Journal of Animal Science/vol.34, No.2; 2024/pp 87-99

Study on antibiotic resistance pathern of *E. coli* in diarrheic calves of Tabriz dairy farms

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Received: (April 8, 2023) Accepted: (December 16, 2023)
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Journal of Animal Science/vol.34 No.2/ 2024/pp 87-99
https://animalscience.tabrizu.ac.ir

DOI: 10.22034/AS.2023.56083.1703

Introduction: Diarrhea is a very important disorder in calves, especially in the first days after birth, due to mortality and economic losses. Among infectious agents, pathogenic strains of *Escherichia coli* (*E. coli*) have been known as the most important causes of diarrhea in calves. Therefore, phenotypic and genotypic studies on *E. coli* strains that cause diarrhea in calves can be helpful for understanding their prevelance control methods.

Material and method: In this study, rectal swabs were collected from 1 to 30-day-old calves with diarrhea during 2021. The antibiotic-resistant pattern of the isolates was determined by the Kirby-Bauer disk diffusion susceptibility test. The prevalence of the pathogenic genes (bundle-forming pilus (*bfp*) and enteropathogenic attachment epithelial cells (*eae*) for enteropathogenic *E. coli* (EPEC) strains and verotoxigenic *E. coli* (VTEC), Shiga toxin (*stx*1 and *stx*2) strains was investigated using PCR.

Results and discussion: Out of 105 samples, 81 isolates (85.05%) of *E. coli* were identified. The resistance of the isolates was observed for penicillin (56.84%), tetracycline (38.3%), sulfamethoxazole-trimethoprim (38.3%), gentamicin (29.6%), ceftazidime (29.2%), colistin (25.9%), imipenem (23.5%), ceftriaxone (22.21%), nalidixic acid (22.2%), ciprofloxacin (14.8%) and ofloxacin (9.9%) were detected in the isolates. Furtheremore, 16% of the isolates had the *eae* gene and (24.7%) had both the *stx*1 and *stx*2 genes. In the present study, the infection rate of calves with diarrhea with VTEC strains was higher than EPEC strains. According to the results of the present study, the presence of antibiotic resistance in *E. coli* isolates indicates the high importance of this bacterium in terms of epidemiology and public health.

Key words: Calves, Diarrhea, *Colibacillosis*, Virulence genes, Kirby-Bauer disk diffusion method

Introduction

Diarrhea is a risky complication in calves and is economically irreparable due to the high treatment costs, low growth rates and increased mortality rates of calves. This disease is caused by the insufficient absorption or increased secretion of fluids into the intestine. Identifying the infectious

and non-infectious agents responsible for diarrhea is crucial and can help to understand how to control this disease (Chandran *et al*. 2013). The most important bacterial agents causing diarrhea in calves are *Escherichia coli* (*E. coli*), Salmonella *spp*. and *Clostridium perfringens* type C and D (Büyükcangaz 2019).

E. coli is a motile and facultative anaerobic bacterium that grows easily on conventional culture media. It is a part of the natural flora of the intestinal tract of animals, but some of its strains cause disease due to pathogenic factors. Diarrhea is one of the most important symptoms of pathogenic strains (Byrne *et al*. 2018). This bacterium is known to be the most important cause of diarrhea in calves and lambs in the first days of life. Enteropathogenic *E. coli* (EPEC), being the main pathogenic agents in developing countries, are the causative agents of diarrhea in calves. These strains do not produce toxins and their virulence is represented in the form of lesions in the villi of the intestinal epithelial cells. The bacterium contacts the apical part of the cytoplasmic membrane and causes the local destruction of the toothbrush tip and the deflection of the apical membrane of enterocytes (Cengiz 2020). This is followed by cup-shaped depressions at the tip of the toothbrush and the deviation of the apical membrane of enterocytes. These lesions reduce the absorption capacity of the mucosa, and disturb the balance of water and electrolytes, resulting in diarrhea (Büyükcangaz *et al*. 2019). These strains have an EPEC adherence factor that is encoded by a 60 mDa plasmid. Strains of EPEC that do not have this factor, have fimbriae with a diameter of 7 nanometers called Bundle forming pilus (bfp) which is encoded by the *bfp* gene. Another factor that contributes to the pathogenesis of these strains is an outer membrane protein encoded by the *eae* gene. These two genes (*eae* and *bfp*), are usually used to identify EPEC strains (Askari *et al*. 2010).

