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Some Bacteriological and Molecular Studies on *ESCHERICHIA COLI* as Causative Agent of Calves Enteritis at Damietta Governorate

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Abstract

The present study carried out to evaluate the presence of diarrheagenic Escherichia as causes of diarrhea in young calves in Damietta governorate during period from November 2015 to March 2016. Bacteriological examination was done on a total of (100) fecal samples collected from young diarrhoeic calves of average age of 2 to 21 days to detect the prevalence of E. *coli* . The samples were grouped according to the age of the calves into 3 age groups as follows: 1st age group (2-7 dayes), 2nd age group (8-14 dayes) and 3rd group (15-21 dayes). The results revealed isolation of 27 strains of E. *coli* (27%). Serotyping of the isolated strains according to somatic (O) and flagellar (H) antigens detected the presence of 6 entertoxigenic E. *coli* (ETEC; 22.22%); 13 enterohemorrhagic E. *coli* (EHEC; 48.15%); 5 enteropathogenic E. *coli* (EPEC; 18.52%) and 3 enterinvasive E. *coli* (EIEC; 11.11%). Application of PCR for the detection of E. *coli* virulence genes including; two Shiga toxins: (Stx1; 88.89%), (Stx2; 51.85%), produced from Shiga toxin producing E. coli (STEC); intimin (eaeA; 29.63%) and hylA (59.26%) genes was done. Each isolate was found to carry one or more virulence genes. Mixed E. *coli* infection is strongly associated with the cases of diarrhea due to the presence of several virulence genes in some isolates. Diarrheic calves were found in high prevalence in the 1st age group (43%) whereas was found in prevalence in the 2nd group (33%) and in 3rd group (24%) .

Antibiogram study revealed that the isolated E. *coli* strains were highly sensitive to Ciprofloxacin and Cefotaxime, moderately sensitive to Gentamicin and resistance to Nalidixic acid and Erythromycine. The present study suggests the importance of maintain strict hygienic measures to prevent spreading any pathogens to healthy calves.

Key Words: diarrheagenic E. *coli*, calves, Virulence genes, Antibiogram.

Introduction

Calf diarrhea is a commonly reported disease in young animal and still a major cause of low productivity and economic loss to cattle producers. In the report of 2007 National Animal Health Monitoring System for U.S. dairy, a half of the deaths in unweaned calves were reported to be attributed to diarrhea **(Yong, 2012).** Diarrhea is

one of the very common disease syndroms in the neonatal calves in different countries and this can have sever impacts both economically and in terms of animal welfare **(Ozkan et al., 2011 and Tajik et al., 2012**). Diarrhea is distinctively characterized by watery feces and frequent bowel movements. Neonatal calf diarrhea is a multifactorial disease which despite diarrheagenic Escherichia coli ades of research in the topics remains the most common cause of neonatal calf mortalities. Several enteropathogens are implicated in neonatal calf diarrhea and their relative prevalence varies geographically but the most common prevalent infections in most areas are Escherichia *coli*, Rotavirus, Coronavirus, Clostridum *Perfringens*, Salmonella and infestation with Cryptosporidium **(Sandgrass and Browing 1993 and mehmet et al., 2001).** These infectious agents attack the lining of the calves gut and cause water loss through the damaged gut wall. Calves can potentially be infected with multiple infection agent at the same time **(Radostits et al., 2003)**. The most common causes of diarrheal diseases in pre-weaned calves are bacteria as E. *coli*, Salmonella sp. and Klebsiella and virus as Coranavirus and Rotavirus **(Razzaque et al., 2009 and Cho and Yoon., 2014)**. Among bacteria, Escherichia *coli* and *Salmonella* are known to be the most common but other bacteria, e.g. campylobacter *spp.* have also been identified as cause of enteric disease and diarrhoea in calves **(Firehammer and Myers, (1981), Prescott and Munroe ,(1982), and Myers et al. 2003)**. The 2 latter groups also contain important human pathogens that may cause outbreaks of food-borne diseases **(De Rycke et al. 1986)** and thus are of high public health important.

Based on the molecular, and pathological criteria, the diarrheagenic Escherichia coli are classified into several pathotypes such as: entertoxigenic E. *coli* (ETEC), enteropathogenic E. *coli* (EPEC), enterohemorrhagic E. *coli* (EHEC), enterinvasive E. *coli* (EIEC), enteroaggregative E. *coli* (EAEC), Diffusely-adherent E. *coli* (DAEC) and vero- or shiga-like toxin producing E. *coli* (VTEC or STEC) **(Nagy and Fekete, 2005)**. Virulence factors from several pathogenic E. *coli* strains might predispose to calves diarrhea.

