# MOLECULAR CHARACTERIZATION OF VIRULENCE GENES IN *ESCHERICHIA COLI* ISOLATED FROM NEWBORN DIARRHEIC CALVES

*Ibrahim E. Eldesouky, *Hisham S. Nada,**Talat H. Sheashe, Asmaa E. Elshishiny

*Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Kafrelsheikh University 33516, Egypt.

** Animal Health Research Institute.

## ABESTRACT

*The present study was designed to elucidate the prevalence and characteristics of pathogenic Escherichia coli strains from diarrheic calves. A total of 120 fecal samples were collected from diarrheic calves from different cow's farms at Kafrelsheikh Governorate, Egypt. E. coli was isolated with a percentage of 20% (24/120). Serogrouping of E.coli isolates showed 12 types belong to different "O" serogroups; the most prevalent serogroups among isolates were O111, O103, O26, O55, O128, O15, O91 and O20. A total of 12 E. coli isolates were screened by multiplex PCR assay for the presence of virulence genes characteristic for E.coli, that is, shiga-toxin producing gene(s) (stx1, stx2), intimin (eaeA) and enterohemolysin (hlyA). Out of the 12 isolates, 11 were positive for one or both stx1and stx2 (91.6%), 8 isolates for hlyA(66.6%) and 4 isolates for eaeA (33.3%). PCR results showed that 9 (75%) isolates carried stx1 gene, 9 (75%) possessed stx2 gene while 7 isolates (58.3%) gave positive amplicon both for stx1 and stx2 genes. All shiga toxin genes (stx1 and/or stx2) positive samples were positive for the hlyA and eaeA genes. Both virulence genes eaeA and hlyA in the same sample were observed in 36.36% of the shiga toxin positive strains. As a result, this study shows that stx1 and stx2 were found as the most common virulence gene markers of E.*

*coli strains isolated from calves with diarrhea.*

## INTRODUCTION

Neonatal calf diarrhea remains an important cause of morbidity and mortality in young calves ***(Constable, 2004)***. Diarrhea in neonatal calves is a syndrome of great etiological complexity that causes substantial economic losses directly through mortality and need for treatment, and indirectly from poor growth ***(C. Herrera-Luna et al., 2009)***. Additionally, diarrhea in livestock is important because of the public health implications. Several infectious agents causing diarrhea in animals are zoonotic and have been associated with food-borne diseases***(Trevejo et al., 2005).***

In addition to the influence of various environmental, managemental, nutritional and physiological factors, numerous infectious agents are capable of causing neonatal calf diarrhea ***(Schumann et al., 1990)***. Multiple enteric pathogens have been recovered from diarrheic calves, their relative prevalence varies geographically but *Escherichia coli*, *Rotavirus*, and *Coronavirus*, *C. perfringens*, *Salmonella* spp. and *Cryptosporidium* spp. are recognized as the most common infectious agents in most areas ***(Garcia et al., 2000; Snodgrass et al., 1986)***.

Bacterial infections are an important cause of morbidity and mortality in neonate diarrhea, and *E.coli* is getting recognized as leading cause ***(Fecteau et al., 1997)***. Calves are most vulnerable to *E. coli* infection where age group appears to be of mostly 1-3 days of age. *E. coli* is a Gram-negative, rod-shaped, flagellated, non-sporulating and facultative anaerobic bacterium of the family *Enterobacteriaceae*. Pathogenic *E.coli* classically classified into 6 different pathotypes based

on their virulence properties: Enterotoxigenic (ETEC), Enterohemorrhagic (EHEC) or Shigatoxin-producing (STEC), Enteroinvasive (EIEC), Enteropathogenic (EPEC), Enteronecrotic (ENEC) and attaching and effacing *E. coli* (AEEC) pathotypes ***(Kaper et al., 2004)***.

The pathogenicity of *E. coli* is associated with a number of virulence factors, including Shiga toxin1 (encoded by the *stx*1 gene), Shiga toxin 2 (encoded by the *stx*2 gene), intimin (encoded by the *eae*A gene), and the plasmid-encoded enterohaemolysin or enterohaemorrhagic *E. coli* haemolysin (*hly*A) which are related to the pathogenesis of STEC strains ***(Kang et al., 2004; Law, 2000)***. In contrast to pathogenic *E. coli* strains, which typically carry certain virulence gene patterns associated with specific pathotypes ***(Frydendahl, 2002)***, commensal *E. coli* strains rarely contain virulence genes***(Boerlin et al., 2005)***.

ETEC infection is the most common type of colibacillosis in young animals especially in calves and piglets ***(Younis et al., 2009)***. Diarrhea in calves is commonly caused by ETEC containing mainly K99 (F5), in addition to Heat-labile enterotoxins (LT) and heat-stable enterotoxins (STa or STb) which consider the most important bacterial virulence factors able to cause severe diarrhea in calves ***(Guler et al., 2008; Kumar et al., 2013; Nguyen et al., 2011)***.

Many previous studies indicate that both healthy and diarrheic calves harbor STEC in their intestine ***(Blanco et al., 1997; Roopnarine et al., 2007)*** and shed the bacteria for several months and in great quantities***(Cray and Moon, 1995; Widiasih et al., 2004)***.

The primary virulence factor in EPEC is the *eae*A gene that encodes intimin, located in the locus of enterocyte effacement (LEE).