Shiga toxin-producing *E. coli* produces characteristic cytotoxins called Shiga toxins. The three different terms which this group is referred to as, are Shiga toxin-producing *E. coli* (STEC), verotoxin-producing *E. coli* (VTEC) and enterohemorrhagic *E. coli* (EHEC). All EHEC strains produce a Shigella-like toxin, encoded by the *stx*1 and *stx*2 genes. They also contain a 97-kDa protein called intimin, encoded by the *eae*A gene (Bibbal *et al*.2014).

Nowadays, due to the excessive and improper use of antimicrobial drugs, especially in breeding animals (as therapeutic and growth stimulants), increasing antimicrobial resistance is known as a serious public health concern. Members of the Enterobacteriaceae family are typically resistant to antibiotics due to numerous inherent and acquired mechanisms (Aslantaş *et al*. 2006). Calf diarrhea causes economic losses both directly (via calf death and medical costs) and indirectly (reduced livestock growth after disease). Among the infectious agents, *E. coli* strains are the most important causes of diarrhea in calves, especially in the first days of life. Many studies have been performed about this infection, which shows the importance of the issue. However, no study has been done in the field of prevalence and molecular identification of pathogenic strains of EPEC and VTEC, their antibiotic resistance in calves with diarrhea in Iran. Since an accurate understanding of the prevalence of a disease indicates the importance of that disease and can help officials in adopting appropriate measures to prevent and control the disease at different levels, in the present study, the causative agents of diarrhea caused by *E. coli* pathogens (EPEC, VTEC) in calves were identified by using PCR.

Materials and methods

Sample collection

The sample size was calculated to provide a sufficient number of *E. coli* positive samples (to evaluate virulence genes in positive cases)

in calves with diarrhea. Therefore, the sample size was determined using the following formula:

$$n = \frac{z^2 \times \hat{p}(1-\hat{p})}{\varepsilon^2}$$

n= Sample size

z= The confidence ratio of 95%

p= Estimated prevalence of 67%

ε= Estimated error of 9%

For this study, 105 swap samples were collected from 1 to 30-day-old calves with diarrhea from industrial, semi-industrial, and traditional farms around Tabriz city, Iran. Samples were collected from the rectum using sterile swabs and put into tubes containing sterile saline normal solution. The samples were immediately transferred in an ice box to the bacteriological laboratory of the Faculty of Veterinary Medicine, University of Tabriz. They were kept in a refrigerator at 4 °C before culture (Büyükcangaz *et al*. 2019).

Identification of *E. coli*

Briefly, samples were cultured on the surfaces of MacConkey agar and EMB (Eosin methylene blue) agar (Merck, Darmstadt, Germany) plates and incubated for 24 h at 37 ° C. Colonies with a metallic green sheen in EMB agar and pink colonies in McConkey medium were used for biochemical tests (Biyne 2015). Three to five presumptive colonies were removed from the EMB agar and inoculated into the tryptic soy agar (TSA) and incubated at 37 °C for 24 h. Confirmatory tests were performed while the colonies such as Methyl Red (MR), Voges-Proskauer (VP), Simmons Citrate agar, and Sulfide indole motility (SIM), oxidase, catalase, sugar fermentation and nitrate reduction. A microbial culture was also used for gram staining. The isolates were cultured in tubes containing Tryptic soy broth (TSB). After incubation at 37 ° C for 24 h, one or two drops of 20% sterile glycerol were added to it and then kept at -20 ° C until the next experiments (Barrow 1993).

Antibiogram test

The Kirby-Bauer disk diffusion method is widely used to determine antibiotic susceptibility patterns in most laboratories. The guidelines of the Clinical and Laboratory Standards Institute (CLSI) were used to perform the test Naves. First, the Müller Hinton agar was prepared and agar plates were incubated for 24 hours at 37 ° C to control possible contamination. Then, standard microbial suspension (10^6 CFU/ml) was prepared for the test. Bacterial-impregnated swabs were streaked on the surface of the Müller Hinton agar. Then, antibiotic discs including nalidixic acid (30 µg), ciprofloxacin (5 µg), penicillin (10 IU), imipenem (10 µg), sulfamethoxazole-trimethoprim (23.75 / 1.25 µg), gentamicin (10 µg), tetracycline (30 µg), ceftriaxone (30 µg), colistin (10 µg), and ofloxacin (5 µg) were placed on the bacterial culture. Finally, the plates were incubated at 35 °C, and the results were evaluated after 18 hours (Biyne 2015).

