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STUDY ON VIRULENCE FACTORS OF ESCHERICHIA COLI ISOLATED FROM CALVES SUFFER FROM DIARRHEA

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ABSTRACT

Diarrhea is an important problem in young calves, especially in suckling beef calves. The mostcommon cause of diarrhea in calves is *E.coli*. The present study was undertaken to screen for presence of virulence factors associated with colibacillosis isolated from one hundred and twenty five fecal samples of calves suffer from diarrhea (<3monthes). All samples were submitted for bacteriological examination, serotyping and virulence tests (Congo red test, serum resistant test, sereny test and haemolytic activity). Finally molecular Identification by using Polymerase chain reaction (PCR) to detect shiga toxin (stx1, stx2) genes. *E.coli* isolates were fifty eight isolates (46.4%). The fecal samples were obtained a Twelve different serotype of *E.coli* (O26:H11, O91:H21, O103:H2, O111:H2, O15:H21, O146:H21, O8,O113:H4, O128:H2, O124, O121:H7 and O55:H7). It was recorded forty two isolates (72.4%) were congo red positive, forty two isolates (72.4%) were serum resistant, eleven isolates (18.96%) made keratoconjunctivitis of Guinea pig and fourteen isolates (24.13%) had hemolytic activity. PCR was performed to detect stx1 and stx2 genes on different serotypes of *E.coli* isolates. The serotypes which positive to stx1 gene only were three (O8,O113:H4,O128:H2) (30%), two serotypes were positive to stx2 only (O15:H21,O146:H21) (20%), four serotypes were positive to both stx1 and stx2 (O26:H11, O91:H21, O103:H2,O111:H2) (40%) and O124 was negative for both stx1 and stx2(10%). This result of *E.coli* strains isolated from diarrheic calves implies that these animals are an important reservoir of *Shiga toxin Escherichia coli* (STEC) strains that are potentially pathogenic toward farm animals.

Key words: *Virulence, Escherichia Coli, calves, Diarrhea*

INTRODUCTION

E.coli found is a normal habitant of the gastrointestinal tract and also found in the environment. However, the infection was happened due to break of the protection barrier, highly pathogenic bacteria type or immunosuppression. Clinical disease due to *E.coli* in calves may be foundas enteric or septicemic disease, being one of the most important causes of high mortalities in dairy calves. (Lofstedt *et al.,* 1999). Some strains develop traits can be harmful to a host animal.

According to virulence factors and the clinical symptoms of the host, pathogenic *E.coli* strains are classified into *Enterotoxigenic E.coli (ETEC),* attaching and effacing *E. coli, enteropathogenic E. coli*, Shiga toxinproducing *E.coli* (STEC), and *necrotoxigenic E. coli* (Deb Roy *et al.,* 2001; Nataro and Kaper, 1998). *Enterotoxigenic E.coli* can cause severe diarrhea in newborn calves by production of heat-stable enterotoxin (STa). The most common observed fimbriae on ETEC from diarrheic calves is K99 (F5) and F41; however, strains with F17 fimbriae have also been isolated (Nagy and Fekete., 1999). *STEC* strains are the main cause of colibacillosis in newborn calves. Although both healthy and diarrheic calves carry *STEC* in their intestine, natural outbreaks and experimental infections have recorded the association of *STEC* with diarrhea and dysentery in young calve (Dean-Nystrom *et al.,* 1997; Sandhu and Gyles, 2002) by production of Shiga toxins. These toxins are subdivided into two groups, Shiga toxin 1 (Stx1) and Shiga toxin 2 (Stx2). Stx1 is a homologous group in which three variants (Stx1, Stx1c and Stx1d) have been describe (Bürk *et al.,* 2003; Zhang *et al.,* 2002). Stx2 is more heterogeneous and consist of several subtypes (Stx2, Stx2c, Stx2d, Stx2e, Stx2f, Stx2g and activatable Stx2d) (Leung *et al.,* 2003; Nataro and Kaper, 1998; Piérard *et al.,* 1998; Schmidt *et al.,* 2000). In addition to toxin production, STEC strainshave other virulence factors such as intimin

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(encoded by the eae gene) (Nataro and Kaper., 1998) the plasmid-encoded enterohemolysin (encoded by the ehxA gene) (Schmidt *et al.,* 1995) and the STEC autoagglutinating adhesion (Saa) (Paton *et al.,* 2001).

