection in comparison with traditional methods [10]. So this study was planned for isolation, identification and molecular characterization of currently circulating *E-coli* between diarrhic calves and contact workers to determine the role of virulence genes and pathotypes in diarrhea in both calves and humans.

Materials and Methods

Ethics statement

The collection of feces samples had been approved by the owner of the farm. Conduct animal experiments in accordance with laboratory regulations. This study was approved by the Ethics Committee of the New Valley University.

Study design

A cross-sectional study was carried out to investigate the prevalence of *E-coli* causing diarrhea in calves and humans by isolation, identification and molecular study and comparison of virulence genes.

Study animals

A total of 161 Holsteins calves with varying ages in four different farms in Egypt were examined clinically for diarrhea as well as all human workers in these farms. The calves were divided into three groups according to their age. Group I aged from 1 day to 1 month including 60 calves (20 clinical samples were collected from farm 1, 8 from farm 2, 15 from farm 3 and 17 clinical samples were collected from farm 4), Group II aged more than 1 month up to 3 month including 55 calves (8 clinical samples were collected from farm 1, 4 from farm 2, 16 from farm 3 and 27 clinical samples were collected from farm 4) meanwhile Group III aged more than 3 month up to 6 month including 46 calves (4 clinical samples were collected from farm 1, 5 from farm 2, 11 from farm 3 and 26 clinical samples were collected from farm 4).

Collection of samples

A total number of 43 fresh fecal samples were collected directly under aseptic condition from the rectum of 43 diarrheic calves (out of 161clinicaly examined calves) suffer from diarrhea which ranged from pasty to watery feces, varying degree of dehydration, off food and weakness (Figure 1) using sterile rectal swabs and 18 stool swab samples from workers [11]. Samples were properly identified and kept in sterilized containers and preserved on ice, transferred to the microbiology Laboratory in The Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) in Abbassia, Cairo for bacteriological examination and molecular examination.

Isolation and identification of *E. coli*

Colonial morphology

All samples were diluted in phosphate buffered saline to be cultured on MacConkey agar (Oxoid; CM0115) and Eosin methylene blue (oxide; CM 69) and incubated at 37˚C for 18 - 24hrs for primary isolation of *E. coli*.

Figure 1: Showed clinical signs and diarrhea in suffered calves.

Microscopic examination

Smears from freshly growing suspected colonies were stained with Gram stain and examined microscopically.

Motility

Motility was assured by growing and spreading of the pure colonies by stabbing in semisolid agar.

Biochemical identification

Pure cultures were examined biochemically by using API 20E identification system according to [12] following the procedures of kit manual.

Genotypic identification

DNA extraction

DNA templates were prepared using Isolate Presto™ Mini gDNA Bacteria kit (Geneaid cat# GBB101).

Primer used

Specific primer for *16s rRNA* gene, (Table 1), was used for molecular identification of *E. coli* isolates.

Genes	Primer Sequence	Product	Reference
16s rRNA	F5-GACCTCGGTTTAGTTCACAGA-3	585 bp	Tonu et al., [13]
	R5-CACACGCTGACGCTGACCA-3		
VT1	F5-CGCTCTGCAATAGGTACTCC-3	256 bp	OIE [14]
	R5-CGCTGTTGTACCTGGAAAGG-3		
VT2	F5-TCCATGACAACGGACAGCAG-3	185 bp	
	R5-GCTTCTGCTGTGACAGTGAC-3		
eaeA	F5-GCTTAGTGCTGGTTTAGGATTG-3	618 bp	
	R5-CCAGTGAACTACCGTCAAAG-3		

Table 1: Primers used for different genes.

Polymerase chain reaction

5µl of genomic DNA, 12.5µl of dream taq green master mix (Thermoscientific #K1081), 1µl of each primer (50 pmole) and 5.5µl of deionized water were added to 0.5ml microfuge tubes. The amplification reactions were performed under following conditions: 94˚C for 4 min, then 29 cycles each at 94˚C for 90 sec, 62˚C for 90 sec and 72˚C for 2 min.; lastly 72˚C for 10 min [13].