PCR assay

DNA extraction

The boiling method was used for DNA extraction. The isolates were cultured on nutrient agar. After incubation at 37 ° C for 24 h, fresh colonies were used for DNA extraction. Three to five colonies of each isolate were completely dissolved in 200 µL of sterile distilled water. The microtubes were placed in a bain-marie (100 ° C) for 10-15 min. Then, the tubes were centrifuged at 14,000 rpm for 10 min. The supernatant was transferred to another sterile tube and used for PCR. To ensure the accuracy of the method, the concentration of template DNA was measured using a nanodrop spectrophotometer in the wavelengths of 260 and 280 nm (Blanco *et al* 2004).

Preparation of primers

The primers designed in this study were also used in previous studies. However, to ensure the specific binding of the primers, the BLAST program was used in the NCBI gene bank. The selected primers were synthesized by Kimia Danesh Tajhiz Company (Iran). The stock primers were prepared in a volume of 100 µmol and kept at -20 ° C. Depending on the daily work, 10 µmol of forward and reverse primers were prepared and used (Kang *et al* 2010).

Table 1- Sequences of the primers used to identify the target genes in *E. coli* isolates from calves with diarrhea

Gene	Primer	Oligonucleotid sequence (5′→3′)	size	Reference
Eae	*eae*F	GGA ACG GCA GAG GTT AAT CTG CAG		
	*eae*R	GGC GCT CAT CAT AGT CTT TC	384	(Heuvelink *et al*. 1998)
bfp	*bfp*F	ACG CCC ACT TCT GAC ACC		
	*bfp*R	CGG ATA TCT AAA TCG CCC AG	900	(Blanco *et al*. 2004)
*stx*1	*stx*1F	ATA AAT CGC CAT TCG TTG ACT AC		(Büyükcangaz *et al*.2019)
	*stx*1R	AGA ACG CCC ACT GAG ATC ATC	180	
*stx*2	*stx*2F	TTA ACC ACA CCC CAC CGG GCA GT		(Büyükcangaz *et al*.2019)
	*stx*1F	ATA AAT CGC CAT TCG TTG ACT AC	255	

PCR procedure

PCR reaction was performed using a thermocycler (MWG AG Biotech Thermal Cycler, USA). The reaction contents for every 25 µL PCR consisted of 12.5 µL of of deionized water. 2 µl of sterile distilled water was used as the negative control. The PCR reaction was performed using the thermal program to amplify the target genes according to (table3). PCR products were subjected to electrophoresis using 1% was stained with Safe Red (Sinagen, Iran). (w/v) agarose gel for 45 minutes. The gel Ultraviolet transillumination (UV Tech, Canada) was used for the visualization of DNA master mix (containing dATP, dTTP, dGTP and dCTP), 2.5 µL of PCR buffer (10X), 2 mmoles of magnesium chloride, 2.5 µL of DNA polymerase enzyme, 1 µl of each of the Forward and Revers primers, 2 µl of template DNA and 8.5 µl. (Khakpour *et al* 2012).

Statistical analysis

Prevalence values were expressed as the percentages and the differences in the SPSS software, version 25.0, was performed for statistical analysis. The prevalence was analyzed by the Chi square test. Statistical significance was established when $P < 0.05$.