Serogrouping of *E.coli* was carried out to give an idea about the most predominant serogroups associated with diarrhea in calves (Blanco *et al.,* 2004; Ugrinovich *et al.,* 2007; Shahrani *et al.,* 2014).

Congo Red Agar test was used to differentiate invasive and noninvasive *E.coli* in poultry¸ this test was used to detect enteroinvasive *E.coli* of bovine origin (Sharma *et al.,* 2006). Antibody and complement have been found the main component that made the bactericidal effect of serum against grame negative bacteria, more over complement activity responsible for bactericidal activity of serum by generating chemostatic factors and directly killing susceptible gram negative bacteria (Taylor, 1983). The ability of *E.coli* to survive in serum and grow in spite of the bactericidal activity of complement has recognized as an important attribution which contributes to its pathogenicity and suggests the possibility of multiplication in Blood stream (John *et al.,* 1989). Hemolytic activity could be used a phenotypic character or virulence factors of *E.coli* serotypes (Gad EL-Said *et al.,* 2005).

So, the aim of this study is detection of *E.coli* from diarrheic calves which can be achieved by isolation, identification, serotyping and detection of virulence gene by PCR.

MATERIALS AND METHODS

Sample Collection (Salvadori *et al.,* 2003)
A total hundred and twenty five fecal samples of diarrheic calves (≤ 3monthes) were collected from different localities of Assiut. The fecal samples were obtained after stimulation of the rectal mucosa then they were collected in sterile plastic tubes and submitted into laboratory on ice packs. Samples were processed within 24 -48 hours. After reception.

Isolation and Identification of *E.coli*: (Quinn *et al.,* 2004)
1- Samples were cultured on nutrient brothand incubated for 24 hours at 37ºC.
2- Isolation: subculture of bacterial growth on MaCconkey agar (Diffco)→ lactose fermenter. (pink colonies). Acolony was picked and cultivated on Eosin methylene blue media (Oxoid)→ green metalic sheen.(Quinn *et al.,* 2004).
3- Identification: Biochemical conformation of the strains was performed by conventional IMVC (Indol, Methyl red, Vogesproskauer and Citrate utilizations tests) urease test, motility triple sugar iron agar (TSI) inculation and according to (Quinn *et al.,* 2004) .

Serological identification of *E.coli*: (Kok *et al.,* 1996)
- The isolates were serologically identified according to (Kok *et al.,* 1996) by using rapid diagnostic E.coli antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types. They include 8 vials of polyvalent in addition to the 44 vials of monovalent antisera and 6 H-sera.

Identification of virulence factors: (Sayed, 2014)
A- *Haemolytic activity (haemolysin)* (Beutin *et al.,* 1989): *E. coli* isolates were inculated onto blood agar containing 5 % sheep blood for detection of enterohaemolysis after 6 hours of incubation at 37C°.

B- *Congo red (CR) binding test* (Panigrahy and Yushen, 1990):
Congo Red Dye binding test (CR test) has been used to differentiate between invasive and non-invasive *E. coli* (Berkhoff and Vinal, 1986; Penigarhy and Yushen, 1990). The medium used for CR dye binding was tryptose agar with 0.2 % galactose and 0.03% CR due to *E coli* isolates were streaks on to CR agar plates and incubated at room temperature for additional 48 hours. The colonies were examined at 18, 24, 48 and 72 hours of incubation. The *E.coli* that produced red colonies between 18 and 72 hours of incubation were recorded as Congo red positive and ones that produced grayish- white colonies and remainded so throughout the incubation period were recorded as Congo red negative. (Penigrahy and Yushen, 1990).

C- serum inactivation assay (Timmis, 1979):
E.coli isolates were incubated at 0.1% (V/V) inoculums level into glucose phosphate broth containing bromothymol blue and 2 % human serum and incubated at 37 C° for 24 hours. The serum resistance was observed by changes in colour from green to yellow.

