

## Antibiogram Results of *Escherichia coli* in Calf Diarrhea and *Escherichia coli* Bacteria in Aksaray Province in The Last Three Months

Ali Evren HAYDARDEDEOĞLU<sup>1</sup>, Melek AYDEMİR<sup>1\*</sup>, Elif Selin ŞENOĞLU<sup>2</sup>, Zeki ARAS<sup>3</sup>

<sup>1</sup>Department of Internal Medicine, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey

<sup>2</sup>Şenoğlu Veterinary Clinic, Selçuklu, Konya, Turkey

<sup>3</sup>Department of Microbiology, Faculty of Veterinary Medicine, Aksaray University, Aksaray, Turkey

### ABSTRACT

*Escherichia coli* is a gram-negative, facultative anaerobic, motile, non-spore-forming rod-shaped bacterium belonging to the Enterobacteriaceae family. Pathogenic *E. coli* are divided into two groups: extraintestinal and intestinal. Intestinal *Escherichia coli* pathotypes: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), Vero- or Shiga-toxin-producing *E. coli* (VTEC or STEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC). Extraintestinal pathogenic *E. coli*: These can be listed as septicemic pathogenic *E. coli*, uropathogenic *E. coli*, avian pathogenic *E. coli*, breast pathogenic *E. coli* and those that cause uterine infections, endometrial pathogenic *E. coli*. Enterotoxigenic *Escherichia coli* (ETEC) is the most common cause of neonatal diarrhea in calves and is a bacterial disease that colonizes the small intestine, produces enterotoxin, and occurs among calves during the neonatal period. The aim of this study is to determine the *E.coli* isolates that cause neonatal calf diarrhea in the Aksaray region between January, February and March 2021. To be used in the study, internal organ samples (liver, heart, lung and mesenteric lymph node) of neonatal calves that died due to diarrhea were taken from 20 different cattle farms in the Aksaray region. One calf that died in each farm was included in the sampling, and a total of 20 calves were sampled. *E.coli* was isolated in 12 (60%) of the internal organ samples covering these three months. According to the antibiogram results of the samples, 50% of the isolates were converted to Amoxicillin and Erythromycin, 33.3% to Tetracycline, 58.3% to Trimethoprim-sulfamethoxazole, 66.6% to Streptomycin, 75% to Flofenicol, Gentamicin and Enrofloxacin, % 83.3 of them were found to be sensitive to Cefloxacin and Cefloxacinium.

**Keywords:** Antibiogram, diarrhea, *E.coli*, neonatal calf

\*\*\*

### Aksaray İli Buzağı İshallerinde *Escherichia coli* ve *Escherichia coli* Bakterisinin Son Üç Aylık Antibiogram Sonuçları

#### ÖZ

*Escherichia coli*, Enterobacteriaceae ailesinde yer alan gram negatif, fakültatif anaerob, hareketli, spor oluşturmeyen çomak şeklinde bir bakteridir. Patojenik *E. coli* ekstraintestinal ve intestinal olmak üzere iki gruba ayrılır. İntestinal *Escherichia coli* patotipleri: enterotoksijenik *E. coli* (ETEC), enteropatojenik *E. coli* (EPEC), Vero- veya Shiga-toksin üreten *E. coli* (VTEC veya STEC), enterohemorajik *E. coli* (EHEC), enteroagregatif *E. coli* (EAEC), enteroinvaziv *E. coli* (EIEC) ve diffuz aderent *E. coli* (DAEC)'dir. Ekstraintestinal patojenik *E. coli* ise; septisemik patojenik *E. coli*, üropatojenik *E. coli*, avian patojenik *E. coli*, meme patojenik *E. coli* ve uterus enfeksiyonlarına neden olanlar, endometriyal patojenik *E. coli* olarak sıralabilir. Enterotoksijenik *Escherichia coli* (ETEC), buzağılarda neonatal ishalin en yaygın nedenidir ve ince bağırsakta kolonize olan, enterotoksin üreten, neonatal dönemde buzağular arasında ortaya çıkan bakteriyel bir hastalıktır. Bu çalışmanın amacı 2021 yılının ocak, şubat ve mart ayları arasında, Aksaray bölgesinde bulunan neonatal buzağı ishaline sebep olan *E.coli* izolatlarını belirlemektir. Çalışmada kullanılmak üzere, Aksaray bölgesindeki 20 farklı sığır işletmesinde ishal kaynaklı ölen neonatal dönemdeki buzağuların iç organ örnekleri (karaciğer, kalp, akciğer ve mezenterik lenf düğümü) alınmıştır. Herbir işletmede ölen bir buzağı örnekleme dahil edilerek, toplam 20 adet buzağıdan örnek alınmıştır. Bu üç ayı kapsayan iç organ örneklerinin 12'sinde (%60) *E.coli* izole edilmiştir. Örneklerin antibiyogram sonuçlarına göre izolatların %50'sinin Amoksisilin ve Eritromisine, %33,3'ünün Tetrasikline, %58,3'ün Trimetoprim-sülfametoksazole, %66,6'sının Streptomisine, %75'inin Flofenikol, Gentamisin ve Enrofloksasine, %83,3'ünün Sefloksasin ve Sefloksasinyuma duyarlı olduğu tespit edildi.