ETEC are common pathotype associated with infectious diarrhea in calves. Neonatal calves are most susceptible to ETEC infection during first 4 days after birth and develop watery diarrhea if infected **(Foster and Smith, 2009)**. They colonize the small intestine by their fimbriae without inducing significant morphological changes and predispose to sever watery diarrhea in newborn calves. Two main virulent factors are included, the fimbriae (pili) and the entertoxins **(Franck et al., 1998)**. Entertoxin producing E. *coli* (ETEC) have been as casutive agent of several important diarrheal diseases in animal and human. These bacteria may produce thermolabile (LT-I and LT-II) and thermostable (STa and STb) entertoxins **(Elwell, 1980)**. The fimbrial adhesion F5 (K99) plays a role

in the colonization of entertoxigenic E. coli in epithelial cells of the small intestine of calves **(Moon et al., 1977)**. Shiga toxin producing E. coli (STEC) are associated with dysentery in calves. They secret two different Shiga toxin: Stx1 and Stx2. Similary; zoonotic E. *coli* O157:H7 it secretes shiga toxins and termed EHEC. They are associated with hemorragic colitis (HC) and hemolytic uremic syndrome (HUS) Other investegation previously isolate E. *coli* O157:H7 strains from fecal samples of calves **(Dastmalchi and Ayremlou, 2012)**.

The present study was carried out to investigate the prevalence of E. *coli* as causative agent of neonatal calf diarrhea in newly born calves in some localites of Damietta governorate, Egypt during November 2015 to March 2016 as well as detection of some virulence genes of the isolated strains.

Materials and Methods

A- Samples collection:

A total of 100 fecal samples were collected from diarrheatic neonatal calves (local, non treated neonatal calves). These were randomaly selected from field cases in different localities at Damietta governorate, Egypt during the period from November 2015 to March 2016 (table 1). Cases were classified into three groups of age: 1st group of age (2-7 days), 2nd group of age (8-14 days), 3rd group of age (15-21 days). Samples were collected in sterile plastic containers, kept in ice box and transported as soon as possible to the laboratory. Samples were subjected to the following bacteriological examination.

Table (1): Number of collected fecal samples from diarrheic calves according the localities of collection and the calves's age in Damietta governorate.

Locality	Age			Total number of Examined calves (No. of collected samples)
	1st group	2nd group	3rd group	
Kafersad	10	9	7	26
Farskour	10	8	6	24
Cinania	7	5	3	15
Zarka	7	4	5	16
KaferElbatekh	9	7	3	19
Total	43	33	24	100

1st group: Calves aged 2-7 days. 2nd group: Calves aged 8-14 days. 3rd group: Calves aged 15-21 days.

1- Isolation and identification of E. *coli*

All samples were inoculated into nutrient broth for 24h at 37°C aerobically. After that, swabs were streaked onto MacConkey agar and Blood agar. Lactose fermented colonies were randomly selected from each isolate and confirmed to be E. *coli* by standrad biochemical tests. Colonies were subculture onto Eosin methylene blue agar for 24h at 37°C for aerobically characteristic metalic sheen colonies of E. *coli*. cultivation and identification where carried out according to **Quinn *et al*., (2002)**.

2- Serodiagnosis of *E. coli*:

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.

3.1- PCR assay:

200 µl of an overnight of purified bacterial culture of isolated E. *coli* strains was mixed with 800 µl of distilled water and boiled for 10 min **(Shah et al., 2009)**. The resulting solution was centrifuged and the supernatant used as the DNA template. Amplification reaction **(Fagan et al., 1999)** were carried out with 30 ng of boiled bacterial suspensions, 10 mM Tris-Hcl (PH 8.4), 0.2 mM concentrations of each 29 deoxynucleoside 59- triphosphate. 3 mM $MgCl_2$, 2 mM concentration of each primers and 4U of AmpliTaq DNA polymerase (Perkin-Elmer).

Distilled water was added to bring the final volume to 50 µl. After PCR reactions, the reaction products were subjected to electrophoresis in a 2 % agarose gel, stained with ethidium bromide and visualized under UV light.

3.2- PCR procedure

Application of PCR for identification of shiga toxins (Stx1 and Stx2), intimin (eaeA) and hylA genes of E. *coli* was performed essentially by using primers (Pharmacia Biotech) as shown in table 2. Briefly, the thermal profile of PCR consisted of initial denaturation at 95°C for 3 min, followed by 35 cycles each of denaturation at 95°C for 20 s, annealing at 58°C for 40 s and extension at 72°C for 90s.The amplified PCR product was electrophoresed on a 2% agarose gel in tris- acetate –EDTA buffer. A100-bp DNA ladder (Qiagen, Germany, GmbH) was used to determined the molecular weight of amplified frament.

Table(2): Primers used for PCR screening.

Primer	Oligonucleotide sequence (5′ → 3′)	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**		
eaeA (F)	**5′** GTGGCGAATACTGGCGAGACT **′3**	890	**Mazaheri et al. (2014)**
eaeA (R)	**5′** CCCCATTCTTTTTCACCGTCG **′3**		
hylA (F)	**5′** ACGATGTGGTTTATTCTGGA **′3**	165	**Fratamico et al. (1995)**
hylA (R)	**5′** CTTCACGTGACCATACATAT **′3**		

5- The Antibiogram: Susceptibility tests were performed using the disc diffusion method on Mueller-Hinton agar (Oxoid) according to **Quinn et al. (1994) and Winn et al. (2006)**.

Results and Discussion

This study aimed to determine the incidence of diarrheagenic E. *coli* in fecal samples of calves collected from different localities covering Damietta governorate, Egypt during November 2015 to March 2016. E. *coli* was isolated from twenty seven samples (27%). The highest rates of diarrhea was observed in calves aged 2-7 days (43%), followed by age 8-14 days (33%) , then calves age 15-21 days (24%) as shown in table (1). Really similar with **Lorino *et al*., (2005), El-Naker *et al*.,(2007), Paul et al., (2010)and lorenz *et al.*, (2011)**, who recorded that the incidence rate of diarrhea in neonatal period was high in the first days of calves age.