Virulence gene detection

Recovered pathogenic isolates were used to detect major virulence genes including *VT1*, *VT2* and *eaeA* genes. Specific primers shown in Table 1.

Polymerase chain reaction

5µl of genomic DNA, 12.5µl of dream taq green master mix (Thermoscientific #K1081), 1µl of each primer (50 pmole) and 5.5µl of deionized water were added to 0.5ml microfuge tubes. The amplification reactions were performed under following conditions: 94˚C for 2 min, then 25 cycles each at 94˚C for 60 sec, 62˚C for 90 sec and 72˚C for 2 min.; lastly 72˚C for 5 min [14].

Results

Prevalence of diarrhea in examined calves

Clinical examination of examined calves showed that, a total 43 out of 161 calves (24 out of 60 from group I calves, 11 out of 55 group II calves and 8 out of 64 group III calves) suffer from diarrhea which ranged from pasty to watery feces, varying degree of dehydration, off food and weakness representing prevalence rates 40%, 20% and 17% respectively. Regarding the prevalence of diarrhea in the farms, it was found that 7 out of 32, 5 out of 17, 9 out of 40 and 22 out of 70 calves suffering from diarrhea from farm 1, 2, 3 and 4 respectively representing prevalence rates 22%, 29% , 21% and 31% respectively as shown in Table 2 and Figure 1.

Isolation and identification of *E-coli*

Colonial morphology

Only 35 out of 43 isolates recovered from diarrheic calves while 10 out of 18 isolates recovered from human workers showed pink-colored smooth colonies on macConkey agar and produced distinct, clear greenish metallic sheen over EMB. All isolates were Gram negative, motile, non-sporulated and medium sized *bacilli*.

Biochemical identification

By using API 20E identification system, the suspected *E. coli* isolates were 23 out of 35 calves isolates while 9 out of 10 human isolates representing recovery rates 66% and 90% respectively. The biochemical reactions were classified into five groups as shown in Table 3. The first group includes 9 out of 23 calve isolates and 5 out of 10 human isolates and gave positive reaction with ONPG, ADH, LDC, ODC, TDA, IND, GLU, MAN, SOR, RHA, SAC, MEL and ARA tests and negative reaction with CIT, H2S, URE, VIP, GEL, INO and AMY tests. The second group includes 5 out of 23 calve isolates and 4 out of 10 human isolates and gave positive reaction with ONPG, LDC, ODC, TDA, IND, GLU, MAN, SOR, RHA, SAC, MEL and ARA tests and negative reaction with ADH, CIT, H2S, URE, VIP, GEL, INO and AMY tests. The third one includes 4 out of 23 calve isolates and gave positive reaction with ONPG, ADH, LDC, ODC, TDA, IND, GLU, MAN, SOR, RHA, MEL and ARA tests and negative reaction with CIT, H2S, URE, VIP, GEL, INO, SAC and AMY tests. The fourth one includes 3 out of 23 calve isolates gave positive reaction with ONPG, ADH, LDC, TDA, IND, GLU, MAN, SOR, RHA, SAC, MEL and ARA tests and negative reaction with ODC, CIT, H2S, URE, VIP, GEL, INO and AMY tests. The last 5th group includes 2 out of 23 calve isolates and gave positive reaction with ONPG, LDC, TDA, IND, GLU, MAN, SOR, RHA, SAC, MEL and ARA tests and negative reaction with ADH, ODC, CIT, H2S, URE, VIP, GEL, INO and AMY tests.

Genotypic identification

All 23 calves isolates and 9 human isolates were identified as *E. coli* using *16s rRNA* gene primer giving a PCR product at the prospected size of 585 bp [13] as shown in Figure 2.

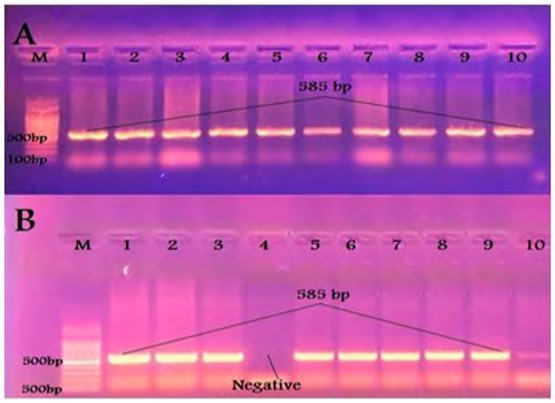

Figure 2: Results of PCR using specific primer of *16S rRNA* gene of *E. coli* (A) Samples taken from diarrheic calves. (B) Samples taken from diarrheic workers.