**Anahtar Kelimeler:** Antibiogram, *E.coli*, ishal, neonatal buzağı

To cite this article: Haydardeedeoğlu A.E., Aydemir M., Şenoğlu E.S., Aras Z. Antibiogram Results of *Escherichia coli* in Calf Diarrhea and *Escherichia coli* Bacteria in Aksaray Province in The Last Three Months. Kocatepe Vet J. (2023):16(4):606-613

Submission: 28.07.2023 Accepted: 16.11.2023 Published Online: 19.12.2023

ORCID ID: AEH: 0000-0002-8473-0072, MA: 0000-0002-4732-8279, ES: 0000-0001-7256-3986, ZA: 0000-0003-4564-2077

\*Corresponding author e-mail: [melekaydemir09@gmail.com](mailto:melekaydemir09@gmail.com)

## INTRODUCTION

*Escherichia coli* is a gram-negative, facultative anaerobic, motile, non-spore-forming rod-shaped bacterium belonging to the Enterobacteriaceae family (Ewers et al., 2004). Pathogenic *E. coli* are divided into two groups, extraintestinal and intestinal, according to the type of disease they cause and their virulence properties (Omerovic et al. 2017).

Basic features of intestinal *Escherichia coli* pathotypes: *E. coli* strains that cause intestinal infections are generally called 'Diarrheal *E. coli* (DEC)', meaning diarrhea. Intestinal pathotypes: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), Vero- or Shiga-toxin-producing *E. coli* (VTEC or STEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC). Extraintestinal pathogenic *E. coli*: These can be listed as septicemic pathogenic *E. coli*, uropathogenic *E. coli*, avian pathogenic *E. coli*, breast pathogenic *E. coli* and those that cause uterine infections, endometrial pathogenic *E. coli* (Carlton et al. 2010).

Calf diarrhea, one of the most critical problems in calf breeding worldwide and in our country, causes economic losses. Although the future of dairy farms depends on many situations, it depends on the calves and heifers taking their place on the farm and their participation in productivity. Globally, calf deaths caused by enterotoxigenic *E. coli* (ETEC) appear to be among the primary causes of death in farms' free calves (El-Seedy et al. 2016). Statistically, this means that an average of 4 calves live healthy on the farm per year. In other words, it is very important to take the necessary precautions to renew 1/4 of the herd every year (Bhat et al. 2012). Because of that, calf loss is a severe economic loss. Calf loss in the world means productivity and economic loss. Calf diarrhea is a cause of high morbidity and mortality worldwide (Wudu 2008). It has been reported that 75% of calf losses before the weaning period are due to diarrhea (Uhde et al. 2008; Bartels et al. 2010).

In the etiology of diarrhea in calves; Bacterial agents such as *Escherichia coli*, *Salmonella spp.*, *Clostridium perfringens*, *Campylobacter jejuni*, *Chlamydia spp.*, viral agents such as *Rotavirus (group A)*, *Coronavirus*, *Adenovirus*, *Parvovirus*, *Astrovirus*, *Calicivirus*, *Bovine Viral Diarrhea (BVD)*, and parasitic agents such as *Coccidia*, *Cryptosporidium*, *Giardia*, *Neoscaris vitulorum* are present (Snodgrass et al. 1986). Hygiene, intensive housing of calves, colostrum management, attention to litter systems may be significant in preventing multiple infectious agents (Larson and Tyler 2005). Studies show that an agent that initiates infection makes the animal vulnerable to the effects of other pathogens. According to many studies, the most common cause of calf diarrhea is shown as Rotavirus, but it is reported that it is seen at a higher rate, especially in dairy farms and calves suckling from the mother. The second common factor is *Cryptosporidium*, but it is more

common in fattening enterprises than dairy enterprises, and *Coronavirus* is reported to be the third common cause. It is stated that the factors that cause calf diarrhea can be determined quickly and minimized with effective treatment (Snodgrass et al. 1986).

Calves with diarrhea are sick at 0-4 weeks of age, especially at 0-2 weeks; It is reported that 80% of them have an infectious cause, 50% of the positive ones have more than one factor, and 31% of them have two factors (Cho et al. 2014). The defense mechanisms of newborn calves against pathogenic factors are not developed. Since there is no antibody transmission from mother to fetus due to the presence of syndesmochorial placenta structure in cattle and the antibodies are too large, calves are born agammaglobulinemic when they are born, and they are not protected against pathogenic factors until they receive a sufficient amount of colostrum (Aldridge et al. 1992; Reber et al. 2006). Immunity generated by the ingestion of colostrum by calves is known as passive immunity. Passive immunity ends with the destruction of maternal antibodies, which lasts for 3-4 weeks (Neto et al. 2004).

*E. coli* is the most important bacterial cause of diarrhea in calves. The environmental conditions of the calf, the type of the causative agent, and the immune status of the calf play an essential role in the formation of infection. In etiology, mainly septicemic and enterotoxigenic (ETEC) (F4, F5 (= K99), F6, F41 antigens) O8, O9, O78, O45, O117, and O35 serotypes and lesser enterohemorrhagic (O157:H7) and necrotoxicogenic *E. coli* are effective. Insufficient, poor quality or no colostrum in the first hours of birth, poor maternal care in the dry period and late separation of them, lack of vitamin A, not paying attention to the cleanliness of the shelters, feeding with milk with mastitis, not paying attention to udder hygiene are the preparatory factors. It is one of the most important diseases of calves worldwide (Radostits et al. 2007). Neonatal calves are susceptible to *E. coli* (K99) infection in the first four days after birth, and watery diarrhea may occur when infected (Foster and Smith 2009).

The outer part of the small intestine, which has a low pH, provides the necessary environment for ETEC invasion. In addition, loss of infected cells, villous atrophy, and damage to the lamina propria are observed in the small intestine. The bacteria reveals the K99<sup>+</sup> antigen for attachment (Francis 1989; Figure 1). In a study, 37 *E. coli* isolated from calves with diarrhea K99 (18.9%), F41 (18.9%), heat-stable enterotoxin a (STA) (18.9%), Shiga toxin 1 (Stx1;13.5%), Shiga toxin 2 (Stx2;5.4%) and intimin (8.1%) genes have been reported to be detected by multiplex PCR (Ok et al. 2009). Although it is generally seen up to two weeks of age, it is more effective in calves less than five days old, and infected animals are also a source of infection. Morbidity varies between 30-70%, mortality is between 50-60% in the first three days of calves, and this rate decreases to 5-10% in 8-

day-old calves. The incubation period is 1-3 days (Radostits et al. 2007).