Calf age significantly affected the prevalence of E. *coli* thus first week of life is the main age for occurrence of diarrhea. These Finding supported by those previously recorded **(Bendali ,*et al*., 1999; Radostits *et al*., (2007) ; Wieler *et al*., (2007) and Guler ,*et al*., 2008)** who found the suscceptibility was higher during the first week of life to E. coli. Age, vaccination of the pregnant dams with Scour Guard 3, colstrum feeding practice,

rotavirus infection and administration of vitamin E/Selenium to pregnant dams were found to be affect significantly the prevalence of ETEC in diarrheic calves **(Younis *et al.*, 2009)**.

Diarrhea is a major cause of mortality in calves under one month old. Diarrheagenic E. *coli* are recognized as the major cause of neonatal calf diarrhea (NCD) with sever lethal outcome and major damage to the livestock industry worldwide. consequently, high mortality rate in calves under 3 weeks-old and up to 3 month old has been reported **(Windeyer *et al.*, 2014)**. Beside, significant loss of other neonatal animal species such as lambs, suckling piglet and foals due to diarrhea has been reported **(Cho and Yoon, 2014)**.

Results of table (3) revealed that the incidence of ETEC was 22.22% (6 of 27 belonging to serotypes O128:H2, strains from these serotypes ETEC strains). Similar result was reported previously by **Bendali *et al.*, (1999) and Acha *et al.*, (2004)** who reported a prevalence rate of 20.3% and 16% respectively. Moreover, in India, PCR could identify higher prevalence (20%) in buffalo calves **Singh *et al.*, (2007)**. On the contrary, lower prevalence (4.7%, 3.86% and 5.8%) of ETEC was recorded by **Akam *et al.* (2004), Kanwar *et al.* (2007) and Oliveira Filho *et al.* (2007)**. In a study carried out in Egypt and Israel, higher prevalence (23%) was also recorded **Perka *et al.*, (2000)**. However, **Younis *et al.*, (2009)** recorded that The prevalence of ETEC was 10.63%. The difference in the prevalence from these variations may be due to variations in number of the sample, region, managment condition and hygienic measures. Diarrhea caused by ETEC is considered the main infectious disease of newborn calves **Martin *et al.*, (2003)**.

Several approaches should be considered for future control such as vaccination of pregnant dams, and fluid therapy **Younis *et al.*, (2009)**. The antibody produced by the cow in response to natural immunization or vaccination are transmitted to calf via the colostrum **Radstits *et al.*, (2007) and Morshedi et al., (2010).** The susceptibility of neonatal calf diarrhea was reported to be from the first 3-4 dayes of life or more than 1week-old. Enterotoxin reduce the intestinal absorption and increase the fluid and electrolyte secretion of small intestinal epithelial cells prediposing to diarrhea **Nagy and Fekete, (2005)**. ETEC are considered the major pathogen-causing diarrhea in new born calves **Younis *et al.*, (2009)**.

In older animals there is a tendency of infection to localized itself in the joints of survivors. Lesions include enlarged, haemorrhagic spleens, and accumulation of syuovial fluid and sometimes pus in affected joints **Blood *et al.*, (1968)**. E. coli produces septicemia and diarrhea in a wide range of hosts including man, avian and animals such

as cattle, piglets, kids, foals, lambs and buffaloas. Pathogenicity of E. coli strains are due to the presence of one or more virulence factors including invasiveness factors like invasins, heat labile, heat stable enterotoxins, verotoxins and colonization factors or adhesins **Smith, (1976)**.

The results of photo. 1 and table (3&4) showed that the shiga toxin virulence genes were the most prevalent in all E. *coli* isolates. In the present study, out of the twenty seven E. coli isolates 24 were positive for Stx1 (88.89%) belonging to serotypes O128:H2, O111:H2, O26:H11, O45:H7, and O91:H21, strains from these serotypes are ETEC, EHEC, EHEC, EPEC and EPEC respectively and 14 were positive for Stx2 (51.85%) belonging to serotypes O111:H2, O26:H11 and O91:H21, strains from these serotypes are EHEC, EHEC and EPEC respectively. Stx1 virulent gene was the predominant virulence gene (88.89%). These resultes agree with **Hashish *et al*., (2016)** who recorded that more than 86.67% of the positive isolates of E. *coli* contained genes for shiga toxin (Stx1) which would suggeste the emergence of a new phenotype causing diarrhea in calves in Egypt. Morever high frequency of E. *coli* isolates carrying the Stx1 gene was observed **Wani *et al*., (2007)**. However, high prevalence of Stx2 gene had been reported by **Irino *et al*., (2005)**. On the not detected , While the Stx2 gene was found to be 93.1% **Karmali *et al*., (2003)**. It is Known that the Stx2 toxins resulted in HUS more frequently than Stx1. Shiga toxin increase the risk for zoonotic STEC infection.

