ORIGINAL ARTICLE

EPIDEMIOLOGICAL STUDY ON *ESCHERICHIA COLI* AND SEROTYPING F5 IN DIARRHEIC CALVES IN INDUSTRIAL FARMS IN EAST-AZERBAIJAN PROVINCE

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ABSTRACT: Calves diarrhea is one of the most common reasons of mortality and losses of animal protein source and economic losses include cachexic carcasses, treatment cost and mortality. There are several agents that cause diarrhea including bacterial, viral, fungi and nutritional which are most common. Escherichia Coli is one of the gastrointestinal tract normal microflora from family of enterobacteriaceae. The aim of present study was to evaluate the presence of E.coli in the fecal samples of calves less than 2 month age in east Azerbaijan province. Present study carried out in the 25 farm on calves less than 2 months age in east Azerbaijan province. Of samples collected from farms, some samples were selected by chance related to different seasons so that 125 samples from each season totally 500 samples of a year. Of 500 samples, 251 samples were positive in term of E.coli. Our data showed the prevalence of E.coli in the calves less than 2 months age is 50.2%. This value was 47.9%, 49.68% and 51.66% in the farms of northwestern, northeastern and southwestern, respectively. Chi-square exam showed no significant difference in prevalence of E.coli in different areas of east Azerbaijan province (P=0.94). Also, of 125 samples collected in each season, it revealed that the positive samples in spring, summer, autumn and winter is 49.6%, 41.6%, 40% and 69.6%, respectively. So, there is statistical difference among groups in term of season (P<0.05). Also, it showed that there is significant difference among groups in term of different age (P<0.05) in which the maximum prevalence was related to 1 week age (68.81%) and minimum was related to week 4 (31.37%). Data showed that the prevalence rate of E.coli is 50.2% of that 3 cases was positive in term of fimbria F5 K99.
KEYWORDS: E.coli, Diarrheic Calves, Epidemiology, East Azerbaijan.

INTRODUCTION

Calves diarrhea is one of the most common reasons of mortality and losses of animal protein source and economic losses include cachexic carcasses, treatment cost and mortality (Dalir Naghadeh and Yarsmaeil, 2008; Pearson and Logan, 1984). There are several agents that cause diarrhea including bacterial, viral, fungi and nutritional which are most common. Near the 40% of calves suffer from diarrhea in own early month of birth (Ongerth and Stibbs, 1989). In our country, the losses are about 20-25% in which 70% is related to the diarrhea (Nadalian and Nourmohammadzadeh, 2008). Escherichia Coli is one of the gastrointestinal tract normal microflora from family of enterobacteriaceae. The genius of Escherichia consists of several species including E.vulneri, E.hermanii, E.fergassonii, E.blttae and E.coli (Hirsh *et al.,* 1999). Antigen structure of enterobacteriaceae is a little complex so that 173 somatic antigen (O) resistance to heat, 80 Capsular antibody susceptible to heat and 156 flagella antigen (H) has been recognized. 5 strains of E.coli cause diarroha which are differentiate from eachother using severity factors, clinical manifestations, epidemiology and different serotypes of O and H in which are including enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enteroadhesive (EAEC) and enterohemorrhagic (EHEC) (Mohon and Manuselis, 1995). Enteropathogenic E.coli has been detected for the first time in early 1940 as an agent of neonates' diarrhea. The previous studies showed that serotyping is related to only O groups. In some studies, the existence of cytotoxin and transitive factors of EPEC has been reported which enable bacteria to attach the epithelial cells. ETEC makes two different enterotoxins: resistance and susceptible to heat. This bacteria cause diarrhea like cholera and is one of the most common agent of diarrhea in human in developed countries. This strain has several antigen including K99, K88 and F41 which attach to epithelial cells and cause diarrhea. The affinity of K99 antigen to enterocytes is decreased by aging. ETEC is invasive and cause syndrome like Shigella infection. It has a Shigella

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like toxin which makes dysentery (Mohon and Manuselis, 1995). This strain invades into the epithelial cells of colon and proliferates. It causes diarrhea by attaching the mucosa layer of intestines and interruption of motility and absorption. EHEC causes hemorrhagic diarrhea, colitis and hemolytic uremia syndrome. This strain causes disease using Shigella like toxins 1 and 2 (Sihvonen and Miettinen, 1985). Pillus and enterotoxins are the most prevalent agents of E.coli virulence (Radostits *et al.,* 2007). Other factors include hemolysin, aerobactin and cytotoxins. The aim of present study was to evaluate the presence of E.coli in the fecal samples of calves less than 2 month age in east Azerbaijan province.