E. coli isolation rates according to age of calves

Regarding to age, the highest isolation rate of *E. coli* were recorded in group I by 77% where 20 out of 24 samples were finally identified as *E. coli* followed by 18% in group II where 2 samples out 11 were identified as *E. coli*. Regarding group III, it recorded 13% representing the lowest isolation rate where only 1 sample out of 8 was identified as *E. coli*. Meanwhile in human samples *E. coli* was recovered from 50% of the collected samples where 9 out of 18 samples were identified as *E. coli* as shown in Table 4.

Isolated *E. coli* virulence genes profiling

The virulence genes profile of *E. coli* isolates was studied and it was found that *VT1*, *VT2* and *eaeA* gens were successfully amplified in 1, 7 and 7 out of 23 calf isolates giving rise the prospected PCR products of 256, 185 and 618 bp respectively as mentioned in OIE [14]. Also only *VT1* and *VT2* in 5 and 4 out of 9 human isolates respectively while *eaeA* gave no positive reaction with the human isolates, as shown in Table 5 and Figures 3-5. Regarding the correlation between virulence genes in the isolated *E. coli*, it was found that, there is only one animal isolate carry both *VT1* and *eaeA* virulence genes while there are 4 animal isolates carry both *VT2* and *eaeA* virulence genes. Meanwhile in human isolates there is no isolates carry more than one virulence gene.

Isolated *E. coli* pathotyping

Depending on the virulence gene profiling of *E. coli* isolates as shown in Table 5, it is clear that, there was 8 out 23 animal isolates were STEC representing 35% of total animal isolates and 7 out of 23 animal isolates were EPEC representing 30% of total animal isolates. Meanwhile, 100% of the human isolates were STEC.

Ages group	Total Ex	Farm (1)		Farm (2)		Farm (3)		Farm (4)		Total D	Age Prev
		Ex	D	Ex	D	Ex	D	Ex	D		
Group I	60	20	4	8	4	15	5	17	11	24	40
Group II	55	8	2	4	1	16	3	27	5	11	20
Group III	46	4	1	5	0	11	1	26	6	8	17
Total	161	32	7	17	5	42	9	70	22	43	27
Farms Prev		22%		29%		21%		31%			

Table 2: Prevalence of diarrhea in examined calves related to age and farms.

Ex: Examined; D: Diseased; Prev: Prevalence

Type of samples	No of Samples	API 20E RESULTS																					No. of Recovered isolates		Recovery rate
		ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX			
Calve isolates	35	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	+	+	+	-	+	-	9	23	66%
		+	-	+	+	-	-	-	+	+	-	-	+	+	-	+	+	+	+	-	+	-	5		
		+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	+	-	+	-	+	-	4		
		+	+	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	+	-	+	-	3		
		+	-	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	+	-	+	-	2		
Human isolates	10	+	+	+	+	-	-	-	+	+	-	-	+	+	-	+	+	+	+	-	+	-	5	9	90%
		+	-	+	+	-	-	-	+	+	-	-	+	+	-	+	+	+	+	-	+	-	4		
Total	45	5 biochemical reactions																					32		

Table 3: Identification of *E coli* isolates using API 20E identification system.

Age group	Diseased	Identified *E. coli*	%
Group I	24	20	77
Group II	11	2	18
Group III	8	1	13
Total	43	23	53
Humans	18	9	50

Table 4: Percent of isolation of *E. coli* according to age of calves.

Species	Total isolates	Virulence gene			Pathotypes	
		Vt1	*Vt2*	*eaeA*	STEC	EPEC
Calves	23	1	7	7	8 35%	7 30%
Humans	9	5	4	0	9 100%	0
Total	32	6	11	7	17 53%	7 22%
Percent						

Table 5: Studying the virulence gene profile of *E. coli* isolated from diarrheic calves and human.