Mixed E. coli infection is strongly associated with the reported cases of diarrhea due to the presence of several virulence genes in some isolates **Hashish *et al*., (2016)**. The shiga toxin produced by E. *coli* strains (STEC) is similar to Shiga-toxin produced Shigella dysenteriae type 1. E. coli producing Stx1 and or Stx2 is a cause of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) in humans. Most cases of HC and HUS are caused by ingestion of foods and drinks contaminated with faeces from cattle, especially ground beef, undercooked hamburgers, salami or other foods like raw milk or homemade cheese from raw milk or when faecal contamination enters the water supply. Less frequent modes of transmission of the infection are cattle to person or person to person direct contact **Shimizu et al., (1987)**.

hylA genes was detected in 16 (59.26%) out of the 27 E. *coli* strains, were (photo. 1 and Table 3 & 4). 13 (48.15%) of hylA strains were also Shiga toxins producing strains belonged to serotypes O111:H2 and O26:H11, strains from these serotypes are EHEC and 3 (11.11%) of hlyA producing strains were eaeA strains belonged to serotype O124 (EIEC) . There was a high association between EHEC and hlyA producing strains. The most important animal species in term of human infection by

EHEC is cattle and high rates of colonization by Stx-positive E. *coli* have been found in bovine herds in many countries **Nataro and Kaper, (1998)**. From our findings suggests that cattle is an important resvoir of STEC and EHEC in Damietta.

EaeA strains not associated with ETEC and EPEC (Table 3).There is a previous report **Aidar,(2000)** of eaeA strains isolated from bovin in Brazil, but these strains were not positive for any enterotoxin or cytotoxin. The eaeA gene that is involved in the attaching and effacing adherence phenotype.

The results recoded in Table (5) revealed the isolated E. coli were highly sensitive to Ciprofloxacin 5 mcg and Cefotaxime 30 mcg at a rate 23/27 (85.19%) and 22/27 (77.78%), respectively. Moderately sensitive to Gentamycin 30mcg at rate 16/27 (59.25%) while these were susceptible to Amoxycillin/Clavulanic acid 20/10mcg, Neomycin 30mcg, Ampicillin/Sulbactam 10/10mcg and Trimethoprim/Sulphamethoxazole 25mcg at rate 10/27 (37.04%), 8/27 (29.63%), 7/27 (25.93%) and 4/27 (14.81%), respectively. Results recorded in Table (5) revealed also that all isolated of E. *coli* resistant to Nalidixic acid and Erythromycin 15mcg.

Conclusion and recommendations

The study indicated diarrheagenic E. coli have role in causing diarrhea in young calves and produce several toxins and colonization factors some of which may be involved in human diseases STEC (Stx1; 88.89%, Stx2; 51.85%). It indicated that the highest rates of diarrhea was obeserved in calves age 2-7 days. Such studies will provide important epidemiological data about this disease. The obtained results revealed that the isolated E. coli strains susceptible to antimicrobial drugs as well as it more resistence to other drugs. Therefore, to prevent and control of E. coli in calves, the following suggestions should be applied:

It is important to isolate any calves experiencing scours and again use strict hygiene principles to keep from spreading any pathogens to the healthy calves. Reduce the load of pathogens in the calf farms through improving host immunity to reduce infection and shedding, attention to cleaning and disinfection of materials within the calf's farm and pay in attention to separate of young calves from each other and from older animals. Care takers should understand what the hazards are and why protocols for cleaning and disinfection are needed. Reduction of pathogen load in the calf environment will reduce the burden of illness on the calves, as well as reduce the potential for pathogens to reach the human population. These results nearly agreed with **Nazir (2004) and Paul et al., (2010**) reported that the isolated E. *coli* was highly sensitive to Enrofloxacin and

Ciprofloxacin, moderately sensitive to Ceflaxin and Amoxicillin, and resistant to Nalidixic acid and Erthromycin.

Table (3): Correlation between serotype and virulence genes of isolated E. *coli* strains from diarrhiec calves (N: 27).

Identified bacterium	Serodiagnosis	No. of strain	%*	Strain characterization	Virulence genes				
					Stx1	Stx2	eaeA	hylA	
E. coli	O128 : H2	6	22.22	ETEC	+	-	-	-	
E. coli	O111 : H2	2	48.15	EHEC	+	+	+	+	
E. coli	O111 : H2	8		EHEC	+	+	-	+	
E. coli	O26 : H11	3		EHEC	+	+	+	+	
E. coli	O91 : H21	1	18.52	EPEC	+	+	-	-	
E. coli	O45 : H7	4		EPEC	+	-	-	-	
E. coli	O124	3	11.11	EIEC	-	-	+	+	
Total	-	27	100	-	24	14	8	16	

*: Calculated according to the total No. of isolates.

Table (4): Detection of Escherichia coli virulence genes in the isolated strains (27) from fecal samples of diarrhiec newborn calves.

Escherichia coli virulence genes	**Positive samples**	
	%*	**No. of genes**
Stx1	88.89	24
Stx2	51.85	14
eaeA	29.63	8
hylA	59.26	16

*: Calculated according to the total No. of isolates.