The LEE genes facilitate intimate adherence to host cells and the formation of the characteristic attaching and effacing (A/E) lesions on intestinal cells, but do not produce Shiga, Shiga-like or verocytotoxins ***(Bolton, 2011)***. However, many STEC also produce intimin, an outer membrane surface adhesin encoded by the chromosomal *eae*A gene***(Mora et al., 2005)***.

Although the role of enterohaemolysin in an intestinal disease is unclear, it has been suggested that enterohaemolysins may enhance the effects of Shiga toxins ***(Caprioli et al., 2005; Holland et al., 1999)***. Consequently, the aim of the present study was to carry out a current molecular characterization of pathogenic *E. coli* strains isolated from a random sampling of neonatal diarrheic calves.

## MATERIALS AND METHODS

### 1. Fecal samples:

A total of 120 fresh fecal samples were collected from diarrheic calves suffering from profuse watery diarrhea aged from one week to 2 months at different localities in Kafr Elsheikh Governorate, Egypt. The health status of each calf was evaluated by clinical examination. Healthy calves had to be free from diarrhea, whereas sick calves showed fever, anorexia, abnormal fecal constancy and/or signs of dehydration and weakness. Fecal samples were taken using sterile rectal swabs after digital stimulation of the rectal mucosa, and then they were collected into sterile plastic tubes and submitted to the laboratory on ice packs as soon as possible for further bacteriological examinations. Samples were

processed within 24–48 h after reception.

## 2. Isolation and identification:

Fecal swab samples were preenriched firstly with Buffered Peptone Water (Oxoid) and incubated at 37°C for 24 hrs., then a loop full from cultivated broth was streaked on MacConkey's agar (Lab M) plates. The inoculated plates were incubated aerobically at 37°C for 24 hrs. Lactose positive colonies were picked up and recultivated on Eosin Methylene Blue (Oxoid) at 37°C for 24 hrs, metallic green colonies were considered as *E. coli*. Several biochemical tests including Triple Sugar Iron Agar (TSI), Indole, Citrate utilization, Voges-Proskauer, urease and Methyl red tests were used for *E. coli* confirmation. All isolates were stored at −70°C in 20% glycerol broth for further description. The suspected colonies were identified morphologically, culturally and biochemically ***(Quinn et al., 2002)***.

## 3. Serological identification:

Antisera of *E.coli* were used for serological identification of somatic "O" and flagellar "H" antigens using slide agglutination test according to ***Kok et al., (1996)***. The diagnostic antisera were purchased from Denka-Seiken (Japan). The O serogroups determination of the *E. coli* isolates was conducted using standard slide agglutination techniques according to the manufacturer's instructions.

## 4. Molecular identification:

### 4.1. Bacterial DNA preparation for PCR:

Among 24 *E. coli* isolates, only 12 *E. coli* isolates were subjected to

PCR for detection of four diarrheic pathogenic genes (*stx*1, *stx*2, *eae*A and *hly*A). Genomic DNA of *E.coli* was extracted through boiling methods. Briefly, an overnight bacterial culture (200µl) was mixed with 800µl of distilled water and boiled for 10 minutes. After boiling, the tubes were immediately placed on ice for 5 minutes followed by centrifugation at 14,000 rpm for 5 minutes. The supernatant containing bacterial DNA was transferred to a new tube, stored at -20°C and processed for PCR detection of the *stx*1, *stx*2, *eae*A and *hly*A genes. The primer pairs used in PCR protocols were selected from published papers based on specificity, compatibility and ability to target the genes of interest. The nucleotide sequences and anticipated molecular sizes of PCR amplified fragments for these gene-specific oligonucleotide primer sets are outlined in Table 1.

**Table (1):** The primers used in PCR for detection of four pathogenic genes in *E. coli*

| References | Product size (bp) | Oligonucleotide sequence (5′ → 3′) http://www.ncbi.nlm.nih.gov/pmc/articles/ PMC140333/table/t2/ - t2fn1 | Primer |
|---|---|---|---|
| ***Dhanashree and Mallya (2008)*** | 614 | 5′ ACACTGGATGATCTCAGTGG ′3 | *stx*1 (F) |
| | | 5′ CTGAATCCCCCTCCATTATG ′3 | *stx*1 (R) |
| ***Dhanashree and Mallya (2008)*** | 779 | 5′ CCATGACAACGGACAGCAGTT ′3 | *stx*2 (F) |
| | | 5′ CCTGTCAACTGAGCAGCACTTTG ′3 | *stx*2 (R) |
| ***Mazaheri et al. (2014)*** | 890 | 5′ GTGGCGAATACTGGCGAGACT ′3 | *eae*A (F) |
| | | 5′ CCCCATTCTTTTTCACCGTCG ′3 | *eae*A (R) |
| ***Fratamico et al. (1995)*** | 165 | 5′ ACGATGTGGTTTATTCTGGA ′3 | *hly*A (F) |
| | | 5′ CTTCACGTGACCATACATAT ′3 | *hly*A (R) |

## 4.2. Detection of virulence genes using multiplex Polymerase Chain Reaction (PCR):

A total of 12 *E. coli* isolates were screened for the presence of the genes using specific primers for *stx*1 and *stx*2 ***(Dhanashree and Mallya,***