Table 2- Thermal program of PCR procedure for target genes in the *E. coli* isolates from calves with diarrhea

Gene	Initial denaturation	Denaturation	Annealing	Extention	Final extension
eae	96˚C for 5 min	95˚C for 20 s	58˚C for 40 s	72˚C for 1 min	72˚C for 10 min
bfp	96˚C for 5 min	95˚C for 20 s	56˚C for 40 s	72˚C for 1 min	72˚C for 10 min
*stx*1	96˚C for 5 min	95˚C for 20 s	55˚C for 40 s	72˚C for 1 min	72˚C for 10 min
*stx*2	96˚C for 5 min	95˚C for 20 s	57˚C for 40 s	72˚C for 1 min	72˚C for 10 min

Results and discussion

Out of the 105 rectal swab samples, 81 isolates of *E. coli* were identified using culture methods, Gram staining, and biochemical tests. Regarding the prevalence of enteropathogenic and verotoxigenic *E. coli* serotypes (EPEC and VTEC) in animals, several studies have been conducted in different regions of the world. The results of the present study showed that one of the main causes of diarrhea in calves in the region is related to *E. coli* strains. In a similar study conducted in Egypt by Reda Tarabes (2020), 95% of the samples were positive for the presence of *E. coli*. Also, in a study in the Alborz province of Iran, *E. coli* were isolated from 60 samples (100%) of calves with diarrhea (Pourtaghi *et al*. 2011). the present study, no *E. coli* was isolated from 24 cases (25.2% of samples) of the calves with diarrhea. This indicates that other agents such as viruses, parasites, and even nutritional factors also contribute to the occurrence of diarrhea. (Reynold et al. 1986) reported that in 31% of the diarrhea cases, no agent was isolated for an intestinal infection. Moreover, other researchers had similar findings in their studies (Dastmalchi 2012). Zahraei in a study on calves with an age of less than one month suffering from diarrhea reported that no bacterial agent was isolated from 15.3% of the samples (Omisakin 2003). diarrhea is of the almost importance in the first week of the life of calves, exposure to this bacterium during this period, if not treated, will lead to the death of the calves and economic losses. Besides consuming colostrum and receiving maternal immunoglobulins, the calf's digestive system is very sensitive in regards to dealing with diseases (Chapman 1993). Therefore, in this research, the prevalence of diarrhea in calves was investigated between two age ranges. It was found that the prevalence rate of diarrhea in the age range of 1-7 days and 7-30 days was 63.6% and 36.4%, respectively. Also, 63% of the calves with diarrhea did not consume colostrum and or had incomplete colostrum consumption, while 37% of calves with diarrhea had a history of colostrum consumption. According to these results, it seems that the prevalence of diarrhea is more common at younger ages (56.6% in the age group of 1-7 days compared to 43.6% in the age group of 7-30 days). Besides the decrease in the levels of maternal antibodies in the calf's serum, the prevalence of diarrhea in the 2-4th weeks can also be related to the change in the diet, because at this age in Iran, farmers usually start feeding the calves with flour, chopped fodder, and other substitutes of milk (Masud et al. 2012).

According to the symptoms of acute diarrhea (lethargy, muscle weakness, severe dehydration, and ataxia) and chronic diarrhea (mild symptoms and watery diarrhea) in calves, in this study, the prevalence of diarrhea, based on severe and mild symptoms, was 72.8% and 27.2%, respectively in terms of gender, the prevalence of diarrhea in male calves was 58% while 42% of female calves showed the clinical signs of diarrhea. The higher occurrence of diarrhea in male calves can be attributed to the lack of attention to this gender, because in most industrial and

semi-industrial cattle farms, male calves are sold after weaning.