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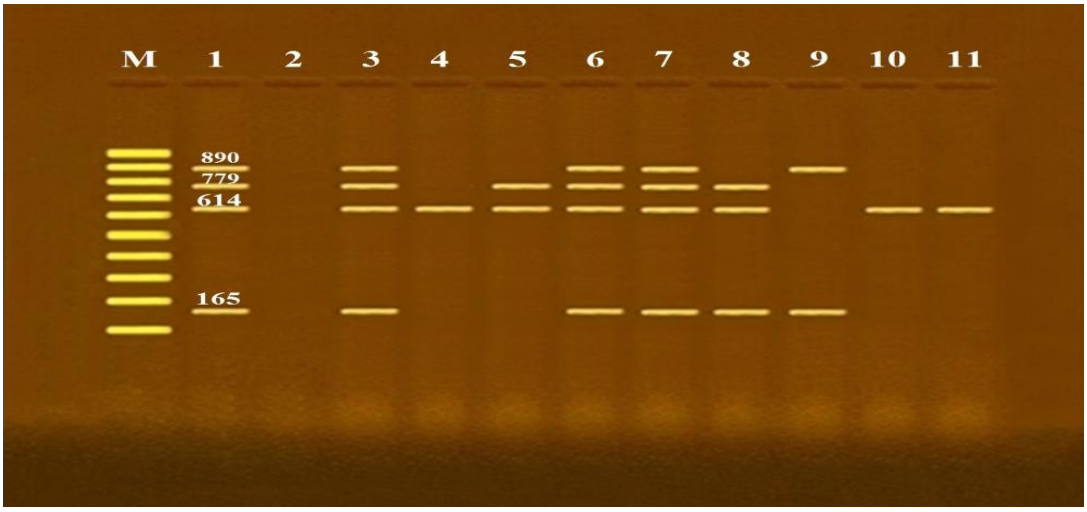
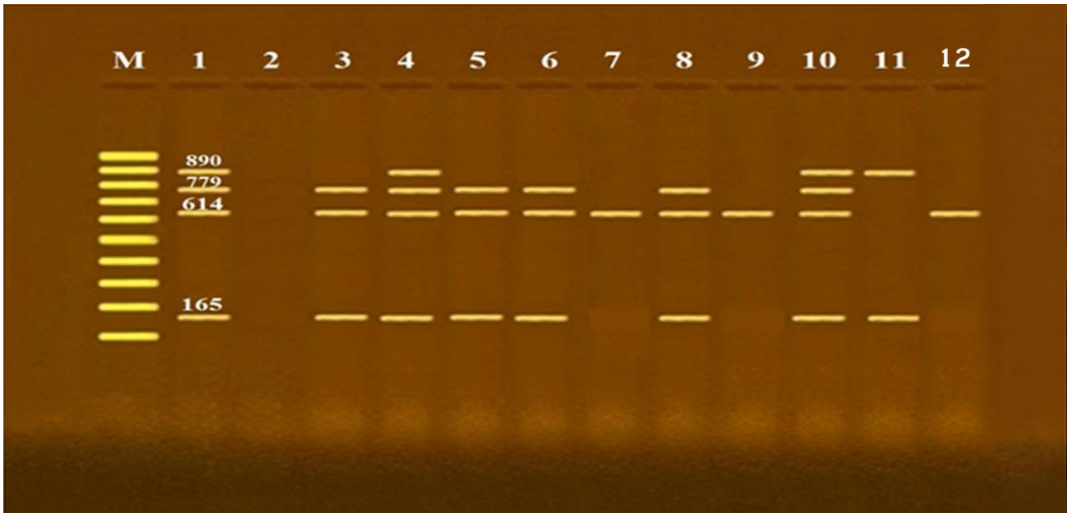
Table (5): In Vitro susceptibility pattern of the isolated Escherichia coli against different antibiotics.

Antibiotics	Isolated Escherichia coli "n.=27"
Amoxycillin/ Clavulanic acid 20/10mcg	10/27 (37.04%)
Ampicillin/ Sulbactam 10/10mcg	7/27 (25.93%)
Ciprofloxacin 5mcg	23/27 (85.19%)
Trimethoprim/Sulphamethoxazole (1.25/23.75) 25mcg	4/27 (14.81%)
Nalidixic acid	-
Cefotaxime 30mcg	22/27 (77.78%)
Gentamycin 30mcg	16/27 (59.25%)
Neomycin 30mcg	8/27 (29.63%)
Erythromycin 15mcg	-