D- Invasiveness test (sereny test) (Sereny, 1955):
E.coli isolates were grown overnight in nutrient broth. The growth in centrifuged at 5000 rpm for 15 min at 4 C° and the bacterial pellet was collected. The concentration was adjusted with 0.9 % normal saline solution to 5×10^5 ml. A volum of approximately 50μl suspension was incubated at the conjunctival sac of guinea pig. Reaction was observed for 96 hours and development of kerato conjunctivitis is recorded as a positive reaction

Polymerase chain reaction (PCR):
Materials used for PCR:
Reagents used for agrarose gel electrophoresis:
Agarose powder, Biotechnology grade (Bioshop^R, Candainc.lot No: OE16323).

It prepared in concentration 2% in 1× TAE buffer.

Tris acetate EDTA (TAE) electrophoresis buffer (50×liquid concentration) (Bioshop®, Candainc. lot No: 9E11854).

The solution diluted to 1× by adding 1 ml stock solution to 49 ml double dist. Water to be used in the preparation of the gel or as a running buffer.

Ethedium bromide solution (stock solution) biotechnology grade (Bioshop ® CandaInc, Lot No: 0A14667):
The stock solution was diluted by 25µl /200ml double distilled water and stored covered at 4ºC. It was used for staining of PCR products that electrophoreses on agarose gel to be visualized by UV light.

Gel loading buffer (6×stock solution) (Fermentas, lot No: ooo56239).
The components were dissolved in sterile double distilled water and stored covered with aluminum foil at room temperature.

DNA ladder (molecular marker):
100 bp (Fermentas, lot No: 00052518).
5X Taq master (Fermentas):
Containing polymerase enzyme, Magnesium chloride ($Mg\ Cl_2$), Deoxy nucleotide triphosphate (dNTP) and PCR grade water.

Primer sequences of E. coli used for PCR identification system:
Application of PCR for identification of shiga toxins (stx1 & stx2) genes of *E.coli* was performed essentially by using primers (Pharmacia Biotech) as shown in the following table:

Primer	Oligonucleotide sequence (5′ → 3′) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC140333/table/t2/ - t2fn1	Product size (bp)	References
stx1 (F)	**5′** ACACTGGATGATCTCAGTGG **′3**	614	**Dhanashree and Mallya (2008)**
Stx1 (R)	**5′** CTGAATCCCCCTCCATTATG **′3**		
Stx2 (F)	**5′** CCATGACAACGGACAGCAGTT **′3**	779	**Dhanashree and Mallya (2008)**
Stx2 (R)	**5′** CCTGTCAACTGAGCAGCACTTTG **′3**		

2. DNA Extraction using QIA amp kit (Shah *et al.*, 2009):
After overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100ºC for 20 minutes. Accurately, 50-200 µl of the culture were placed in Eppendorf tube and the following steps were carried out:

2.1. Equal volume from the lysate (50-200 µl) was added, addition of 20-50µl of proteinase K, then incubation at 56 ºC for 20-30 min. After incubation, 200 µl of 100% ethanol was added to the lysate.

2.2. The solution was added to the column and centrifuged at 8000 rpm for 1 min. then the filtrate was discarded.

2.3. The sediment was washed using AW1 buffer (200 µl), the column was centrifuged at 8000 rpm / 1 min, and the filtrate was discarded.

2.4. Washing was applied by using the AW2 buffer (200µl), the column was centrifuged at 8000 rpm / 1 min. and the filtrate was discarded.