Enterotoxin production is one of the essential factors that determine pathogenicity in *E. coli* strains. ETECs produce mainly two types of enterotoxins: thermolabile toxin (LT), which becomes inactive at 60°C for 30 minutes, and thermostable toxin (ST), which can withstand 100°C for 15 minutes. Enterotoxin type differs according to animal species. While more LT is synthesized in strains that spread in calves and cattle, ST synthesis varies according to species (Debroy and Maddox 2001; Hossain et al. 2007; Rigobelo et al. 2006). Enteropathogenic (EPEC) *E. coli* is reported to cause diarrhea due to the destruction of microvilli and the release of verotoxin after it invades the small and large intestines (Pospischil 1989). When calves are born in heavily contaminated environmental conditions, virulent pathogens may settle in the distal part of the intestines and cause disease before normal adult intestinal flora is formed (Fecteau et al. 2009). Although bacteria survive and are ingested in the small intestines, they must interact with the fimbriae on the microvilli of the small intestine through fimbrial antigens, defined as "K antigens", in order to become biologically active. K-antigens in the capsule of gram (-) bacteria are characterized by the presence of LPS with O antigens on the cell wall, and they do this with their flagella with H antigens (Foster and Smith 2009; Hunt 2010).

The presence of uncontrollable inflammation in infected extravascular tissues plays a role in the pathogenesis of sepsis and septic shock (Buttenschoen et al. 2010). Dehydration with electrolyte loss due to *E. coli*, sepsis due to elevated LPS, and related changes are observed in calf diarrhea (Bicknell and Noon 1993; Roberts et al. 2011).

Septicemia or coli-septicemia in newborn (neonatal) calves progresses rapidly in the first 2-6 days of their lives and often results in death (Fecteau et al. 2009). Diarrhea of newborn calves due to *E. coli* is a disease characterized by diarrhea that is usually mucous, watery, yellow, grayish or green, sometimes bloody, and, if left untreated, progressive dehydration, resulting in increased electrolyte loss and death (Bicknell and Noon 1993; Figure 2). In the very early stages of the disease, clinical symptoms are not evident and exhibit similar symptoms to other disease symptoms (Fecteau et al. 2009). Hyperdynamic and hypodynamic phase changes are observed depending on the increase in LPS. The absence of the sucking reflex is a mental state picture that mainly occurs between moderate depression and coma, and it is also reported as non-specific clinical findings (Roberts et al. 2011).

In cases of diarrhea, significant fluid-electrolyte losses occur in animals. As a result of diarrhea, a significant amount of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> and HCO<sub>3</sub><sup>-</sup> is lost in the feces. While this causes a decrease in blood pH, plasma HCO<sub>3</sub><sup>-</sup> value, Na and Cl concentrations in calves, it causes an increase in the base deficit and plasma K

concentration. In calves with diarrhea, blood volume decreases due to excessive extracellular fluid loss, it affects the urinary excretion of K, and the excretion of H ions decreases due to disruptions in renal functions and acid-base balance. In addition, metabolic acidosis occurs due to the rapid increase in H<sup>+</sup> ions in the blood. In calf diarrhea, H<sup>+</sup> ions, which increase excessively in the plasma, pass into the intracellular fluid, causing K<sup>+</sup> ions, 98% of which are in the cell, to pass into the extracellular fluid. As a result, hyperkalemia occurs (Özkan 2017).

Depending on the systemic inflammatory response syndrome (SIRS) that occurs with sepsis, one or multiple organ failures and changes in clinical and laboratory findings due to this occur (Nguyen et al. 2007). Diarrhea, mental changes, stagnation, hyperemia of mucous membranes, low blood pressure, increase in heart rate, coldness in extremities, loss of appetite, hypovolemia, decrease in urine output, high fever (sometimes low fever), increase in respiratory rate (Jacobi 2002; Cunnington and Nadel 2008; Fecteau et al. 2009), abnormal changes in coagulation, leukocytosis/leukopenia, thrombocytopenia, elevated (sometimes decreased) blood sugar and hyperlactatemia (Nguyen et al. 2007), disseminated intravascular coagulation (DIC) and multi-organ failure (CSF) (Zeerleder et al. 2005) symptoms are observed.

Shock consists of 4 different mechanisms that cause clinical manifestations of acute circulatory failure (Weil and Henning 1979). Decreases in venous recycling (internal or external fluid losses) resulting from decreased circulating volume is the first mechanism. The second is severe arrhythmias (such as ventricular tachycardia or severe AV blocks) or decreased heart contraction (infarction, ischemia, myopathy, myocarditis). The third mechanism is obstruction due to pneumothorax, pulmonary embolism, or cardiac tamponade. Finally, the fourth reason is unstable tissue perfusion due to loss of vascular rhythm (as a result of anaphylaxis, sepsis, or spinal injury) (Vincent and de Backer 2013).