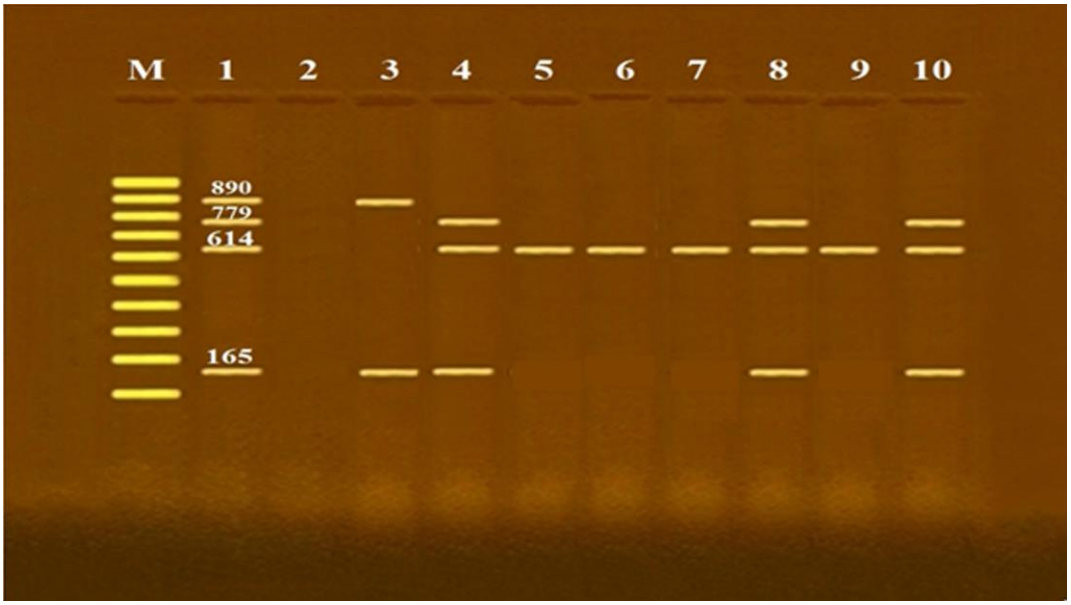
A



B



C



Photograph (1): Agarose gel electrophoresis of multiplex PCR of (614 bp), stx2 (779 bp), eaeA (890 bp) and hlyA (165 bp) genes for characterization of *nteropathogenic E. coli*. *1= positive control, 2= negative control and M= DNA ladder

References

Acha S.J.; Kuhn I.; Jonsson P.; Mbazima G.; Katouli M. and Mollby R., (2004): Studies on calf diarrhea in Mozambique: prevalence of bacterial pathogens. Acta Veterinari Scandnavia 45: 27–36.

Aidar L.; Penteado A.S.; Trabulsi L.R.; Blanco, J.E.; Blanco M.; Blanco J. and Pestana de Castro A.F. (2000) Subtypes of intimin among non-toxigenic Escherichia coli from diarrheic calves in Brazil. Can. J. Vet. Res., 64(1): 15-20.

Akam A.; Khelef D.; Kaidi R.; Othmani A.; Lafri M.; Tali-Maamar H.; Rahal K.; Tahrat N.; Chirila, F., Cozma, V. and Abdul-Hussain, M.S., (2004): Frequency of Cryptosporidium parvum, Escherichia coli K99 and Salmonella spp. isolated from healthy and unhealthy calves in six breeding farms from Mitidja, Algeria (Preliminary results). Revista Scientia Parasitologica 5, 13–21.

Bendali, F.; Bichet, H.; Schelcher F. and Sanaa, M. (1999): Pattern of diarrhea in newborn beef calves in south-west France. Veterinary Research 30: 61–74.

Blood DC.; Henderson A.J.; Radosintins, R.; Arundel J.H. and Gay C.C. (1968): 5th end. A text book of the diseases of cattle, sheep, pigs and hourses, PP. 467.

Cho Y.I. and Yoon K.J. (2014) An overview of calf diarrhea-infectious etiology, diagnosis and intervention. J. Vet. Sci., 15: 1-17.

Cruickshank R.; Dguid J.P. and Swain R.H.A. (1975): 12th ed. Medical Microbiology, Vol. 11 Churchill, Livingstone, Edinburg, london and New York.

Dhanashree, B. and Mallya, S. (2008): Detection of shiga-toxigenic *Escherichia coli* in diarrhoeagenic stool and meat samples in Mangalore, India. Indian J. Med. Res., 128: 271-277.

Dastmalchi S.H. and Ayremlou N. (2012) Characterization of Shiga toxin-producing Echerichia coli (STEC) in feces of healthy and diarrheic calves in Urmia region, Iran J. Microbiol., 4: 63-69.

De Rycke J.; Bernard S.; Laporte J.; Naciri M.; Popoff M.R. and Rodolakis M.R. (1986) Prevalence of various enteropathogens in the feces of diarrheic and healthy calves. Ann. Rech. Vét. 17, 159-168.

El-Naker, Y., El-Sawalhy, A., Youssef, M. and Zeidan, S. (2007): Some studies on neonatal calf diarrhea in Egypt, Part 1: Causative agents and some epidemiological aspects Bull. Amin. Hlth. Prod. Afr., 56: 161-190.

Elwell L.P. (1980) Plasmid-mediated factors associated with virulence of bacteria to animals. Ann. Rev. Microbiol., 34: 465-496.

Fagan, P.; Hornitzky, M.; Bettelheim, K. and Djordjevic, S. (1999):

Detection of Shiga-Like Toxin (stx1 and stx2), Intimin (eaeA), and Enterohemorrhagic *Escherichia coli* (EHEC) Hemolysin (EHEC hlyA) Genes in Animal Feces by Multiplex PCR. Appl. Environ. Microbiol., 65 (2): 868–872.

Firehammer B.D. and Myers L.L. (1981) Campylobacter fetus subsp. jejuni: its possible significance in enteric disease of calves and lambs. Am. J. Vet. Res. 42, 918-922.

Foster D.H. and Smith G.W. (2009) Pathophsiology of diarrhea in calves. Vet Clin. North Am. Food Anim. Pract., 25: 13-36.