2.5. The column was placed in a new clean tube then, 25-50 µl from the Elution buffer was added, centrifuged at 8000 rpm/1min. Then the column was discarded. The filtrate was put in clean tube containing the pure genomic DNA. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Amplification reaction of E. coli (Fagan *et al.*, 1999):
The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR assays were carried out in 1 ml of nucleic acid template prepared by using reference EHEC isolates (approximately 30 ng of DNA), 10 mMTris-HCl (pH 8.4), 10 mMKCl, 3 mM MgCl2; 2 mM concentrations of each primer, 0.2 mM concentrations of each 29-deoxynucleoside 59-triphosphate, and 4 U of Ampli Taq DNA polymerase (Perkin-Elmer). Amplification conditions consisted of an initial 95°C denaturation step for 3 min followed by 35 cycles of 95°C for 20 sec, 58°C for 40 s, and 72°C for 90 sec. The final cycle was followed by 72°C incubation for 5 min. The reference strains were *E. coli* O157:H7 Sakai (positive for stx1, stx2 and eaeA) and *E. coli* K12DH5α (a non pathogenic negative control strain) that does not posses any virulence gene. Amplified DNA fragments were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment size.

Table 1: Component of PCR Master Mix.

Component	Volume / Reaction
Emerald Amp GT PCR mastermix (2x premix)	**12.5*µl***
PCR grade water	**2.5 *µl***
Forward primer (20 pmol)	**1 *µl***
Reverse primer (20 pmol)	**1 *µl***
Template DNA	**6 *µl***
Total	**25 *µl***

Table 2: Cycling conditions of the different primers during PCR.

Target gene	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension
stx1	95˚C 3 min.	95˚C 20 sec.	58˚C 20 sec.	72˚C 1.5 min.	72˚C 5 min.
stx2	95˚C 3 min.	95˚C 20 sec.	58˚C 20 sec.	72˚C 1.5 min.	

RESULTS

Typical *E.coli* colonies were isolated from 58 fecal samples (46.4%) out of 125 fecal samples followed by *Enterobacteraerogenes* were foundin 14 fecal samples (11.2%).While *Proteuemirabili* were isolated from10 fecal samples (8%), *Klebsiella pneumonia* were found in 8 fecal samples (6.4%), *Hafnia species* were recorded in 6 fecal samples (4.8%), *Citrobacterfreundii* were isolated from 4 fecal samples (3.2%) and 25 fecal samples had untyped bacteria as shown in table (3)

Table 3: Different types of bacteria isolated from fecal samples of calves suffer from diarrhea.

Types of isolates	No of isolates	Percentage of isolates
E.coli	58	46.4%
Enterobacteraerogenes	14	11.2%
Proteuemirabili	10	8%
Klebsiella pneumonia	8	6.4%
Hafnia species	6	4.8%
Citrobacterfreundii	4	3.2%
Untyped	25	20%
Total	125	100%

The isolates of 58 *E.coli* were serologically identified by using rapid diagnostic *E.coli* antisera sets .It was recorded that O26:H11 was the most serotype found in *E.coli* isolates (17.24%) followed by O111:H2 (13.7%). While O128:H2, O91:H21,O121:H7,O55:H7 had the same percent (10.3%), but O146:H21, O124 were recorded (6.9%) finally, O113:H4.O103:H2,O15 and O8 were found with percent (3.4%). As in table (4).

Table 4: Different serotypes of E.coli isolated from fecal samples of calves suffer from diarrhea.

Serogroups	No.	%
O26:H11	10	17.24%
O111:H2	8	13.7%
O128:H2	6	10.3%
O91:H21	6	10.3
O121:H7	6	10.3
O55:H7	6	10.3
O146:H21	4	6.9%
O124	4	6.9%
O113:H4	2	3.4%
O103:H2	2	3.4%
O15	2	3.4%
O8	2	3.4%
Total	58	100%

In Congo red binding test: We found that 80% of O26:H11 were positive for Congo red test followed by 75% of O111:H2, but 66.67% of each O128:H2, O91:H21 and O55:H7 only gave positive reaction. All of O121:H7, O113:H4, O1.3:H2, O15 and O8 were positive to Congo red test. Only O124 serotype did not respond to Congo red test. As in table (5)

In serum resistant test: We found that the results of serum resistant test had the same results of Congo red binding test. As in table (5)

In invasiveness test: In this study 20% of O26:H11 made conjunctivitis in guinea pig, 25% of O111:H2 gave positive reaction, 16.67% of O128:H2 had irritation in conjunctiva of guinea pig, while 33.3% of each O91:H21 and O121:H7 were positive to Sereny test and 50% of each O113:H4 and O103:H2 gave positive results of invasiveness test but O55:H7, O146:H21, O124, O15 and O8 gave no reaction in this test. As in table (5)