MATERIALS AND METHODS
Present study carried out in the 25 farm on calves less than 2 months age in east Azerbaijan province. Of samples collected from farms, some samples were selected by chance related to different seasons so that 125 samples from each season totally 500 samples of a year. Samples were transferred to laboratory in the sterile tubes and near the ice. The mediums used for isolation of bacteria were eosin methylene blue (EMB) and macconkey agar. Also, differential diagnostic mediums were triple sugar iron agar, urea agar and simmon citrate agar. For serotyping, the isolated colonies of E.coli were cultured in the nutrient agar medium. After the preparing the suspension of bacteria in the distilled water, specific antiserum of E.coli K99 was added and the agglutination test was performed. For detecting the fimbria F5 the rapid agglutination test was exerted.

RESULTS
Of 500 samples, 251 samples were positive in term of E.coli. Our data showed the prevalence of E.coli in the calves less than 2 months age is 50.2%. This value was 47.9%, 49.68% and 51.66% in the farms of northwestern, northeastern and southwestern, respectively. Chi-square exam showed no significant difference in prevalence of E.coli in different areas of east Azerbaijan province (P=0.94). Table 1.

Table 1: prevalence rate of E.coli in the fecal samples of calves obtained from different areas of East Azerbaijan province

Area	No. of samples	Positive	Prevalence (%)
Northwestern	96	46	47.91
Northeastern	159	79	49.68
Southwestern	120	62	51.66
Southeastern	125	64	51.2
Total	500	251	50.2

Also, of 125 samples collected in each season, it revealed that the positive samples in spring, summer, autumn and winter is 49.6%, 41.6%, 40% and 69.6%, respectively. So, there is statistical difference among groups in term of season (P<0.05). Table 2.

Table 2: prevalence rate of E.coli in the fecal samples of calves obtained from different seasons

Season	No. of samples	Positive	Prevalence (%)
Spring	125	62	49.6
Summer	125	52	41.6
Autumn	125	50	40
Winter	125	87	69.6
Total	500	251	50.2

Also, it showed that there is significant difference among groups in term of different age (P<0.05) in which the maximum prevalence was related to 1 week age (68.81%) and minimum was related to week 4 (31.37%). Table 3.

Table 3: prevalence rate of E.coli in the fecal samples of calves obtained from different ages

Age (week)	No. of samples	Positive	Prevalence (%)
1	93	64	68.81
2	115	56	48.69
4	134	50	37.31
6	101	51	50.49
8	57	30	52.63
Total	500	251	50.2

Data showed that the prevalence rate of E.coli is 50.2% of that 3 cases was positive in term of fimbria F5 K99.

DISCUSSION
Calves diarrhea is one of the most prevalent diseases in the cow industry and mortality rate during 1-60 days old. The age range was less than 2 month in present study in which the E.coli was evaluated. In a study by Snodgrass *et al.,* (1982a) and Snodgrass et al., (1982b) on calves with 1 month age, 4% enterotoxigenic E.coli was isolated. Based on previous studies, 3-10% of strains isolated from calves diarrhea had F5 factor (Sherwood *et al.,* 1983). In a study, 10% of fecal samples were E.coli positive which is ETEC K99. In some countries, the prevalence rate of ETEC K99 is about 30-40% (Quinn *et al.,* 1994), however, the prevalence rate of ETEC K99 and F5 positive is around 3-6%, this is may be due to geographical situation of this area (Sherwood *et al.,* 1983). Based on the results of our study, of 500 samples 251 samples (50.2%) were positive in term of E.coli. however, 3% was F5 and K99 positive in the age range 1-7 days old which is compatible with the results of other

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countries. Based on the fact that existence of K99 and F5 factors has direct relation to the heat-resistance toxin and distention of intestinal arch, so, these are the important factor for detection of E.coli ETEC in the Scotland and England (Sherwood *et al.,* 1983; Meyers, 1984). Garcia *et al.,* (2000) isolated 16.7% F5 from the fecal samples of calves in the Spain. In a study by Lotfollahzadeh *et al.,* (2004) it has been showed that 40.8% of isolates related to diarrhea in calves is related to E.coli in which maximum prevalence is related to 23-30 day old and minimum is related to 15-21 day old. It is against of our study that we showed maximum prevalence rate (68.81%) is related to calves with 1-7 day old and minimum in 4 week old (31.37%). Ghaemmaghami *et al.,* (1999) reported the prevalence of E.coli K99 in the calves less than 1 month age around 30%.

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