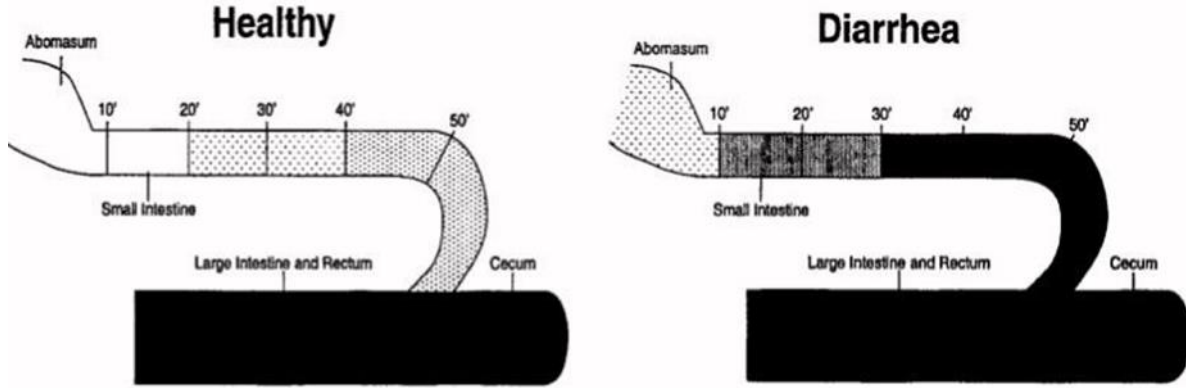
Several mechanisms have been proposed for the development of multi-organ failure. These are: tissue or cell hypoxia, stimulation of tissue apoptosis, translocation of microorganisms or their compounds from the gastrointestinal tract, immune system disorders and mitochondrial dysfunction (Osterbur et al. 2014). Presumably, MODS develops due to decreased oxygen availability and use, changes in cellular metabolism, and cardiovascular dysfunction leading to tissue hypoxia. Tissue hypoxia occurs due to metabolic acidosis and decreased oxygen ratio (Evans and Smithies 1999). Pulmonary dysfunction is a picture of persistent hypoxemia due to increased permeability of pulmonary vessels, pulmonary epithelial damage, development of micro-thrombosis, pulmonary edema, and a decrease in surfactant production (Ware and Matthay 2000). In addition, the development of oliguria and azotemia shapes renal dysfunction. Acute

renal failure develops due to deterioration of renal blood flow resulting from microvascular alteration with the development of hypotension (Evans and Smithies 1999). Gastrointestinal dysfunction primarily presents with ileus, but the loss of the normal barrier function of the gastrointestinal mucosa may also occur. In addition, losses in the mucosal barrier together with bacterial translocation or absorption of endotoxin contribute to the pathogenesis of MODS (Rombeau and Takala 1997).

Commonly occurring central nervous system dysfunction may be characterized by depression. However, due to severe damage to neurons, septic

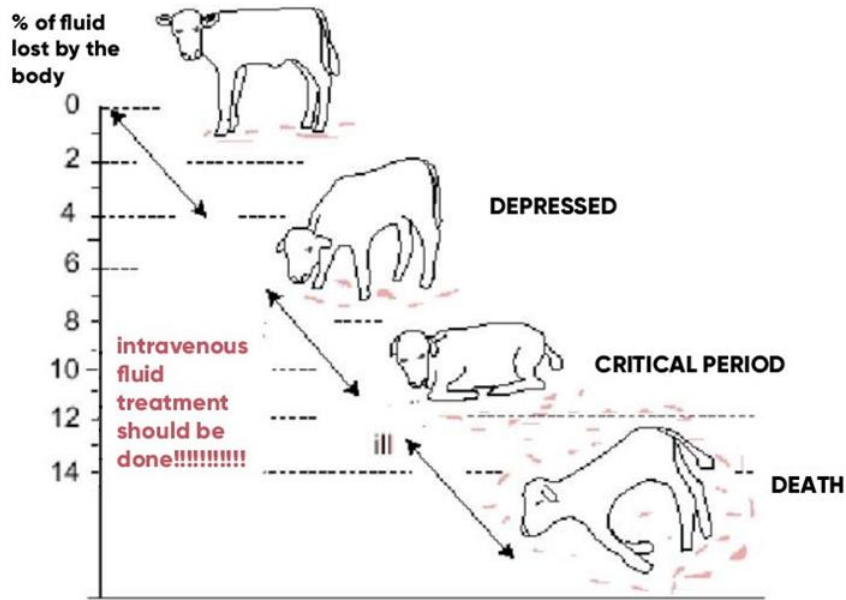
encephalopathy may also occur (Papadopoulos et al. 2000).

Since there are many serotypes of *E. coli* infection, the agent's identification is important in selecting the vaccine to protect from the disease. In cases of calf diarrhea, the Latex Agglutination Test is frequently used to identify *E. coli* K99<sup>+</sup> (Cho et al. 2010). PCR testing helps identify complex to isolate viruses in cell cultures or bacteria that take a long time to grow. In addition to the above-mentioned diagnostic methods, the diagnosis of enteropathogens can also be made with immunochromatographic test kits (Çitil et al. 2004).



**Figure 1:** Diagram of distribution and concentration of *E. coli* bacteria in the healthy intestinal tract and diarrheal intestinal tract of a calf. It shows that the number of *E. coli* in the large intestine of diarrheal and healthy calves are similar, but it increases the number of *E. coli* in the small intestines of diarrheal calves, especially in the distal jejunum and ileum (Constable, P.D. 2009).

**Şekil 1:** Sağlıklı ve ishallerli buzağuların bağırsak sisteminde *E. coli* bakterisinin dağılımı ve konsantrasyonunun diyagramı. İshallerli ve sağlıklı buzağuların kalın bağırsağında *E. coli* sayısının benzer olduğunu ancak ishallerli buzağuların ince bağırsaklarında, özellikle distal jejunum ve ileumda *E. coli* sayısını arttırdığını göstermektedir (Constable, P.D. 2009).



**Figure 2:** Changes in the percentage of dehydration, taking into account the clinical symptoms and health of calves (Wattiaux 2005).

**Şekil 2:** Buzağuların klinik semptomları dikkate alınarak dehidrasyon yüzdesindeki değişiklikler gösterilmiştir (Wattiaux 2005).