Haemolytic activity: In this test only 10% of O26:H11 had a haemolytic activity on blood agar, 37.5% of O111:H2 gave positive reaction, while 33.3% of each O128:H2 and O91:H21 were recorded as positive results, 16.67% of each O121:H7 and O55:H7 made a haemolysis on blood agar and 50% of each O113:H4, O103:H2, O15 and O8 gave positive results. Finally O146:H21 and O124 had no effect on blood agar. As in table (5)

Table 5: Relationship between different serogroups and phenotypic virulence factors of E.coli isolated from fecal samples of calves suffer from diarrhea.

Serogroups	No.	Congo Red binding	Serum resistance	Invasiveness activity	Haemolytic activity
		No. of +ve (%)	No. of +ve (%)	No. of +ve (%)	No. of +ve (%)
O26:H11	10	8(80%)	8(80%)	2(20%)	1(10%)
O111:H2	8	6(75%)	6(75%)	2(25%)	3(37.5%)
O128:H2	6	4(66.67%)	4(66.67%)	1(16.67%)	2(33.3%)
O91:H21	6	4(66.67%)	4(66.67%)	2(33.3%)	2(33.3%)
O121:H7	6	6(100%)	6(100%)	2(33.3%)	1(16.67%)
O55:H7	6	4(66.67%)	4(66.67%)	0	1(16.67%)
O146:H21	4	2(50%)	2(50%)	0	0
O124	4	0	0	0	0
O113:H4	2	2(100%)	2(100%)	1(50%)	1(50%)
O103:H2	2	2(100%)	2(100%)	1(50%)	1(50%)
O15	2	2(100%)	2(100%)	0	1(50%)
O8	2	2(100%)	2(100%)	0	1(50%)
Total	58	42(72.4%)	42(72.4%)	11(18.96%)	14(24.13%)

Ten different serotypes of *E.coli* isolates were taken for multiplex PCR using shiga toxin 1(stx1) (614 bp) and shiga toxin 2(stx2) (779 bp) Genes for characterization of shiga toxin *E.coli* (STEC). The results were recorded as in table (6)

Table 6: Stx1 and Stx2 genes profile of ten serotypes *E.coli* strains isolated from calves suffer from diarrhea.

Virulence genes.	Positive serogroups	Number of +veserogroups
Stx1	O8, O113:H4,O128:H2	3(30%)
Stx2	O15 ,O146:H21	2(20%)
Stx1 and stx2	O26:H11, O91:H21, O103 ,O111:H2	4(40%)
Negative stx1 ,stx2	O124	1(10%)