Since the administration of antibiotics in calves with diarrhea is of high importance in the occurrence of clinical signs as well as the observation of false results in microbial culture, therefore, the history of antibiotic receiving was investigated in the affected calves. The prevalence of diarrhea in calves with a history of antibiotic receiving was 29.6% while 70.4% of (30.9%), and industrial cattle breeding (8.6%). Season of occurrence is also very important in the prevalence rate of diarrhea. In the current study which was performed from the spring to the winter of calves with diarrhea did not consume any antibiotics.The prevalence of diarrhea in calves was also investigated in terms of the type of cattle breeding, which was divided into traditional (60.5%), semi-industrial The difference in the prevalence of *E. coli* can be related to various reasons, such as the immunization of pregnant cows before giving birth, compliance with hygienic principles in cattle breeding, as well as feeding colostrum to newborn calves in sufficient quantities and at the right time. As it was determined in this study, the highest rate of prevalence of diarrhea (60.5%) was observed in traditional cattle farms, followed by semi-industrial cattle farms (30.9%) and industrial cattle farms (8.6%) (Table4). One of the most important reasons for the low rate of infection in industrial and semi-industrial farms is the observance of hygiene principles in calving and the regular cleaning of the bedding, and the regular observance of feeding times (especially with colostrum). In terms of colostrum consumption after birth, the prevalence rate in calves that did not consume colostrum or were incompletely fed was higher compared to calves that consumed colostrums completely (Bonyadian 2017). lower level, adult cows with diarrhea, is significantly higher than in healthy cows. especially calves with diarrhea and at a in a study conducted by (Asadian et al. 2015) in Shahrekord (Iran) on 102 fecal samples of calves with diarrhea and 16 fecal samples of healthy calves, out of 35 isolates suspected of infection with enterohemorrhagic *E. coli*, 6 isolates (17.14%) were identified as O157:H7. Studies show that cattle are the main reservoir of verotoxigenic serotypes and other strains of *E. coli*. The prevalence of these serotypes in newborn livestock, Furtheremore, the frequency of verotoxigenic strains was reported as 10.9%. In the study of (Bibbal *et al*. 2014) in France, the prevalence of *eae*A and *stx* genes was estimated as 73.3% and 88.79%, respectively.

Also, (Dastmalchi et al. 2012) investigated the prevalence of *stx*1, *stx*2, and *eae*A genes on healthy and diarrheal calves in Urmia (Iran) using Multiplex PCR and the results indicated that 23.1% of the samples had the *stx*1 gene, 26.92% of samples contained the *stx*2 gene and 26.92% of samples had the *eae*A gene.

Table 3- Prevalence rates of *E. coli* in calves with diarrhea based on age, gender, consumption of colostrum, antibiotic consumption, clinical symptoms, season, and type of cattle breeding

Factor		No.	(%)	*P*
Age	1-7 days	50	61.7	0.765
	7-30 days	31	38.3	
Gender	Female	34	42	0.486
	Male	47	58	
Consumtion of colostrum		30	37	0.063
	Positive	51	63	
Receiving of Antibiotic	Negative	24	29.6	
	Positive	57	70.4	0.010*
Clinical symptoms	Negative	59	72.8	
		22	27.2	
Season	Acute	32	39.5	0.000*
	Chronic	21	25.9	
Cattle		19	23.5	
	Spring	9	11.1	0.223
husbendry	Summer	49	60.5	
	Autumn	25	30.9	0.406
	Winter	7	8.6	
	Traditional			
	Semi-industrial			
	Industrial			

*There is a significant difrences among prevalence rates of *E. coli* in calves with diarrhea based on antibiotic consumption and clinical symptoms.

The results of (Vani et al. 2003) showed that (4.16%) of the samples from calves and lambs with diarrhea had the *eae*A gene, (2.5%) contained the *stx*1 gene, and (2.3%) had the *stx*2 gene (Masud et al. 2012). In the present study, results of the PCR test showed that out of 81 *E. coli* isolates, 13 isolates (0.16%) contained the *eae* gene, and no positive isolates were found for the *bfp* gene. Also, out of the 81 isolates, 20 isolates (24.7%) had both genes (*stx*1, *stx*2), 27 isolates (33.3%) were positive for *the stx*1 gene only and 31 isolates (37.3 %) only contained the *stx*2 gene. Also, 13% of the isolates had the *eae* gene and no positive isolates were identified for the *bfp* gene (Table 5). It was also found that 10 isolates (12.3%) contained *stx*1 and *eae* genes, 14 isolates (17.3%) contained *stx*2 and *eae* genes, and 18 isolates (22.2%) contained *stx*1, stx2, and eae (Figures 2, 3, and 4).