***2008)***, *eae*A***(Mazaheri et al., 2014)*** and *hly*A ***(Fratamico et al., 1995)*** using multiplex PCR. The PCR assay was carried out in a total volume of 50 µl of mixture containing 10 mM Tris-HCl (pH 8.4), 10 mM KCl, 3 mM MgCl2; 0.2 mM dNTPs, 2mM of each virulence gene-specific primer, 4 U Taq polymerase (Promega) and 2 µl DNA template. Amplification conditions consisted of an initial denaturation step at 95°C for 3 min, followed by 35 cycles of amplification (denaturation at 95°C for 20 sec, annealing at 58°C for 40 sec, and extension at 72°C for 90 sec) and final extension at 72°C for 5 min. Amplified PCR products were separated by electrophoresis on 2% agarose gel with Tris Acetate EDTA (TAE) buffer (Bioshop,Canada). The gel was stained with ethidium bromide (Bioshop,Canada) and visualized under UV transluminator. A 100-bp DNA ladder (Fermentas, USA) was used as molecular weight marker. *E. coli* strains for each gene and distilled water were used as positive and negative controls respectively.

## RESULTS

### 1. Prevalence and serotyping of *E.coli* recovered from diarrheic calves:

In the present study, out of the examined 120 diarrheic calves, 24 *E. coli* isolates were identified with an incidence of 20%. The isolates were tested against 33 different O serogroups commonly associated with pathogenic bovine *E. coli*. Based on serotyping of *E. coli* isolates, Table (2) showed that the recovered *E.coli* strains belonged to 12 different serogroups and revealed the predominance of serotypes; O26 : H11 (5 strains, 20.8%), O111 : H4( 4 strains, 16.7%), O55 : H7, O114 : H21,

O119 : H4, O125 : H21, O124 (2 strains each, 8.3%), O103, O20, O15, O91 : H21, O128 : H2( one strain each, 4.1%)

**Table (2):** Serovars distribution of *E.coli* isolates among diarrheic calves

| Strains pathotype | No. of strains | % | Serotype | *E.coli* isolates (n=24) | |
|---|---|---|---|---|---|
| | | | | No. | % |
| EHEC | 10 | 41.6% | O111 : H4 | 4 | 16.7% |
| | | | O26 : H11 | 5 | 20.8% |
| | | | O103 | 1 | 4.1% |
| EPEC | 9 | 37.5% | O55 : H7 | 2 | 8.3% |
| | | | O114 : H21 | 2 | 8.3% |
| | | | O119 : H4 | 2 | 8.3% |
| | | | O20 | 1 | 4.1% |
| | | | O15 | 1 | 4.1% |
| | | | O91 : H21 | 1 | 4.1% |
| ETEC | 3 | 12.52% | O128 : H2 | 1 | 4.1% |
| | | | O125 : H21 | 2 | 8.3% |
| EIEC | 2 | 8.3% | O124 | 2 | 8.3% |

## 2. Molecular characterization of *E. coli* isolates:

A total of 12 samples from 24 *E. coli*-positive isolates were analyzed by multiplex PCR to detect 4 virulence genes. The PCR assay correctly determined the presence or absence of *E. coli* virulence genes as it is illustrated in Figure (1).

It has been found that *stx*1and *stx*2 genes were present in similar proportions and identified more frequently than *eae*A and *hly*A genes in diarrheic calves. Out of the 12 isolates, 11 were positive for one or both *stx*1and *stx*2 (91.6%), 8 isolates for *hly*A (66.6%) and 4 isolates for *eae*A (33.3%). All Shiga toxin genes (*stx*1 and/or *stx*2) positive samples were positive for the *hly*A and *eae*A genes. Both virulence genes *eae*A and *hly*A in the same sample were observed in 36.36% (4/11) of the Shiga toxin positive genes strains, while *eae*A gene was observed in all strains positive for *hly*A and Shiga toxin positive (Figure 2). One isolate doesn`t have any of the target genes. As shown in Table (3), the virulence gene profile of the *E.coli* isolates from diarrheic calves was found in 12 diverse combinations, from which isolates with the genetic profile *stx*1,

*stx*2 and *hly*A was the most prevalent. The *eae*A gene was detected in 4 (33.3%) isolates of these two isolates were positive for *stx*1 plus *stx*2, one for *stx*2 and one for *stx*1.

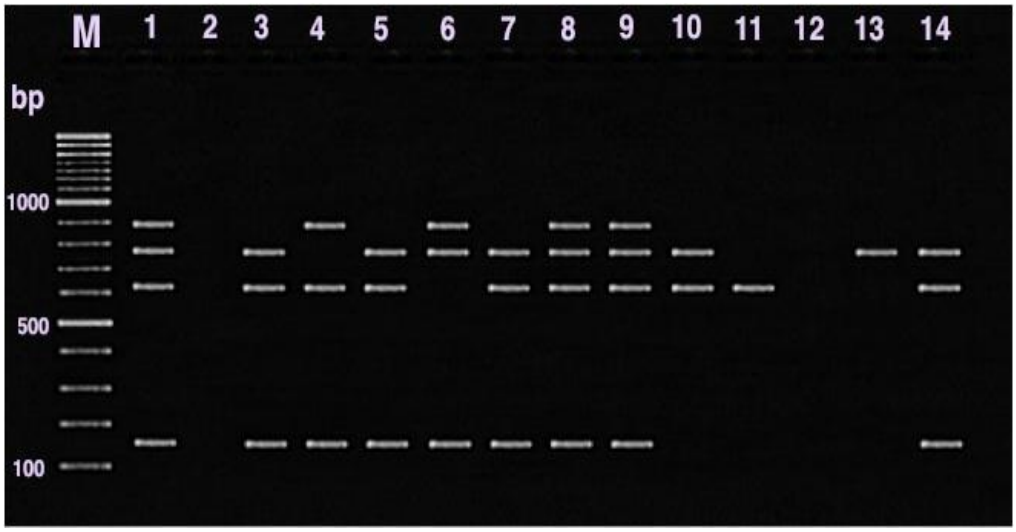


**Fig. (1)**: Representative gels for multiplex-PCR amplification of DNA extracted from selected *E. coli* isolates from diarrheic calves showing the presence of diverse virulence genes, *stx*1 (614 bp), *stx*2 (779 bp), *eae*A (890 bp) and *hly*A (165 bp).