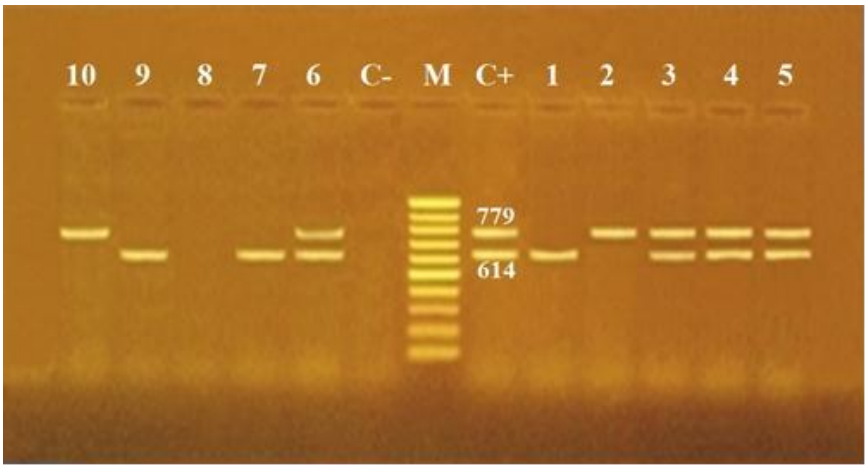

Figure (1): Agarose gel electrophoresis of multiplex PCR using shiga toxin 1(stx1) (614 bp) and shiga toxin 2(stx2) (779 bp) Genes for characterization of Enterotoxigenic *E.coli*.
Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *E. coli* for stx1, stx2. Lane C-: Control negative. Lanes 1 (O8), 7 (O113) & 9 (O128): Positive *E. coli* strains for stx1 gene. Lanes 2 (O15) & 10 (O146): Positive *E. coli* strains for stx2 gene. Lanes 3 (O26), 4 (O91), 5 (O103) & 6 (O111): Positive E. coli strains for stx1 and stx2 genes.

DISCUSSION

Diarrhoea due to the *Enterotoxigenic E.coli* (ETEC) is one of the most frequent bacterial diseases in neonatal calves and the predominant pathogen cultured from calves with septicaemia. Detailed studies of the virulence factors produced by *E.coli* strains in farm animals are needed. In this study 125 fecal samples of diarrhetic calves were used, coliforms were detected in one hundred fecal samples with the incidence of 80% (table 3). The incidence of *E.coli* is (46.4%) followed by *Enterobacteraerogenes* (11.2%), *Proteuemirabili* (8%), *Klebsiella pneumonia* (6.4%) *Hafnia species* (4.8%), *Citrobacterfreundii* (3.2%).

Serogrouping of *E.coli* was carried out to give an idea about the most important and predominant serogroupes found in diarrhetic calves. In thisstudy the obtained results showed that isolated *E.coli* belonged to 12 different serotypes O26:H11(10)O111: H2(8), O128:H2(6), O91:H21(6), O121:H7(6), O55:H7(6), O146:H21(4), O124(4), O113:H4(2), O103:H2(2), O15(2), O8(2). This results agree with (Blanco *et al.,* 2004) who found different serotypes of shiga toxin *E.coli* including (O113,O26,O91,O8); Shahrani *et al.* (2014) recorded different serotypes includind (O128,O113,O26,O121)

In table (5): These findings suggest that CR dye binding could be used as a phenotypic marker to distinguish between invasive and non-invasive isolates. In this study we found 42 strains (72.4%) positive Congo red binding test. Such findings agree with Berkhoff and Vinal (1986) who found that about half of the *E. coli* was CR positive, which were obtained from environmental and cloacal origin. Likewise, Panigarhy and Yushen (1990) also found 13/ 21 (61.9 %) *E.coli*were CR positive. Mousaet al. (2010) also found 90.9% of *E .coli* isolated from diarrheic sheep and 90% isolated from goat were positive to Congo red test; Sayed (2014) also found 72.2% *E.coli* positive Congo red.

It is assumed that serum resistance is an important virulence property of *E.coli* strains. The obtained results in this study showed that 42(72.4%) strains of *E.coli* positive to serum resistance as in table 5. Da Rocha *et al.* (2002) found 88.9% of E.coli positive to serum resistance; Sayed, (2014) found 83.3% of *E.coli* positive to serum resistance.

The term invasive *E.coli* referred to those strains of *E.coli* which able to induce keratoconjunctivitis in eyes of Guinea pig (Sereny test, 1955). The obtained results in this study showed that 11 isolates of *E.coli* (18.96%) made positive reactions of Sereny test (keratoconjunctivities) in Guinea pigs as in table 5. Sharma *et al.* (2006) found the incidence of positive results were (58.69%) Amira *et al.* (2013) found the positive results were (40.5%); Sayed (2014) found the positive results were (33.3%).

Some of *E.coli* strains have hemolytic action, in our study 14(24.13%) E.coli isolates had hemolytic activity table 5. Salvadori *et al.* (2003) found (9.8%) of *E.coli* serotyped positive to hemolysis on blood agar. While *Gad* El-Said (2005) recorded that (81.25%) of *E.coli* isolates show hemolytic activity.