## MATERIALS and METHODS

This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k).

### Samples

To be used in the study, internal organ samples (liver, heart, lung and mesenteric lymph node) were taken from neonatal calves that died due to diarrhea in 20 different cattle enterprises in the Aksaray region between January, February and March 2021. One calf that died in each farm was included in the sampling, and samples were taken from a total of 20 calves. Before death, the animals were found to show different symptoms of *E. coli* infections. Dominant clinical findings; septicemia, enteritis, dehydration, weakness, acidosis. All samples were transferred to the laboratory in an icebox with minimal delay for bacteriological examination to detect *E. coli* isolates.

### Cultivation

Samples collected for the isolation of the *E. coli* pathogen were inoculated into 5% sheep blood agar (Oxoid) and MacConkey Agar (Oxoid) and incubated at 37°C for 18-24 hours. Lactose fermenter (pink) colonies were streaked on eosin methylene blue agar

and confirmed as *E. coli* using standard biochemical tests according to Collee et al. (1996).

### Antibacterial Susceptibility Test

The standard disc diffusion method determined the antibiotic susceptibility of isolated strains (CLSI 2012). Isolates incubated in tryptic soy broth (TSB, Oxoid) at 37°C for 18-24 hours were seeded on Mueller-Hinton Agar (Oxoid), and antibacterial susceptibility test discs were placed 3 cm apart and incubated at 37°C for 24 hours. For this purpose, Amoxicillin (25 µg), Enrofloxacin (5 µg), Trimethoprim-sulfamethoxazole (1.5 µg-23.5 µg), Gentamicin (10 µg), Tetracycline (30 µg), Streptomycin (10 µg), Erythromycin (15 µg), Florfenicol (30 µg), Ciprofloxacin (5 µg) and Cefquinome (30 µg) antibiotic discs (Oxoid) were used.

## RESULTS

*E. coli* was isolated from 12 (60%) of a total of 20 internal organ samples. As a result of the antibacterial susceptibility test, 50% of the isolates were Amoxicillin and Erythromycin, 33.3% Tetracycline, 58.3% Trimethoprim-sulfamethoxazole, 66.6% Streptomycin, 75% Florfenicol, Gentamicin and Enrofloxacin, and 83.3% were found to be sensitive to Cefquinome and Ciprofloxacin (Figure 3).

Antibiotic	S	I	R
Amoxicillin (25 µg)	6	2	4
Enrofloxacin (5 µg)	9	1	2
Trimethoprim- sulfamethoxazole (25 µg)	7	2	3
Gentamicin (10 µg)	9	1	2
Tetracycline (30 µg)	4	4	4
Streptomycin (10 µg)	8	2	2
Erythromycin (15 µg)	6	3	3
Florfenicol (30 µg)	9	2	1
Ciprofloxacin (5 µg)	10	1	1
Cefquinome (30 µg)	10	1	1

S: sensitive; I: moderately sensitive; R: resistant

**Figure 3:** Antibacterial resistance in all *E. coli* strains isolated from calves  
**Şekil 3:** Buzağılardan izole edilen tüm *E. coli* suşlarındaki antibakteriyel direnç

## DISCUSSION

Neonatal calf diarrhea remains one of the most important problems facing livestock farming, causing major economic losses (Garcia et al. 2000). Calf diarrhea; It still causes significant economic losses in our country due to reasons such as growth retardation, deaths and treatment costs. Today, it continues to be a cause of high morbidity and mortality globally (Wudu et al. 2008; El-Seedy et al. 2016). Calf diarrhea is difficult to control effectively. Because the prevention and control of calf diarrhea depends on multiple factors such as the environment, pathogenic microorganisms and the disease-causing power of the factors, nutrition and passive transfer (Waltner-Toews et al. 1986; Balıkçı 2012; Cho and Yoon 2014).

Identification of the likely causative pathogen in diarrheal outbreaks is important to enable targeted preventive measures such as vaccination and the identification of possible risk factors or sources of infection (Izzo et al. 2011). It has been reported that 75% of calf losses before weaning and 26% in the neonatal period are due to diarrhea (Uhde et al. 2008; Bartels et al. 2010; Cho and Yoon 2014). *E. coli* are the most common pathogens screened in calves under 2 months of age (Achá et al. 2004). Their prevalence varies depending on the geographical location of farms, farm management practices, and herd size (Cho and Yoon 2014).

In this study, internal organ samples taken from calves that died due to diarrhea during the neonatal period in 20 different farms were used. *E. coli* was isolated from 12 (60%) of a total of 20 internal organ samples. In internal organ samples of other calves, bacterial infections such as *Clostridium* and *Campylobacter species* (Myers et al. 1984), which require specific or enriched culture media, can be attributed to fatal diarrhea caused by bacterial infections that cannot develop in culture media (Cho et al., 2010). At the same time, since the washout period of antibiotics used for treatment in calves with diarrhea was not taken into account, residues of antibiotics were associated with false negative results in microbiological cultures.

The prevalence of the *E. coli* isolate in our current study was similar to that reported by Awad et al. (1979) 80% and Haggag and Khaliel (2002) 82% show compatibility with their studies; The prevalence rates obtained in other studies are respectively compared to Azzam et al. (2006) 5%, El-Shehedi et al. (2013) 35.83%, Osman et al. (2013) 63.6% and Hassan (2014) 50%, which were found to be lower compared to our study. These differences in prevalence rates of *E. coli* in calves with diarrhea may be attributable to geographic locations and management practices, with ETEC infection occurring primarily through ingestion of contaminated food or water, ETEC infection being among the primary pathogens in the neonatal period, hygienic measures, as well as hygienic measures (Cho and Yoon 2014). Despite the increasing availability of

vaccines against other *E. coli*-associated pathogens and the continued emphasis on optimizing colostral transfer of passive immunity, improved treatment protocols for calf diarrhea are required.