Lane M: 100 bp DNA marker. Lane 1: Control positive *E. coli* reference strain (O157:H7, Sakai) for *stx*1, *stx*2, *eae*A and *hly*A genes. Lane 2: Control negative *E. coli* (K12DH5α). Lanes 3, 5, 7 & 14 (*E.coli* O15, O26, O91 & O128): Positive strains for *stx*1, *stx*2 and *hly*A genes. Lane 4 (*E.coli* O20): Positive strain for *stx*1, *eae*A and *hly*A genes. Lane 6 (*E.coli* O55): Positive strain for *stx*2, *eae*A and *hly*A genes. Lanes 8 & 9 (*E.coli* O103 & O111): Positive strains for *stx*1, *stx*2, *eae*A & *hly*A genes. Lane 10 (*E.coli* O114): Positive strain for both *stx*1 & *stx*2 genes. Lane 11 (*E.coli* O119):

Positive strain for *stx*1 gene only. Lane 12 (*E.coli* O124): Negative strain for *stx*1, *stx*2, *eae*A and *hly*A genes. Lane 13 (*E.coli* O125): Positive strain for *stx*2 gene only.

**Table (3):** Distribution of virulence genes of *E. coli* strains isolated from diarrheic calves

| Virulence gene | *E.coli* strains | | O Serotypes *E.coli* |
|---|---|---|---|
| | No. | % | |
| *stx*1 | 9/12 | 75% | O111,26,103,114,119,20,15,91,128 |
| *stx*2 | 9/12 | 75% | O111,26,103,55,114,15,91,128,125 |
| *eae* A | 4/12 | 33.3% | O111,103,55,20 |
| *hly*A | 8/12 | 66.7% | O111,**26**,103,55**,**20,15,91,128 |
| *stx*1 and *stx*2 | 7/12 | 58.3% | O111,26,103,114,15,91,128 |
| *stx*1, *hly*A | 7/12 | 58.3% | O111, 26, 103,20, 15, 91,128 |
| *stx*2, *hlyA* | 7/12 | 58.3% | O111,26, 103, 55, 15, 91, 128 |
| *stx*1, *stx*2, *eae*A | 2/12 | 16.6% | O111,103 |
| *stx*1, *stx2,hly*A | 6/12 | 50% | O111,26,103, 15,91,128 |
| *stx*1,*eae*A,*hly*A | 3/12 | 25% | O111,103,20 |
| *stx*2,*eae*A,*hly*A | 3/12 | 25% | O111,103, 55 |
| *stx*1,*stx*2, *eae*A and *hly*A | 2/12 | 16.6% | O111, O103 |

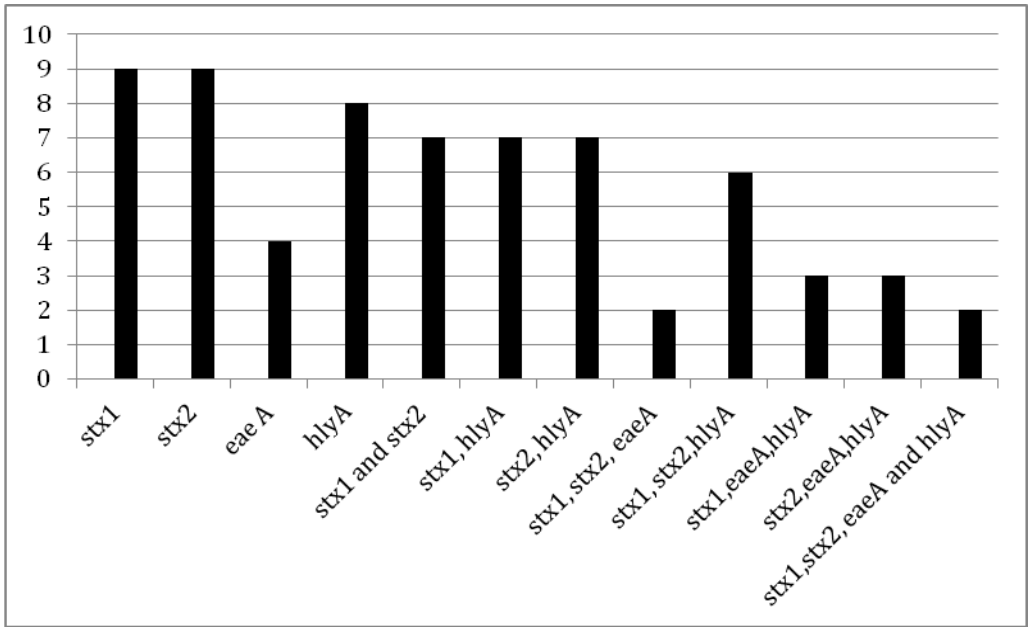


**Fig. (2):** Distribution of virulence genes of *E.coli* strains isolated from diarrheic calves

## DISCUSSION

Diarrhea is a frequent and growing concern in young calves, especially in the first week of age ***(Picco et al., 2015)***. Studies of the virulence factors produced by *E. coli* strains in farm animals are relevant mainly because colibacillosis is an important cause of economic loss on farms. At the same time, *E. coli* isolated from animals may also have genes related to virulent strains for humans, zoonoses-associated or not ***(Leomil et al., 2003)***. The main concern of the paper was to investigate some virulence genes associated with *E.coli* recovered from diarrheic calves. In order to categorize *E. coli* pathotypes, the presence of virulence characteristics needs to be identified. The use of PCR-based technology to identify virulence genes has become widely adopted to