Francis D.H.; Allen S.D. and White R.D. (1989) Influence of bovine intestinal fluid on the expression of K99 pili by Escherichia coli. Am. J. Vet. Res., 50: 822-826.

Franck S.M.; Bosworth B.T. and Moon H.W. (1998): Multiplex PCR forenterotoxigenic, attaching and effacing, and Shiga toxin-producing *Escherichia coli* strains from calves. J Clin Microbiol, 36: 1795-1797.

Fratamico, P.; Sackitey, S.; Wiedmann, M. and Deng, M. (1995): Detectio of *Escherichia coli* O157:H7 by multiplex PCR. J. Clin. Microbiol. 33: 2188– 2191.

Guinee P.A.M.; Agterberg C.M. and Jansen W.H. (1972) Escherichia coli O antigen typing by means of a mechanized microtechnique. Appl. Microbiol., 24: 127-131.

Guler L.; Gunduz, K. and Ok, U. (2008): Virulence factors and antimicrobial susceptibility of Escherichia coli isolated from calves in Turkey. Zoonoses and Public Health 55: 249–257.

Hashish E.A.; El Damaty H.M.; Tartor Y.H and Abdelaal A.M. (2016): Epidemiological Study of diarrheagenic *Escherichia coli* virulence genes in newborn calves. Pak. Vet. J., 2016, 36(1): 54-58.

Irino K.; Kato M.A.; Vaz T.M.; Ramos I.I.; Souza M.A. and Cruz A..S. (2005): Serotypes and virulence markers of shiga toxin-producing Escherichia coli (STEC) isolated from dairy cattle in Sao State Brazil Vet. Microbiol., 105: 29-36.

Kanwar, R.; Randhir, S.; Filia, G.; Arora A. and Sandhu, K.S. (2007): factors of Escherichia coli and Salmonella spp. isolated from diarrhoeic calves and their antibiogram pattern. Indian Journal of Animal Sciences 77: 1069–1073.

Karmali M.A.; Mascarenhas M.; Shen S.H.; Ziebell K. and Johnson S. (2003): Association of genomic O island 122 of Escherichia coli EDL 933 with verocytotoxin-producing Escherichia coliserotypes that are linked to epidermic and or serious disease. J. Clin. Microbiol., 41: 4930-4940.

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.

Lorenz, I., Fagan, J. and More, S.J. (2011): Calf health from birth to weaning. Managment of diarrhea in preweaned calves. Ir. Vet. J. 64(1): 9.

Lorino, T., Daudin, J.J., Robin, S. and Sanaa, M. (2005): Factors associated with time to neonatal diarrhoea in French beef calves. Prev Vet Med; 68: 91-102.

Martin M.J.; Martin-Sosa S.; Alonso J.M. and Hueso P. (2003): Enterotoxigenic Escherichia coli strains bind bovine milk gangliosides in a ceramide dependent process. Lipids 38: 761–768.

Mazaheri, S.; Ahrabi, S. and Aslani, M. (2014): Shiga Toxin-Producing *Escherichia Coli* Isolated From Lettuce Samples in Tehran, Iran. Jundishapur J. Microbiol., 7 (11): 1-6.

Mehmet C.; Banur B.; Timur G. and Hakki E. (2001) Prevalence of Rotaviruses, Escherichia coli K99 and O157: H7 in healthy dairy cattle herds in Van, Turky. Turk. J. Vet. Anim. Sci., 25: 191.

Moon H.W.; Nagy B.; Isaacson R.E. and Qrskov I. (1977) Occurrence of K99 antigen on Escherichia coli isolated from pigs and colonization of pig ileum by K99 enterotoxigenic E.coli from calves and pigs. Infect. Immun., 15: 614-620.

Morshedi A.; Rabbani M.; Zahraei SalehiT.; Rezazadeh, F. and Taghipoor Bazargani T. (2010) Evaluation of antibodies levels against rotavirus and coronavirus in the colostrum of non vaccinated cows in southern Tehran Iran. Int. J. Vet. Res., 4(4): 217-219.

Myers L.L.; Radostits O.M.; Gay C.C.; Blood C.D. and Hinchcliff K.W. (2003) Veterinary Mededcine: a textbook of the diseases of cattle, Sheep, pigs, goats and horses. 10th Edt. Baillier Tindal, England.

Nazir K.H. (2004) Molecular base of diverrsified E.coli isolates potentiating antibiotic resistant pattern and compromising epidemiology. M.S. Thesis, Department Microbiology and Hygiene, Faculty and Veterinary Science, Bau, Mymensingh, p. 43.

Nagy B. and Fekete P.Z. (2005) Entertoxigenic Escherichia coli in veterinary medicine. Int. J. Med. Microbiol., 295: 443-454.

Nataro P.J. and Kaper J.B (1998) Diarrheagenic Esherichia coli. Clin. Microbiol. Rev., 11: 142-201.

Oliveira Filho J.P.; Silva D.P.G.; Pacheco M.D.; Mascarini L.M.; Ribeiro M.; Alfieri A.A.; Alfieri A.F.; Stipp D.T.; Barros B.J.P. and Borges, A.S. (2007): Diarrhea in Nelore calves: clinical and etiologic study. PesquisaVeterinaria Brasileira. Colegio 27:419–424.