The main factor in the treatment of calves with diarrhea during the neonatal period is the administration of intravenous fluids and oral electrolyte solutions. In addition, the effectiveness of antimicrobial drugs in the treatment of calf diarrhea is controversial. Calves with diarrhea are more likely to fail or partially fail the passive transfer pass. Therefore, it should not be forgotten that diarrhea is more likely to be bacteremic and antimicrobial agents may be indicated for treatment. The active ingredients of antibiotics used in treatment should be made more specific through laboratory diagnosis and antibiogram tests (Berge et al. 2005).

According to the results of antibiogram tests performed against different *E. coli* serogroups and untyped groups, it was determined that *E. coli* showed different degrees of sensitivity to antibiotics. While mostly *E. coli* isolates show high resistance to the majority of antimicrobials, only; showed high sensitivity to marbofloxacin, spectinomycin and neomycin. The reason for this is that most of the animals are probably unconsciously treated with antimicrobial drugs, resulting in the development of high resistance (Berge et al. 2005; Duse et al. 2015).

In our study, an antibiogram test was performed to determine whether the antibiotics used against the *E. coli* agent in the Aksaray region were sensitive. It determined the antibiotic susceptibility of strains isolated by the standard disc diffusion method (CLSI 2012). The isolates, which were incubated in tryptic soy medium (TSB, Oxoid) at 37°C for 18-24 hours, were planted on Mueller-Hinton Agar (Oxoid) and antibacterial susceptibility test discs were placed at 3 cm intervals and incubated at 37°C for 24 hours. According to the antibiogram results of the samples, 50% of *E. coli* isolates were treated with Amoxicillin and Erythromycin; 33.3% Tetracycline; 58.3% to Trimethoprim-sulfamethoxazole; 66.6% to Streptomycin; 75% to Flofenicol, Gentamicin and Enrofloxacin; 83.3% of them were determined to be sensitive to Cefloxacin and Cefloxacinium.

## CONCLUSION

Every year in the Aksaray region, many calves die during the neonatal period due to *E. coli* and calf losses cause serious damage to the country's economy. In addition, antibiotics that are still used unconsciously today and their treatment costs continue to cause problems that cannot be ignored. Determining the enteropathogen that causes calf diarrhea and choosing the right antibiotic in treatments through antibiogram testing against this pathogenic agent have an important place in treatments. In this way, it is believed that preventing antibiotic resistance, avoiding unnecessary antibiotic use and reducing mortality rates with correct

treatment will also contribute to the country's economy. 20 calves were used in our study, and studies with more animals are needed to evaluate the prevalence rates of *E.coli* isolates in the Aksaray region and to determine the resistance and sensitivity to commonly used antibiotics.

**Conflict of interest:** The authors declared that there are no actual, potential or perceived conflicts of interest for this article.