PCR for detection of virulence factor producing by *STEC* in calves is very important finding to demonstrate predominate of *E. coli* serotype that mainly effect the calves and causes severe diarrhea. PCR analysis of ten different serotypes of *E.coli* strains represented the presence of stx1 and stx2

genes in ten *E.coli* serogroupes (O26:H11, O91:H21, O103:H2, O111:H2, O15:H21, O146:H21, O8, O113:H4, O128:H2, O124). Using Shiga toxin1 (stx1) (614 bp) and Shiga toxin 2(stx2) (779 bp) Genes for characterization of shiga toxin *E.coli*. The serotypes which positive to stx1gene only were three (O8,O113:H4, O128:H2) (30%), two serotypes were positive to stx2 only (O15:H21, O146:H21) (20%), four serotypes were positive to both stx1 and stx2 (O26:H11, O91:H21, O103:H2,O111:H2) (40%) and O124 was negative for both stx1 and stx2 (10%). Salvadori *et al.* (2003) recorded stx1 were 20(9.8%) and stx2 were 13(6.34%) out of 205 *E.coli* isolates. Nguyen *et al.* (2010) found stx1 were 46(13.3%), stx2 were 73(21.15%) and both stx1 and stx2 were 58(16.81%). But our study disagree with Luna (2008) who found that stx1 gene positive in 12.2% of total samples, stx2 positive in 7.8% but both stx1 and stx2 were found in 15.2%.

CONCLUSION

The high number of STEC strains isolated from diarrheic calves implies that these animals are an important reservoir of STEC strains that are potentially pathogenic toward farm animals.

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دراسه عن عوامل الضراوة للأشيرشيا كولاي المعزولة من العجول التي تعاني من الإسهال

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يعتبر الاسهال من اهم المشاكل التي تصيب العجول الصغيره خاصه عجول التسمين الرضيعه. من اهم اسبابه الاشيرشيا كولاي. لقد اجريت هذه الدراسه لمعرفه عوامل الضرواه المصاحبه للاسهال في مائه وخمسه وعشرون عينه تم تجميعها من العجول التي تعاني من الاسهال تحت سن ثلاثة اشهر. العينات تم تجميعها من اماكن مختلفه في مصر. تم اجراء الفحص البكتريولوجي والسيرولوجي واختبارات الضرواه (الكونغو الاحمر ، اختبار مقاومة السيرم ، اختبار السيريني ، اختبار مدى قدرة الميكروب على تحلل الدم). واخيرا تم اجراء تفاعل البلمره المتسلسل باستخدام جينين (stx1,stx2). تم عزل ٥٨ عتره من الاشيرشيا كولاي من عينات الاسهال التي تم جمعها كما تم الحصول على ١٢ نوع مختلف من الاشيرشيا كولاي المعزوله (O26:H11,O91:H21, O103:H2,O111:H2, O15:H21, O146:H21, O8,O113:H4, O128:H2, O124,O121:H7 and O55:H7). وجد ان ٤٢ عتره من الاشيرشيا كولاي المعزوله ايجابيه لاختبارات الكونغو الاحمر واختبار مقاومة السيرم بنسبه ٧٢,٤٪ اما اختبار السيريني فكان ١١عتره من الاشيرشيا كولاي المعزوله ايجابيه بنسبة ١٨,٩٦٪ والذي يقوم بعمل التهاب ملتحمة العين لخنزير غينيا كما وجد ١٤ عتره من البكتريا المعزوله بنسبة ٢٤,١٣٪ له قدره على تحلل الدم. اما في تفاعل البلمره المتسلسل فقد وجد بعد استخدام الانواع المختلفه للاشيرشيا كولاي المعزوله ان ٣ منها لديها جين stx1 بنسبه ٣٠٪ وهم (O8,O113:H4,O128:H2) اما الانواع التي لديها جين stx2 فكانت نوعان وذلك بنسبه ٢٠٪ (O15:H21,O146:H21) اما التي لديها جين stx1,stx2 كانت ٤ انواع بنسبة ٤٠٪ وهم (O26:H11,O91:H21,O103:H2,O111:H2).

هذه النتائج تبين مدى اهميه العجول الصغيره التي تعاني من الاسهال كحامل الميكروب (STEC) ونقله الي الحيوانات الاخرى .