distinguish pathogenic *E. coli* strains from normal gut flora. The prevalence rate of pathogenic *E. coli* in our study was much lower than previous studies ***( Osman et al., 2013; Shahrani et al., 2014)***with prevalence 63.6%, 76.45% respectively, while a similar rate was recorded by ***(C. Herrera-Luna et al., 2009)*** with17%. In the current study, it has been found that the incidence of EHEC, EPEC, ETEC and EIEC pathotypes were 41.6%, 37.5%, 12.52% and 8.3%, respectively (Table 2). Four virulence factors including phage-encoded cytotoxins, called shiga toxin 1 (*stx*1) and shiga toxin 2(*stx*2), the protein intimin (*eae*A) and the plasmid-encoded enterohaemolysin or enterohaemorrhagic *E. coli* haemolysin (*hly*A) ***(Law, 2000)*** which are related to the pathogenesis of *E.coli* strains were investigated. Globally, fecal samples from diarrheic calves carried the genes *stx*1, *stx*2, *eae*A, *hly*A and combinations of *stx*1 and *stx*2, *stx*1 and *eae*A, and *stx*2 and *eae*A genes in their feces ***(C. Herrera-Lunaet al., 2009; Nguyen et al., 2011)*** have been reported.

STEC strains belonging to serogroups O15, O20, O103, and O157 have previously been found to be associated with diarrhea and enteritis in calves in Belgium ***(Mainil et al., 1993)***, Spain***(Orden et al., 1999)***, and the United States***(Dean-Nystrom et al., 1997)***. Our results showed that O111, O103, O26, O55, O128, O15, O91 and O20 were the most common serogroups, while O125 was the less common.

Diarrhea in calves is commonly caused by ETEC ***(Jay et al., 2004)***. More recently, AEEC and STEC have also been identified as causes of diarrhea and dysentery in calves ***(Franck et al., 1998; Mainil et al., 1990)***. STEC has been implicated as an etiological factor of calf diarrhea***(Dean-Nystrom et al., 1997; Orden et al., 1998; Sandhu and***

***Gyles, 2002; Wieler et al., 1996)***, and these animals form a principle reservoir of STEC that is pathogenic for humans***(Armstrong et al., 1996; Boerlin et al., 1999)***. In the present study, we found that a total of 11 isolates (91.6%) were positive for the *stx* genes. Similarly, high percentages (40% or more) of *stx* gene positive *E. coli* strains have been reported in India ***(Arya et al., 2008)***. The results thus obtained are compatible with that of others studies carried out in in Germany***(Wieler et al., 1996)***, USA ***(Holland et al., 1999)*** and Brazil***(Leomil et al., 2003)*** and demonstrated the higher prevalence of shiga toxin producing *E. coli* in the faeces of young calves, but comparably higher than that of ***(Osek et al., 2000)*** with 7.6% in Poland. Several studies have described that most STEC from diarrheic calves only produce *stx*1, whereas *stx*2-positive strains are the dominant types in healthy calves ***(Mainil et al., 1993; Orden et al., 1998; Wieler et al., 1996)***. This is in contrast to our observation, wherein the *stx*2 gene (9 isolates) was detected as frequently as *stx*1 (9 isolates). However higher prevalence rate of *stx*1 than *stx*2 of *E.coli* isolated from diarrheic calves (13.5% and 5.4% respectively) was recorded by ***(Guler et al., 2008)*** on Turkey.

In the current study, the *eae*A gene was detected in 33.3% (4/12) of isolates. Similar prevalence of *eae*A gene was also found in other studies ***(Aidar-Ugrinovich et al., 2007; Blanco et al., 2004)***. The low prevalence of *eae*A gene has been reported in many studies ***(Fremaux et al., 2006; Hornitzky et al., 2005)***, these variations are likely due to geographical differences. The importance of this data lies in the fact that *eae*A-positive strains are considered more virulent for humans than *eae*A-negative strains. The distribution of *eae*A gene was observed in all samples

positive for shiga toxin producing *E. coli* strains It has been reported that in calves the *eae*A gene and intimin have a defined role in causing the attaching and effacing (AE) lesions ***(Dean-Nystrom et al., 1997)***.

Moreover, the data presented here showed that there was higher prevalence (66.7%) of the *hly*A gene with uniform distribution in *stx*1 and/ or *stx*2 positive isolates. This could have resulted from the fact that it can be easily transferred among bacterial isolates since it is plasmid encoded ***(Schmidt et al., 1999)***.