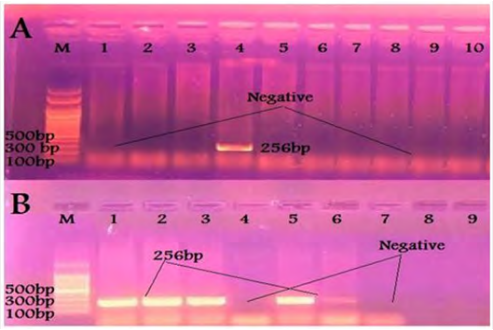

Figure 3: Results of PCR using specific primer of *VT1* gene of *E. coli* (A) Samples taken from diarrheic calves. (B) Samples taken from diarrheic workers.

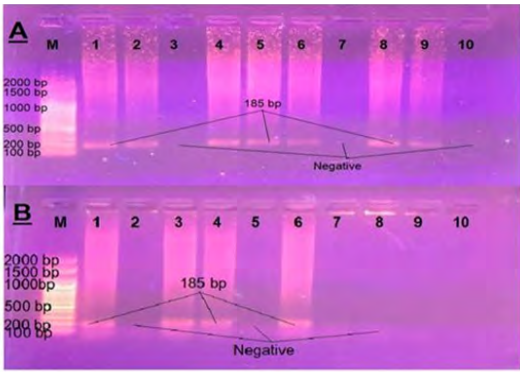

Figure 4: Results of PCR using specific primer of *VT2* gene of *E. coli* (A) Samples taken from diarrheic calves. (B) Samples taken from diarrheic workers.

Discussion

Diarrhea is a major problem in calves causing high mortality rates and high economic Impact. The multifactorial nature of neonatal calf diarrhea makes this condition hard to control effectively. Therefore, prevention and control of such condition must be based on a good understanding of those problem complexities during the calving period before disease outbreaks [15].

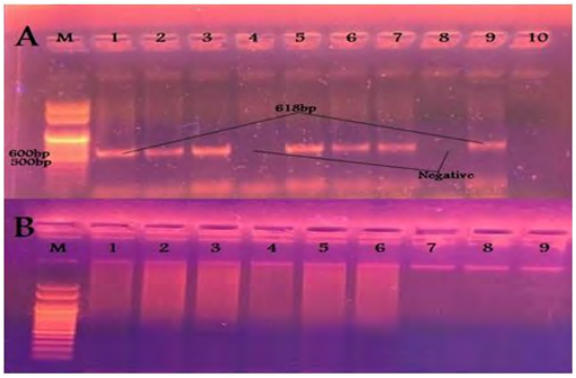

Figure 5: Results of PCR using specific primer of *eaeA* gene of *E. coli* (A) Samples taken from diarrheic calves. (B) Samples taken from diarrheic workers.

Due to high distribution of *E. coli* strains in infectious calf diarrhea, this work was planned for isolation, identification and molecular characterization of *E. coli* and detection of virulence gens that play a role in *E. coli* pathogenesis. In this study a total number of 161 calves of different ages in different four farms in Egypt were clinically examined for diarrhea as well as related human workers in these farms. The clinical examination showed that, a total 43 out of 161 calves (24 out of 60 from calves aged from 1 day to 1 month, 11 out of 55 calves aged more than 1month up to 3 month and 8 out of 64 calves aged more than 3 month up to 6 month) suffer from diarrhea which ranged from pasty to profuse watery feces, varying degree of dehydration, off food and weakness representing prevalence rates 40%, 20% and 17% respectively. Regarding the prevalence of diarrhea in the farms, it was found that 7 out of 32, 5 out of 17, 9 out of 40 and 22 out of 70 calves suffering from diarrhea from the different 4 farms representing prevalence rates 22%, 29%, 21% and 31% respectively as shown in Table 2 and Figure 1. Regarding to age, our finding revealed that the highest prevalence rate of diarrhea was reported in the youngest age of calve group and the prevalence rate decreased with old age. Our finding confirmed by Garcia et al., [16] who stated that calves are at greatest risk of diarrhea within the first month of life and the incidence of diarrhea decreases with age increase. on the contrary Olaogun et al., [17] concluded that higher percent of diarrhea observed in old calves than young ones in his study on the prevalence of diarrhea in calves in 12 farms in Oyo and Ogun states in South Western Nigeria and also reported that a total 120 out of 825 calve up to 6 month old (14.5%) showing signs of diarrhea. Different prevalence percent was investigated by Achá et al., [18] in 8 dairy farms in Mozambique at 4 occasions during 2 consecutive years. He found that a total 63 out of 1241 calves up to 6 months of age (5%) had signs of diarrhea, two farms had an overall higher prevalence (13% and 21%) of diarrhea.