Ozkan C.; Altug N. Yuksek N.; Kaya A. and Akgul Y. (2011) Assessment of electrocardiographic findings, serum nitric oxide, cardiac troponins and some enzymes

in calves with hyperkaliemia related to neonatal diarrhoea. Revue Med. Vet., 162: 171-176.

Perka K.; Moussa A.A.; Tromp A.M.; Reda I.M.; Refai M.; Friedman A.; Farid A.F.; Gallily O.; Salah S.M. and Saif, L. (2000): Neonatal diarrheal disease of dairy cattle in Egypt and Israel. Israel Journal of Veterinary Medicine 55, 13–18.

Paul S.K.; Khan M.S. R.; Rashid M.A.; Hassan J. and Mahmud S.M.S. (2010): Isolation and characterization of Escherichia coli from buffalo calves in some selected areas of Bangladesh. Bangl. J. Vet. Med., 8(1): 23-26.

Prescott J.F. and Munroe D.L. (1982) Campylobacter jejuni enteritis in man and domestic animals. J. Am. Vet. 42, 164-165.

Quinn P.J.; Carter M.S.; Markey B. and Carter G.R. (1994): Clinical Veterinary Microbiology. Mosby Year Book, Europe Limited.

Quinn P.J.; Markey B.K.; Carter M.E.; Donnelly W.J.C.; Leonard F.C. and Maguire D. (2002): Veterinary microbiology and microbial disease. Blackwell; P. 133-16.

Radostits O.M.; Gay C.C.; Blood C.D. and Hinchcliff K.W. (2003) Veterinary Mededcine: a textbook of the diseases of cattle, Sheep, pigs, goats and horses. 10th Edt. Baillier Tindal, England.

Radostits O.M.; Gay C.C.; Hinchcliff K.W. and Constable, P.D.(2007) Veterinary medicine, 10th edition. WB Saunders Co., London, UK. pp: 851-875 and 1286-1305.

Razzaque, M.A.; Abbas S.; Al-Mutawa T. and Bedair M. (2009) Mortality of pre-weaned calves inKuwait's dairy herds, its causes and impact of interventions. Int. Vet. Med. 5: 1-12.

Sandgrass D.R. and Browing G. (1993) Enteric vaccines for farm animals and horses. In vaccines for veterinary application oxford bulterworth-Heinemann.59-81.

Shah, D., Shringi, S., Besser, T. and Call, D. (2009): Molecular detection of foodborne pathogens, Boca Raton: CRC Press, In Liu, D. (Ed). Taylor & Francis group, Florida, USA, Pp. 369-389.

Shimizu M.; Sakano T.; Yamamoto J. and Kitajima K. (1987) Incidence and some characteristics of fimbriae FY and 31A of ESchEerchia coli isolates from calves with diarrhea in japan. Microbiol. Immunol., 31: 417-426.

Smith H.W. (1976) The sensitivity of strains of Bacterium coli isolated from cases of calf scours to certain chemotherapeutic agent. Veterinary Records, 66:43.

Singh, M., Singh, R., Arora, A.K.and Sandhu, K.S., (2007): Molecular characterization of Escherichia coli isolated from diarrhoeic neonatal calves. Indian Journal of Animal Sciences 77: 1063–1068.

Tajik J.; Nazifi S.; Naghib, S.M. and Ghasrodasht, A.R. (2012) Comparison of electrocardiographic parameters and serum electrolytes and microelements between signle infection of rotavirus and coronavirus and concurrent infectionof cryptosporidium parvum with rotavirus and coronavirus in diarrheic dairy calves. Comp. Clin. Pathol., 21: 241-244.

Wani S.A.; Hussain I.; Nabi A.; Fayaz I. and Nishikawa Y. (2007): variants of eae and Stx genes of topical enteropathogenic Escherichia coli and non-O157 shiga toxin-producing EScherichia coli from calves. Lett. Appl. Microbiol., 45: 610-615.

Windeyer M.C.; Leslie K.E.; Godden S.M.; Hodgins D.C.; Lissemore K.D. and LeBlanc S.J. (2014) Factors associated with morbidiry, mortality and growth of dairy heifer calves up to 3 month of age. Prev. Vet. Med., 113: 231-240.

Winn, W.; Allen, S.; Janda, W.; Koneman, E.; Procop, G.; Schreckenberger, P. and Woods, G. (2006): Koneman'a color Atlas and Textbook of Diagnostic Microbiology. 6th Ed., Lippincott William of Wilkins: London and New York.

Wieler, L.H., Sobjinski, G., Schlapp, T., Failing, K., Weiss, R., Menge, C., and Baljer,G. (2007): Longitudinal prevalence study of diarrheagenic Escherichia coli in dairy calves. Berl Munch Tierarztl Wochenschr 120, 296–306.

Yong-il Cho (2012) Ecology of calf diarrhea in cow-calf operations Ph. D. Thesis (Microbiology) Iowa State University, Ames, Iowa.

Younis EE; Ahmed AM.; El-Khodery SA, Osman SA and El-Naker YF. (2009) Molecular screening and risk factors of entertoxigenic Escherichia coli and Salmonella spp. in diarrheic neonatal calves in Egypt. Res. Vet. Sci., 87: 373-37