**Authors' Contributions:** The authors declared that they contributed equally to the article.

**Ethical approval:** This study is not subject to HADYEK's permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

**Acknowledgement:** I thank all the authors for their contribution to this study.

**Explanation:** This study was previously presented as an undergraduate graduation paper (2020).

## REFERENCES

- Acha, S. J., Kühn, I., Jonsson, P., Mbazima, G., Katouli, M., & Möllby, R. (2004). Studies on calf diarrhoea in Mozambique: prevalence of bacterial pathogens. *Acta Veterinaria Scandinavica*, 45(1), 1-10.
- Aldridge, B., Barry, F., & Adams, M.A. (1992). Role of colostral transfer in neonatal calf mangement: failure of acquisition of passive immunity. *The Compendium on continuing education for the practicing veterinarian*, 14, 265-269.
- Balıkçı, E. (2012). Neonatal ishali buzağlarda Rotavirus, Coronavirus, E. coli K99 ve Cryptosporidium parvum'un hızlı test kitleri ile teşhisi ve enteropatojen ile maternal immünite ilişkisi. *Fırat Üniversitesi Sağlık Bilimleri Veteriner Dergisi*, 26(2), 73-78.
- Berge, A. C. B., Atwill, E. R., & Sischo, W. M. (2005). Animal and farm influences on the dynamics of antibiotic resistance in faecal *Escherichia coli* in young dairy calves. *Preventive veterinary medicine*, 69(1-2), 25-38. <https://doi.org/10.1016/j.prevetmed.2005.01.013>
- Bhat, S.A., Juyal, P.D., & Singla, L.D. (2012). Prevalence of cryptosporidiosis in neonatal buffalo calves in Ludhiana district of Punjab, India. *Asian J Anim Vet Adv*, 7(6), 512-520.
- Bartels, C.J., Holzhauer, M., Jorritsma, R., Swart, W.A., & Lam, T. J. (2010). Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. *Preventive veterinary medicine*, 93(2), 162-169. <https://doi.org/10.1016/j.prevetmed.2009.09.020>
- Bicknell, E., Noon, & T.H. (1993). Neonatal calf diarrhea. *Anim Care Health Maint*, 19-23. <https://doi.org/10.1016/B978-141603591-6.10021-1>
- Buttenschoen, K., Radermacher, P., & Bracht, H. (2010). Endotoxin elimination in sepsis: physiology and therapeutic application. *Langenbeck's archives of surgery*, 395(6), 597605.
- Carlton L. G., John F. P., Glenn S., Charles O. T. (2010); Pathogenesis of Bacterial Infections in Animals, p.267 – 307. <https://doi.org/10.35864/evmd.530084>
- Cho, Y.I., Kim, W.I., Liu, S., Kinyon, J.M., & Yoon, K.J. (2010). Development of a panel of multiplex real-time polymerase chain reaction assays for simultaneous detection of major agents causing calf diarrhea in feces. *J Vet Diagn Invest.*, 22 (4), 509-517.
- Cho, Y.I., & Yoon, K.J. (2014). An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. *Journal of veterinary science*, 15 (1), 1-17.
- CLSI National Committee for Clinical Laboratory Standards, (M02-A11). Performance Standards for antimicrobial Susceptibility Testing, Pennsylvania Wayne. 2012, Vol. 32.
- Constable, P.D. (2009). Treatment of calf diarrhea: antimicrobial and ancillary treatments. *Veterinary Clinics of North America: Food Animal Practice*, 25(1), 101-120. <https://doi.org/10.1016/j.cvfa.2008.10.012>
- Collee, J. G., Fraser, A. G., Marmion, B. P., & Simmons, A. (1996). *Practical medical microbiology*. vol. 1. Churchill Livingstone, New York, 131-149.
- Cunnington, A., & Nadel, S. (2008). New therapies for sepsis. *Curr Top Med Chem.*, 8, 603614.
- Çitil, M., Arslan, M.Ö., Güneş, V., Erdoğan, H.M. (2004). Neonatal buzağı ishallerinde *Cryptosporidium* ve *Eimeria* enfeksiyonlarının rolü. *Kafkas Üniv. Vet. Fak. Derg*, 10 (1), 5964.
- Debroy, C., & Maddox, C.W. (2001). Identification of virulence attributes of gastrointestinal *Escherichia coli* isolates of veterinary significance. *Animal Health Research Reviews*, 2 (2), 129-140.
- Duse, A., Waller, K. P., Emanuelson, U., Unnerstad, H. E., Persson, Y., & Bengtsson, B. (2015). Risk factors for antimicrobial resistance in fecal *Escherichia coli* from preweaned dairy calves. *Journal of dairy science*, 98(1), 500-516. <https://doi.org/10.3168/jds.2014-8432>
- El-Seedy, F. R., Abed, A. H., Yanni, H. A., & Abd El-Rahman, S. A. A. (2016). Prevalence of *Salmonella* and *E. coli* in neonatal diarrheic calves. *Beni-Suef University journal of basic and applied sciences*, 5(1), 45-51.
- Evans, T.W., & Smithies, M. (1999). ABC of dysfunction: Organ dysfunction. *Br Med J*, 318(7198), 1606-1609. <https://doi.org/10.1136/bmj.318.7198.1606>
- Fecteau, G., Smith, B.P., & George, L.W. (2009). Septicemia and meningitis in the newborn calf. *Veterinary Clinics of North America: Food Animal Practice*, 25(1), 195-208. <https://doi.org/10.1016/j.cvfa.2008.10.004>
- Foster, D.M., & Smith, G.W. (2009). Pathophysiology of diarrhea in calves. *Veterinary Clinics of North America: Food Animal Practice*, 25 (1), 13-36. <https://doi.org/10.1016/j.cvfa.2008.10.013>
- Francis D.H, Allen S.D, & White R.D, (1989): Influence of bovine intestinal fluid on the expression of K99 pili by *Escherichia coli*. *American journal of veterinary research*, 50(6), 822-826.
- Garcia, A., Ruiz-Santa-Quiteria, J. A., Orden, J. A., Cid, D., Sanz, R., Gómez-Bautista, M., & De La Fuente, R. (2000). Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. *Comparative immunology, microbiology and infectious diseases*, 23(3), 175-183. [https://doi.org/10.1016/S0147-9571\(99\)00071-5](https://doi.org/10.1016/S0147-9571(99)00071-5)
- Hossain, M.T., Siddique, M.P., Hossain, F.M.A., Zinnah, M.A., Hossain, M.M., Alam, M.K., & Choudury, K.A. (2008). Isolation, identification, toxin profile and antibiogram of *Escherichia coli* isolated from broilers and layers in Mymensingh district of Bangladesh. *Bangladesh Journal of Veterinary Medicine*, 6 (1), 1-5.