Table 4- The prevalence rate of virulence genes in *E. coli* isolated from calf diarrhea

	***E. coli* isolates**	***eae***	***bfp***	***Stx*1**	***Stx*2**	***Stx*1, 2**	***Stx*1, *eae***	***Stx*1,2, *eae***
No.	81	13	0	27	31	20	10	18
%	85.05	16	0	33.3	37.3	24.7	12.3	22.2

Generally, the results showed that the infection rate of affected calves with VTEC strains was higher than that of EPEC strains. In terms of EPEC infection rate, 9 isolates (19.1%) were identified in male calves and 4 isolates (11.8%) in female calves. Regarding VTEC strains, 14 isolates (29.8%) were detected in male calves and 6 isolates (17.6%) were found in female calves. Moreover, 71.6% of the calves (58 calves) were between 1-7 days old and 28.4% (23 calves) were between 7-30 days old. In terms of infection with EPEC strains, 8 isolates (13.1%) were found at the age of 1-7 days and 5 isolates (21.7%) were detected at the age of 7-30 days. Regarding VTEC strains, 9 isolates (15.5%) were at the age of 1-7 days and 11 isolates (47.8%) were at the age of 7-30 days (Tables 5 and 6).

Table 5- Prevalence rates of EPEC from calves with diarrhea based on age, gender, and receiving of antibiotics

Receiving of antibiotic				**Gender**				**Age**			
positive		**Negative**		**Male**		**Female**		**7-30 years old**		**1-7 years old**	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
6	25	7	12.3	9	19.1	4	11.8	5	21.7	8	13.8
	P=0.079				*P*=0.183				*P*=0.133		

Table 6- Prevalence rates of VTEC from calves with diarrhea based on age, gender, and receiving of antibiotics

Receiving of antibiotic				**Gender**				**Age**			
positive		**Negative**		**Male**		**Female**		**7-30 years old**		**1-7 years old**	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
7	29.2	13	22.8	14	29.8	6	17.6	11	47.8	9	15.5
	P=0.544				*P*=0.231				*P*=0.472		

The high prevalence rate (24.7%) of verotoxigenic *E. coli* in cases of calf diarrhea is significant since VTEC is known as the cause of important economic diseases in veterinary medicine, such as bacillary mastitis and bacillary diarrhea. Therefore, the feces of calves with diarrhea are one of the most important sources of disease transmission in terms of epidemiology. Furtheremore, the high percentage of contamination in calves is

noteable in terms of the risk of human infection, since calves are considered as the disease reservoir for humans. There is no specific information about the ratio of *stx*1 and *stx*2. Most observations show that the *stx*2 gene is more dangerous and some studies also reported that the strains containing the *stx*2 gene are more likely to be pathogenic than the strains containing *stx*1 (Bonyadian 2017). Moreover, it has been reported that *stx*2 is 400 times more toxic than *stx*1. In the present study, no specific pattern was observed in the prevalence of the virulence genes in this bacterium, but the *stx*2 gene was identified in the VTEC serotype.

According to results from previous studies, it is apparent that most healthy calves are negative for serotype O157:H7. However, the prevalence of this serotype in different regions was estimated from 0.1% to 62%. In studies conducted in Europe and America, the level of contamination has been reported from 0 to more than 10% (Chapman et al.1993). This difference in the prevalence rate occurs for various reasons; For example, the prevalence of diarrhea in calves is higher in hot seasons compared to cold seasons. Considering that Tabriz is one of the cold and mountainous regions of the country (Iran), sampling was performed in all four seasons of the year. In this study, the prevalence rate of diarrhea was reported as 41.7% in spring and 25% in summer. In a similar study, (Chapman et al. 1993) estimated that the prevalence of VTEC strains in England was 15.7% and they reported that the prevalence of VTEC strains is higher in spring and summer than in cold seasons. In the study of (Aslan Tas et al. 2006), the prevalence of this agent in Turkey was 13.6% and the highest prevalence rate was reported in July and November while the lowest prevalence rate was found in February (Reynolds 1986). In another study by (Omisakin et al. 2002) in England, the prevalence of *E. coli* in 589 samples collected from the rectum of cows before slaughter was reported as 7.5% from May to July. In other studies, the prevalence of serotypes was different based on the samples, the time of sampling, the health state of the animal regarding diarrhea, the methods used for the examination and the time and geographical regions of sampling. For example, studies from 1998 to 1999 indicated the prevalence rate of verotoxigenic serotypes in Germany and England as 1% to 4% (Chapman et al. 1993).