In conclusion, it is evident that this study has shown high prevalence of strains carrying shiga toxin genes; this implies that *stx* genes, which are encoded on bacteriophages, are continuing to expand in the *E. coli* population. Moreover, the high number of STEC strains isolated from diarrheic calves suggests that these animals are an important reservoir of STEC strains that are potentially pathogenic toward farm animals and humans.

## REFERENCES

- ***Aidar-Ugrinovich, L., Blanco, J., Blanco, M., Blanco, J.E., Leomil, L., Dahbi, G., Mora, A., Onuma, D.L., Silveira, W.D., Pestana de Castro, A.F., (2007)***: Serotypes, virulence genes, and intimin types of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) isolated from calves in Sao Paulo, Brazil. International journal of food microbiology 115, 297-306.
- ***Armstrong, G.L., Hollingsworth, J., Morris, J.G., Jr., (1996)***: Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemiologic reviews 18, 29-51.

- ***Arya, G., Roy, A., Choudhary, V., Yadav, M.M., Joshi, C.G., (2008)***: Serogroups, atypical biochemical characters, colicinogeny and antibiotic resistance pattern of Shiga toxin-producing *Escherichia coli* isolated from diarrhoeic calves in Gujarat, India. Zoonoses and public health 55, 89-98.
- ***Blanco, M., Blanco, J.E., Blanco, J., Mora, A., Prado, C., Alonso, M.P., Mourino, M., Madrid, C., Balsalobre, C., Juarez, A., (1997)***: Distribution and characterization of faecal verotoxin-producing *Escherichia coli* (VTEC) isolated from healthy cattle. Veterinary microbiology 54, 309-319.
- ***Blanco, M., Padola, N.L., Kruger, A., Sanz, M.E., Blanco, J.E., Gonzalez, E.A., Dahbi, G., Mora, A., Bernardez, M.I., Etcheverria, A.I., Arroyo, G.H., Lucchesi, P.M., Parma, A.E., Blanco, J., (2004)***: Virulence genes and intimin types of Shiga-toxin-producing *Escherichia coli* isolated from cattle and beef products in Argentina. International microbiology : the official journal of the Spanish Society for Microbiology 7, 269-276.
- ***Boerlin, P., McEwen, S.A., Boerlin-Petzold, F., Wilson, J.B., Johnson, R.P., Gyles, C.L., (1999)***: Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. Journal of clinical microbiology 37, 497-503.
- ***Boerlin, P., Travis, R., Gyles, C.L., Reid-Smith, R., Janecko, N., Lim, H., Nicholson, V., McEwen, S.A., Friendship, R., Archambault, M., (2005)***: Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. Applied and environmental microbiology 71, 6753-6761.

- ***Bolton, D.J., (2011)***: Verocytotoxigenic (Shiga toxin-producing) *Escherichia coli*: virulence factors and pathogenicity in the farm to fork paradigm. Foodborne pathogens and disease 8, 357-365.
- ***C. Herrera-Luna1, D.K., G. Lapan2, S. Revilla-Fernandez2,, B. Haschek1, I.S.-S., K. Moestl3, W. Baumgartner1, (2009)***: Characterization of virulence factors in *Escherichia coli* isolated from diarrheic and healthy calves in Austria shedding various enteropathogenic agents. Veterinarni Medicina 54, 1-11.
- ***Caprioli, A., Morabito, S., Brugere, H., Oswald, E., (2005)***: Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. Veterinary research 36, 289-311.
- ***Constable, P.D., (2004)***: Antimicrobial use in the treatment of calf diarrhea. Journal of veterinary internal medicine / American College of Veterinary Internal Medicine 18, 8-17.
- ***Cray, W.C., Jr., Moon, H.W., (1995)***: Experimental infection of calves and adult cattle with Escherichia coli O157:H7. Applied and environmental microbiology 61, 1586-1590.
- ***Dean-Nystrom, E.A., Bosworth, B.T., Cray, W.C., Jr., Moon, H.W., (1997)***: Pathogenicity of *Escherichia coli* O157:H7 in the intestines of neonatal calves. Infection and immunity 65, 1842-1848.
- ***Dhanashree, B., Mallya, P.S., (2008)***: Detection of shiga-toxigenic *Escherichia coli* (STEC) in diarrhoeagenic stool & meat samples in Mangalore, India. The Indian journal of medical research 128, 271-277.

- ***Fecteau, G., Van Metre, D.C., Pare, J., Smith, B.P., Higgins, R., Holmberg, C.A., Jang, S., Guterbock, W., (1997)***: Bacteriological culture of blood from critically ill neonatal calves. The Canadian veterinary journal. La revue veterinaire canadienne 38, 95-100.
- ***Franck, S.M., Bosworth, B.T., Moon, H.W., (1998)***: Multiplex PCR for enterotoxigenic, attaching and effacing, and Shiga toxin-producing *Escherichia coli* strains from calves. Journal of clinical microbiology 36, 1795-1797.
- ***Fratamico, P.M., Sackitey, S.K., Wiedmann, M., Deng, M.Y., (1995)***: Detection of *Escherichia coli* O157:H7 by multiplex PCR. Journal of clinical microbiology 33, 2188-2191.
- ***Fremaux, B., Raynaud, S., Beutin, L., Rozand, C.V., (2006)***: Dissemination and persistence of Shiga toxin-producing *Escherichia coli* (STEC) strains on French dairy farms. Veterinary microbiology 117, 180-191.
- ***Frydendahl, K., (2002)***: Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. Veterinary microbiology 85, 169-182.
- ***Garcia, A., Ruiz-Santa-Quiteria, J.A., Orden, J.A., Cid, D., Sanz, R., Gomez-Bautista, M., de la Fuente, R., (2000)***: *Rotavirus* and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. Comparative immunology, microbiology and infectious diseases 23, 175-183.