A total number of 43 fresh fecal samples were collected directly under aseptic condition from the rectum of diseased calves (out of 161clinicaly examined calves) as well as 18 stool swab samples from human workers then transferred to the microbiological laboratory for bacteriological and molecular examination. The colonial and morphological isolation revealed that, only 35 out of 43 isolates recovered from diarrheic calves while 10 out of 18 isolates recovered from human workers showed pink-colored smooth colonies on macConkey agar and produced distinct, clear greenish metallic sheen over EMB and all isolates were Gram negative, motile, non- sporulated, medium sized *bacilli*. On the other hand the biochemical identification using API 20E identification system revealed that 23 isolates out of 35 suspected calves' isolates and 9 out of 10 suspected human isolates representing recovery rates 66% and 90% respectively proved to be *E. coli* as shown in Table 3. All 23 calves and 9 human isolates were genotypically identified as *E. coli* by amplification of universal *16s rRNA* gene giving the prospected PCR product of 585 bp as confirmed by Tonu et al., [13] as shown in Figure 2. Higher percent of *E. coli* were isolated by Elseedy et al., [19] who concluded that out of 127 collected fecal samples from diarrheic calves, 119 (93.7%) bacterial isolates were recovered, including 23 (18.1%) *Salmonella* serovars and 96 (75.6%) were *E. coli* strains. On contrary, a lower *E. coli* isolation percent (10%) was isolated by Olaogun et al., [17] from three farms in Oyo and Ogun States, Nigeria and 45% by Paul et al., [20] in some selected areas of Bangladesh. Also among 84 diarrheic calves samples, 30 (35.71%) *E. coli* were isolated by Masud et al., [21]. The difference in the prevalence of *E. coli* were explained by Cho and Yoon [15] who concluded its relation to geographical location of the farms, farm management practices and herd size.

Regarding to age, the highest isolation rate of *E. coli* were recorded in group I that aged from 1 day to 1 month by 77% where 20 out of 24 samples were finally identified as *E. coli* followed by 18% in group II that aged more than 1 month up to 3 month where 2 samples out 11 were identified as *E. coli*. Regarding group III that aged more than 3 month up to 6 month, it recorded 13% representing the lowest isolation rate where only 1 sample out of 8 was identified as *E. coli*. Meanwhile in human samples *E. coli* was recovered from 50% of the collected samples where 9 out of 18 samples were identified as *E. coli* as shown in Table 4. Anwarullah et al., [12] reported that the highest isolation rate of *E. coli* was recorded in old age group than young one representing 1.33%, 4%, 9.33% in similar groups respectively.

E. coli can be classified into six pathogroups based on virulence scheme: Enterotoxigenic *E. coli* (ETEC), Shiga Toxin-producing *E. coli*, Enteropathogenic *E. coli*, Enteroinvasive *E. coli*, Enteroaggregative *E. coli* and Enterohaemorrhagic *E. coli* [22]. Although there are a wide range of different virulence factors that may play a role in the pathogenesis of *E. coli*, the present study investigated the presence of only 3 virulence genes encoding putative virulence factors and the obtained 23 isolates of *E. coli* were screened for the presence of certain Virulence factors, shiga toxin production (*VT1* and *VT2* genes), attaching and effacing character (*eae A* gene) and it was found that *VT1*, *VT2* and *eaeA* gens were successfully amplified in 1, 7 and 7 out of 23 calve isolates giving rise the prospected PCR products of 256, 185 and 618 bp respectively as described in OIE [14]. Also only *VT1* and *VT2* in 5 and 4 out of 9 human isolates respectively while *eaeA* gave no positive reaction with the human isolates as shown in Table 5. Regarding the correlation between virulence genes in the isolated *E. coli*, it was found that, there is only one calf isolate carry both *VT1* and *eaeA* virulence genes while there are 4 calves isolates carry both *VT2* and *eaeA* virulence genes. Meanwhile in human isolates there is no isolates carry more than one virulence gene.