Antibiotic resistance is one of the main challenges in the treatment of diarrhea with *E. coli* strains. In the present study, the antimicrobial resistance pattern of *E. coli* isolates was studied by the disk diffusion method, and the highest levels of resistance were detected to penicillin (56.8%). The antimicrobial resistance of the isolates was recorded as follows: sulfamethoxazole-trimethoprim (38.3%), gentamicin (29.6%), ceftazidime (29.2%), colistin (25.9%), tetracycline (24.7%), imipenem (23.5%), ceftriaxone (22.2%), nalidixic acid (22.2%), ciprofloxacin (14.8%) and ofloxacin (9.9 %) (Table 7). In other studies, almost similar results were achieved and multi-drug resistance has been widely observed in *E. coli* isolates from animals, including calves

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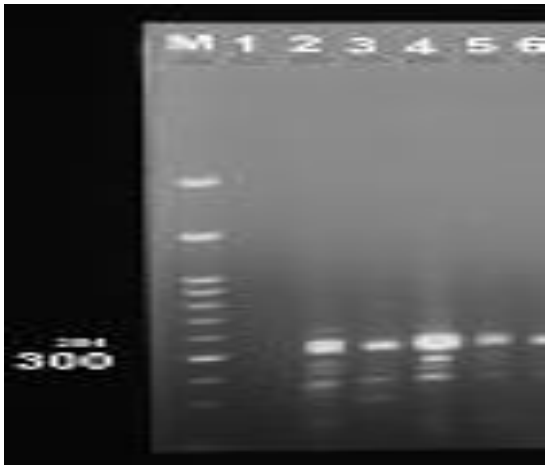

Figure 6- PCR results for the *eae* gene in *E. coli* isolates from calves with diarrhea M: marker size, lane 1: negative control, Lanes 2 to 6: positive sample of *eae* gene at384 bp

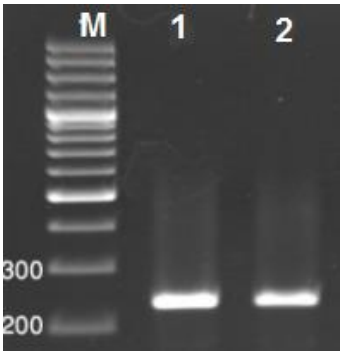

Figure 7- PCR result of *stx*2 gene in *E. coli* isolates from calves with diarrhea

M: marker size, lane 1: positive control, Lanes 2: positive sample, Lanes 4: negative control of *stx*2 gene at255

Table 7- Antibiogram resistance pattern of *E. coli* isolates from calves with diarrhea

Antibiotic	Disk	Resistant		Moderate		Sensitive	
		No.	%	No.	%	No.	%
Penicillin	P:10 µg	46	56.8	11	13.6	24	29.6
Sulfamethoxazole-Trimethoprim	ST 23.75 / 1.25 µg	31	38.3	21	25.9	29	35.8
Gentamycin	GM:10 µg	24	29.5	19	23.5	38	46.9
Ceftazidime	CAZ:30 µg	21	29.2	15	20.8	36	50.0
Tetracycline	TE:30 µg	31	38.3	21	25.9	29	35.8
Colistin	CL:10 µg	21	25.9	25	30.9	35	43.2
Ciprofloxaci	CP:5 µg	12	14.8	43	53.1	26	32.1
Imipenem	IPM:10 µg	19	23.5	32	39.5	30	37.0
Ofloxacin	OFX:5 µg	8	9.9	45	55.6	28	34.6
Nalidixic Acid	NA:30 µg	18	22.2	26	32.1	37	45.7
Ceftriaxone	CRO:30 µg	18	22.2	30	37.0	33	40.7

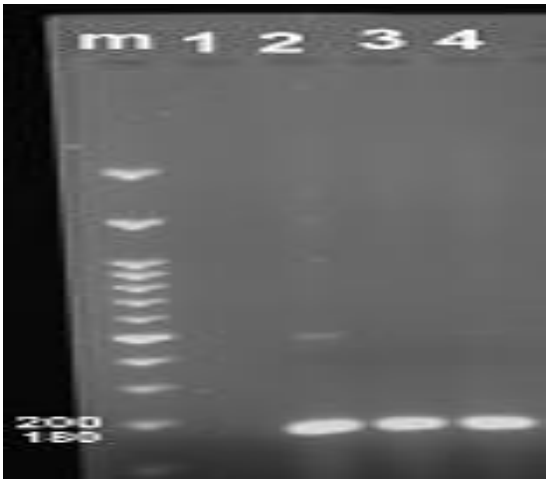

Figure 8- PCR result of *stx*1 gene in *E. coli* isolates from calves with diarrhea