- Hunt, J.M. (2010). Shiga toxin-producing *Escherichia coli* (STEC). *Clin Lab Med., Clinics in laboratory medicine*, 30 (1), 21-45. <https://doi.org/10.1016/j.cll.2009.11.001>
- Izzo, M. M., Kirkland, P. D., Mohler, V. L., Perkins, N. R., Gunn, A. A., & House, J. K. (2011). Prevalence of major enteric pathogens in Australian dairy calves with diarrhoea. *Australian veterinary journal*, 89(5), 167-173. <https://doi.org/10.1111/j.1751-0813.2011.00692.x>
- Jacobi, J. (2002). Pathophysiology of sepsis. *Am J Health-Syst Pharm.*, 59: 3-8. [https://doi.org/10.1093/ajhp/59.suppl\\_1.S3](https://doi.org/10.1093/ajhp/59.suppl_1.S3)
- Larson, R.L., & Tyler, J.W. (2005). Reducing calf losses in beef herds. *Vet Clin North Am Food Anim Pract.*, 21 (2), 569-584. <https://doi.org/10.1016/j.cvfa.2005.02.009>
- Myers, L. L., Firehammer, B. D., Border, M. M., & Shoop, D. S. (1984). Prevalence of enteric pathogens in the feces of healthy beef calves. *American journal of veterinary research*, 45(8), 1544-1548.
- Neto, R.M., Packer, I.U., & Doprado, G.V.B. (2004). Colostral Immunoglobulins Absorption in Canchim and Nelore Calves. *Revista Brasileira de Zootecnia*, 33 (6), 1544-1547. <https://doi.org/10.1590/S1516-35982004000600021>
- Nguyen, S.T., Nguyen, D.T., Le, D.Q., Le Hua, L.N., Nguyen, T.V., Honma, H., & Nakai, Y. (2007). Prevalence and first genetic identification of *Cryptosporidium* spp. in cattle in central Vietnam. *Veterinary parasitology*, 150(4), 357-361. <https://doi.org/10.1016/j.vetpar.2007.09.010>
- Ok, M., Güler, L., Turgut, K., Ok, Ü., Şen, I., Gündüz, I.K., & Güzelbekteş, H. (2009). The studies on the aetiology of diarrhoea in neonatal calves and determination of virulence gene markers of *Escherichia coli* strains by multiplex PCR. *Zoonoses and public health*, 56(2), 94-101. <https://doi.org/10.1111/j.1863-2378.2008.01156.x>
- Omerovic, M., Müştak, H. K., & Kaya, İ. B. (2017). *Escherichia coli* Patotiplerinin Virulens Faktörleri. *Etlık Veteriner Mikrobiyoloji Dergisi*, 28(1), 1-6. <https://doi.org/10.35864/evmd.530084>
- Osterbur, K., Mann, F.A., Kuroki, K., & Declue, A. (2014). Multiple organ dysfunction syndrome in humans and animals. *J Vet Intern Med*, 28 (4), 1141-1151. <https://doi.org/10.1111/jvim.12364>
- Özkan, C. (2017). İshalli Buzağlarda Hiperkalemi ve Tedavi Yaklaşımları. *Van Buzağı Hastalıkları sempozyumu*, 59-69.
- Papadopoulos, M.C., Davies, D.C., Moss, R.F., Tighe, D., & Bennett, E.D. (2000). Pathophysiology of septic encephalopathy: a review. *Crit Care Med*, 28 (8), 3019-3024.
- Pospischil, A. (1989). Pathologie und Pathogenese infektiöser Durchfallerkrankungen beim Kalb, *Vet*, 5: 27-32.
- Radostits, O.M., & Gay, C.C. (2007). Traumatic reticuloperitonitis In: Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, PD Eds. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*. 10th edn. Elsevier Health Sciences, Philadelphia, PA, USA, 337-352.
- Reber, A.J., Lockwood, A., Hippen, A.R., & Hurley, D.J. (2006). Colostrum induced phenotypic and trafficking changes in maternal mononuclear cells in a peripheral blood leukocyte model for study of leukocyte transfer to the neonatal calf. *Vet Immunol Immunopathol*, 109: 139-150. <https://doi.org/10.1016/j.vetimm.2005.08.014>
- Rigobelo, E.C., Gamez, H.J., Marin, J.M., Macedo, C., Ambrosin, J.A., & Ávila, F.A.D. (2006). Virulence factors of *Escherichia coli* isolated from diarrheic calves. *Arq Bras Med Vet Zootec*, 58 (3), 305-310. <https://doi.org/10.1590/S0102-09352006000300003>
- Roberts, J.A., Roberts, M.S., Semark, A., Udy, A.A., Kirkpatrick, C.M., Paterson, D.L., Roberts, M.J., Kruger, P., & Lipman, J. (2011). Antibiotic dosing in the „at risk“ critically ill patient: Linking pathophysiology with pharmacokinetics/pharmacodynamics in sepsis and trauma patients. *BMC Anesthesiology*, 11 (1): 1471-1453.
- Rombeau, J.L., & Takala, J. (1997). Summary of round table conference: gut dysfunction in critical illness. *Intensive care medicine*, 23(4), 476-479.
- Smith, G.W. (2009). Treatment of calf diarrhea: oral fluid therapy. *Veterinary Clinics of North America: Food Animal Practice*, 25(1), 55-72.
- Snodgrass, D.R., Terzolo, H.R., Sherwood, D., Campell, I., & Menzies, J.D. (1986). Aetiology of diarrhoea in young calves. *Vet Rec*, 119; 31-34.
- Uhde, F.L., Kaufmann, T., Sager, H., Albini, S., Zanoni, R., Schelling, E., & Meylan, M. (2008). Prevalence of four enteropathogens in the faeces of young diarrhoeic dairy calves in Switzerland. *The Veterinary record*, 163(12), 362-366. <https://doi.org/10.1136/vr.163.12.362>
- Vincent, J.L., & de Backer, D. (2013). Circulatory shock. *N Engl J Med*, 369, 1726-34. <https://pubmed.ncbi.nlm.nih.gov/24171518/>
- Waltner-Toews, D., Martin, S.W., & Meek, A.H. (1986). An epidemiological study of selected calf pathogens on Holstein dairy farms in southwestern Ontario. *Canadian Journal of Veterinary Research*, 50(3), 307.
- Ware, L.B., & Matthay, M.A. (2000). The acute respiratory distress syndrome. *N Engl J Med*, 342 (18), 1334-1349.
- Wattiaux, M.A. (2005). Heifer raising - birth to weaning. *Neonatal diarrhea*. Babcock Institute for International Dairy Research and Development, 2005.
- Weil, M.H., & Henning, R. (1979). New Concepts in the Diagnosis and Fluid Treatment of Circulatory Shock: Thirteenth Annual Becton, Dickinson and Company Oscar Schwidetsky Memorial Lecture. *Anesthesia & Analgesia*, 58 (2), 124-132.
- Wudu, T., Kelay, B., Mekonnen, H.M., & Tesfu, K. (2008). Calf morbidity and mortality in smallholder dairy farms in Ada'a Liben district of Oromia, Ethiopia. *Tropical animal health and production*, 40(5), 369-376.
- Zeerleder, S., Hack, C.E., & Wuillemin, W.A. (2005). Disseminated intravascular coagulation in sepsis. *CHEST Journal*, 128 (4), 2864-2875.