- ***Guler, L., Gunduz, K., Ok, U., (2008)***: Virulence factors and antimicrobial susceptibility of *Escherichia coli* isolated from calves in Turkey. Zoonoses and public health 55, 249-257.
- ***Holland, R.E., Wilson, R.A., Holland, M.S., Yuzbasiyan-Gurkan, V., Mullaney, T.P., White, D.G., (1999)***: Characterization of *eae*+ *Escherichia coli* isolated from healthy and diarrheic calves. Veterinary microbiology 66, 251-263.
- ***Hornitzky, M.A., Mercieca, K., Bettelheim, K.A., Djordjevic, S.P., (2005)***: Bovine feces from animals with gastrointestinal infections are a source of serologically diverse atypical enteropathogenic *Escherichia coli* and Shiga toxin-producing *E. coli* strains that commonly possess intimin. Applied and environmental microbiology 71, 3405-3412.
- ***Jay, C.M., Bhaskaran, S., Rathore, K.S., Waghela, S.D., (2004)***: Enterotoxigenic K99+ *Escherichia coli* attachment to host cell receptors inhibited by recombinant pili protein. Veterinary microbiology 101, 153-160.
- ***Kang, S.J., Ryu, S.J., Chae, J.S., Eo, S.K., Woo, G.J., Lee, J.H., (2004)***: Occurrence and characteristics of enterohemorrhagic *Escherichia coli* O157 in calves associated with diarrhoea. Veterinary microbiology 98, 323-328.
- ***Kaper, J.B., Nataro, J.P., Mobley, H.L., (2004)***: Pathogenic *Escherichia coli*. Nature reviews. Microbiology 2, 123-140.
- ***Kok T., Worswich D. and Gowans E. (1996):*** Some serological techniques for microbial and viral infections. In Practical Medical

Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill Livingstone, UK.

- ***Kumar, A., Taneja, N., Singhi, S., Shah, R., Sharma, M., (2013)***: Haemolytic uraemic syndrome in India due to Shiga toxigenic *Escherichia coli.* Journal of medical microbiology 62, 157-160.
- ***Law, D., (2000)***: Virulence factors of *Escherichia coli* O157 and other Shiga toxin-producing E. coli. Journal of applied microbiology 88, 729-745.
- ***Leomil, L., Aidar-Ugrinovich, L., Guth, B.E., Irino, K., Vettorato, M.P., Onuma, D.L., de Castro, A.F., (2003)***: Frequency of Shiga toxin-producing *Escherichia coli* (STEC) isolates among diarrheic and non-diarrheic calves in Brazil. Veterinary microbiology 97, 103-109.
- ***Mainil, J.G., Bex, F., Jacquemin, E., Pohl, P., Couturier, M., Kaeckenbeeck, A., (1990)***: Prevalence of four enterotoxin (STaP, STaH, STb, and LT) and four adhesin subunit (K99, K88, 987P, and F41) genes among *Escherichia coli* isolates from cattle. American journal of veterinary research 51, 187-190.
- ***Mainil, J.G., Jacquemin, E.R., Kaeckenbeeck, A.E., Pohl, P.H., (1993)***: Association between the effacing (eae) gene and the Shiga-like toxin-encoding genes in *Escherichia coli* isolates from cattle. American journal of veterinary research 54, 1064-1068.
- ***Mazaheri, S., Salmanzadeh Ahrabi, S., Aslani, M.M., (2014)***: Shiga toxin-producing *Escherichia coli* isolated from lettuce samples in tehran, iran. Jundishapur journal of microbiology 7, e12346.

- ***Mora, A., Blanco, J.E., Blanco, M., Alonso, M.P., Dhabi, G., Echeita, A., Gonzalez, E.A., Bernardez, M.I., Blanco, J., (2005)***: Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Research in microbiology 156, 793-806.

- ***Nguyen, T.D., Vo, T.T., Vu-Khac, H., (2011)***: Virulence factors in *Escherichia coli* isolated from calves with diarrhea in Vietnam. Journal of veterinary science 12, 159-164.

- ***Orden, J.A., Ruiz-Santa-Quiteria, J.A., Cid, D., Garcia, S., de la Fuente, R., (1999)***: Prevalence and characteristics of necrotoxigenic *Escherichia coli* (NTEC) strains isolated from diarrhoeic dairy calves. Veterinary microbiology 66, 265-273.

- ***Orden, J.A., Ruiz-Santa-Quiteria, J.A., Cid, D., Garcia, S., Sanz, R., de la Fuente, R., (1998)***: Verotoxin-producing *Escherichia coli* (VTEC) and eae-positive non-VTEC in 1-30-days-old diarrhoeic dairy calves. Veterinary microbiology 63, 239-248.

- ***Osek, J., Gallien, P., Protz, D., (2000)***: Characterization of shiga toxin-producing *Escherichia coli* strains isolated from calves in Poland. Comparative immunology, microbiology and infectious diseases 23, 267-276.

- ***Osman, K.M., Mustafa, A.M., Elhariri, M., Abdelhamed, G.S., (2013)***: The distribution of *Escherichia coli* serovars, virulence genes, gene association and combinations and virulence genes encoding

serotypes in pathogenic *E. coli* recovered from diarrhoeic calves, sheep and goat. Transboundary and emerging diseases 60, 69-78.