M: marker size, lane 1: negative control, Lanes 2 to 6: positive sample of *stx*1 gene at180 bp

Conclusion

The cattle breeding industry is considered as a crucial industry in the agriculture and economy of northwest Iran. According to the findings of the present work and previous studies, it can be concluded that the prevalence rates of EPEC and VTEC are various in different regions of the world. Some of these differences can be attributed to several factors such as the management system, age of the calves, relative prevalence of infection in the region, better health care is needed in cattle breeding. The relatively high percentage of VTEC among the calves

In the present study, the relatively high prevalence of VTEC (24.7%) and EPEC (16%) in calves with diarrhea necessitates the need to confront this disease. According to the present work, the high percentage of the *stx*2 gene caused reduction of weight gain and finally increaisinig of mortality and decreasing of productivity in calves.

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مطالعه الگوی مقاومت آنتی بیوتیکی *ا شریشیا کولی* در گوساله‌های مبتلا به اسهال در گاوداری های تبریز

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چکیده :

زمینه مطالعاتی: اسهال ناشی از سویه های پاتوژن(EPEC, VTEC) در گوساله ها بویژه در نخستین روزهای پس از تولد، به علت تلفات و خسارات اقتصادی حاصله از اهمیت زیادی برخوردار است. **روش کار:** در طی این تحقیق ۱۰۵ سوآب رکتوم از گوساله های ۱ تا 30 روزه مبتلا به اسهال در مدت یک سال(بهار . تابستان . پاییز و زمستان ۱۴۰۰) در شهرستان تبریز جمع آوری شد .نمونه ها بر روی محیط های مک کانکی آگار و ائوزین-متیلن بلو کشت داده شدو با استفاده از رو شهای استاندارد میکروبشناسی ، تعداد ۸۱ ایزوله E.COLI شناسایی وبرای تعیین میزان مقاومت آنتی بیوتیکی به روش انتشار در دیسک(کربی- بائر) به محیط کشت مولر- هینتون انتقال داده شد و شیوع ژن های بیماری زای *اشریشیا کلی* انتروپاتوژنیک (eae , bfp) و *اشریشیا کلی* وروتوکسیژنیک (STx1,STx2) با استفاده از تکنیک PCR بررسی شد. **نتایج:** بیشترین میزان مقاومت آنتی بیوتیکی برای پنی سیلین (۵۶.۸۴٪)، تتراسایکلین (۳۸.۳٪) ، تری متوپریم -سولفومتاکسازول (۳۸.۳٪)، جنتامایسین(۲۹.۶٪)، سفتازیدیم(۲۹.۲٪)، کلیسیتین(۲۵.۹٪)، ایمی پنم(۲۳.۵٪)، سفتریاکسون(۲۲.۲۱٪)، نالیدیکسیک اسید(۲۲.۲٪)، سیپروفلوکساسین(۱۴.۸٪)، افلوکساسین(۹.۹٪) به ترتیب گزارش شد. در بررسی ژنهای حدت از ۸۱ نمونه اشریشیاکلی جدا شده ۱۶.۰٪ دارای هردو ژن (eae , bfp) و ۲۴.۷٪ دارای هردو ژن (STx1,STx2) می باشند. **نتیجه گیری نهایی:** نتایج نشان داد که درصد بالایی از نمونه های *اشریشیا کلی* جداشده از اسهال گوساله ها مقاوت آنتی بیوتیکی نسبت به تعدادی از آنتی بیوتیک های مورد استفاده دارند و همچنین درصد قابل توجه ای از این جدایه ها حاوی ژنهای حدت می باشند که این موضوع از نظر اپیدمیولوزی و بهداشت عمومی قابل تامل می‌باشد.

واژگان کلیدی : گوساله ؛ اسهال؛ *کلی باسیلوز* ؛ ژن های حدت؛ روش انتشار در دیسک کربی – بائر