Depending on the virulence gene profiling of *E. coli* isolates, it is clear that, there was 8 out 23 animal isolates were STEC representing 35% of total animal isolates and 7 out of 23 animal isolates were EPEC representing 30% of total animal isolates. Meanwhile, 100% of the human isolates were STEC. The overall prevalence of STEC in all samples and farms was 53%. Different prevalence of STEC among calves was studied and was 20% by Shaw et al., [23] 37.5%

by Fukushima and Seki [24] 12.14% by Tahamtan et al., [25] 17.7% by Irshad et al., [26] and 22.7% by Kohansal and Asad [27].

Dastmalchi and Ayremlou [28] reported that, prevalence was 19.6% among diarrheic calves, 23.1% carried *VT1* gene, 26.92% possessed *VT2* gene while 13 isolates (50%) gave positive amplicon for both *VT1* and *VT2* genes. Shahrani et al., [29] and Akter et al., [30] concluded that *VT1* is the most prevalent than *VT2*. Prevalence of 51% among calves was obtained by Wang et al., [31] which was closely contact to our finding. Higher prevalence 83% among diarrheic calves was obtained by Pervez et al., [32]. The highest STEC prevalence was detected in age under one month as shown in tables 4 and 5. Similar results was observed by Cobbold and Esmarchelier [33], they concluded that calves as young as 48 to 72h old excrete STEC. Humans can become infected with STEC by ingesting contaminated food or water or by transmission from infected animals or humans [34]. Prevalence of STEC infection in human was 15% by Salmanzadeh-Ahrabi et al., [35], 2.3% by Rajendran et al., [36], 1.7% by van Duynhoven et al., [37], 7% was reported in patients with diarrhoea in Morogoro, Tanzania in 2006 by Raji et al., [38] and 56% by Matussek et al., [39]. The overall prevalence of virulence-associated gene VT1 only, *VT2* only, *VT1* and *VT2* and *eaeA* were 10.7%, 20.8%, 68.5%, 3.9%, respectively as reported by Wang et al., [31]. Pervez et al., [32] reported that 10% of the isolates of calves were positive for *VT1* gene while *eaeA* not detected. The *VT1* gene was detected more frequently in calves than in adult animals [40].

Some factors that contribute to the presence and spread of STEC in a herd are the management practices, stress, diet, population density, geographic region and season [41]. Contact with feces of cattle, direct contact with the animals or their environment and consumption of contaminated beef, milk, dairy products, water, unpasteurized apple juices and vegetables are possible routes for STEC human exposure and disease [42]. So, measures to prevent direct contact with animal fecal material in the environment include the wearing of protective clothing, increased hand washing and targeted education of the population at risk regarding possible sources of STEC infection.

So, from the previously mentioned results, we discerned that there is a correlation between *E. coli* pathotypes isolated from animals and contact humans but, further study is needed and will be done to study the sequence analysis of amplified genes to discuss deeply the direct relation between human and animal pathotypes. Other studies may be recommended to cover other different virulence genes of different *E. coli* pathotypes like Enterotoxigenic (ETEC), Enteroaggregative (EAEC) and enteroinvasive *E. coli*.

Also, our finding can concluded to the risk of increasing the prevalence of pathogenic *E. coli* increase with the decrease in ages of susceptible calves, control and management of the diarrhea in calves should referenced to the actual situation of the exact farm regarding antibiotic therapy, autovaccination of calves and housing parameters. Cross infection of pathogenic *E. coli* between infected animals and contact workers is highly possible. Biosafety and biosecurity measures should be implemented to minimize the risk of calves' infection and cross infection between human and animals.

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