- ***Picco, N.Y., Alustiza, F.E., Bellingeri, R.V., Grosso, M.C., Motta, C.E., Larriestra, A.J., Vissio, C., Tiranti, K.I., Terzolo, H.R., Moreira, A.R., Vivas, A.B., (2015)***: Molecular screening of pathogenic *Escherichia coli* strains isolated from dairy neonatal calves in Cordoba province, Argentina. Revista Argentina de microbiologia 47, 95-102.
- ***Quinn P.J., Carter M.E., Markey B.K., Donnelly W.J.C. and Leonard F.C. (2002):*** Veterinary Microbiology and Microbial diseases. MPG. Book 1st ed. Bodmin, Cornwall, UK.
- ***Roopnarine, R.R., Ammons, D., Rampersad, J., Adesiyun, A.A., (2007)***: Occurrence and characterization of verocytotoxigenic *Escherichia coli* (VTEC) strains from dairy farms in Trinidad. Zoonoses and public health 54, 78-85.
- ***Sandhu, K.S., Gyles, C.L., (2002)***: Pathogenic Shiga toxin-producing *Escherichia coli* in the intestine of calves. Canadian journal of veterinary research = Revue canadienne de recherche veterinaire 66, 65-72.
- ***Schmidt, H., Geitz, C., Tarr, P.I., Frosch, M., Karch, H., (1999):*** Non-O157:H7 pathogenic Shiga toxin-producing *Escherichia coli*: phenotypic and genetic profiling of virulence traits and evidence for clonality. The Journal of infectious diseases 179, 115-123.
- ***Schumann, F.J., Townsend, H.G., Naylor, J.M., (1990)***: Risk factors for mortality from diarrhea in beef calves in Alberta. Canadian journal

of veterinary research = Revue canadienne de recherche veterinaire 54, 366-372.

- ***Shahrani, M., Dehkordi, F.S., Momtaz, H., (2014)***: Characterization of *Escherichia coli* virulence genes, pathotypes and antibiotic resistance properties in diarrheic calves in Iran. Biological research 47, 28.
- ***Snodgrass, D.R., Terzolo, H.R., Sherwood, D., Campbell, I., Menzies, J.D., Synge, B.A., (1986):*** Aetiology of diarrhoea in young calves. The Veterinary record 119, 31-34.
- ***Trevejo, R.T., Barr, M.C., Robinson, R.A., (2005)***: Important emerging bacterial zoonotic infections affecting the immunocompromised. Veterinary research 36, 493-506.
- ***Widiasih, D.A., Ido, N., Omoe, K., Sugii, S., Shinagawa, K., (2004)***: Duration and magnitude of faecal shedding of Shiga toxin-producing *Escherichia coli* from naturally infected cattle. Epidemiology and infection 132, 67-75.
- ***Wieler, L.H., Vieler, E., Erpenstein, C., Schlapp, T., Steinruck, H., Bauerfeind, R., Byomi, A., Baljer, G., (1996)***: Shiga toxin-producing *Escherichia coli* strains from bovines: association of adhesion with carriage of eae and other genes. Journal of clinical microbiology 34, 2980-2984.
- ***Younis, E.E., Ahmed, A.M., El-Khodery, S.A., Osman, S.A., El-Naker, Y.F., (2009)***: Molecular screening and risk factors of enterotoxigenic *Escherichia coli* and Salmonella spp. in diarrheic neonatal calves in Egypt. Research in veterinary science 87, 373-379.

## الملخص العربي

أجريت هذه الدراسة لتوضيح مدى خصائص وانتشار سلالات بكترياالقولوني العصوي الممرض (الايشريشيا كولاي ) المسببة للإسهال في العجول. تم تجميع عدد ١٢٠ عينة براز من عجول مصابة بالإسهال من مزارع أبقار مختلفة بمحافظة كفرالشيخ. عُزلت الايشريشيا كولاي بنسبة ٢٠٪ من العجول المصابة واظهر الفحص السيرولوجي للمعزولات عن وجود ١٢ نوع تنتمي الي مختلف السلالات ( الانتيجنO) وكانت السلالات ( *O111, O103, O26, O55, O128, O15, O91, O20…* ) الأكثر انتشارا بين العزلات. تم فحص عدد ١٢ عينة باستخدام تفاعل البلمرة المتسلسل المتعدد للكشف عن أربعة جينات مرتبطة بالضراوة وهي الشيجاتوكسين ١،٢ والانتيمين والهيمولايسين.

أظهرت النتائج أن ١١ عينة من أصل ١٢ كانت تحمل جينات الشيجاتوكسين (٩٠٪) بينما ثمان معزولات كانت تحمل الهيمولايسين وأربع معزولات كانت تحمل جين الانتيمين. اظهر الفحص ايضا ان تسع معزولات كانت تحمل الشيجاتوكسين ١ وتسع معزولات تحمل الشيجاتوكسين ٢ من بينهم سبع معزولات تحمل كلا الجينين معا. كانت كل المعزولات التي تحمل الشيجا توكسين ايجابية أيضا لكل من الهيمولايسين والانتيمين أوضحت هذه الدراسة أن جينات الشيجاتوكسين هي الأكثر ارتباطا بالميكروب القولوني العصوي المعزول من عجول مصابة بالإسهال وكذلك جينات الهيمولايسين والانتيمين.