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Microbiological characterization and genetic analysis of bacteria isolated from blood cultures and fecal samples in calves with symptoms of septicemia and diarrhea

Caracterización microbiológica y análisis genético de bacterias aisladas de hemocultivos y muestras fecales en terneros con síntomas de septicemia y diarrea

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ABSTRACT

Diarrhea in calves can be caused by bacteria, viruses, and parasites. Among bacteria, *Escherichia coli* is considered responsible for the appearance of enteric diarrhea and septicemia in these animals, conditions that require immediate attention. Among *E. coli* infections of calves, more focus is placed on intestinal pathogenic (InPEC) infections, and extra–intestinal pathogenic (ExPEC) infections are ignored. This study aims to reveal which *E. coli* pathotype causes the infection as molecular and serotype and to reveal the differences according to the age groups of the factors in the herd. Blood and fecal samples of 10 calves aged 3–15 d with diarrhea were analyzed. The primary agent causing enteritis was determined by examining the stool samples with BoviD–5 Ag. Then, samples were subjected to culture and identification processes. It was determined that the stool samples had 2/10 with *E. coli* K99, 4/10 with rotavirus, and 4/10 with mixed rotavirus infections and *Cryptosporidium* spp. *E. coli* was detected from all blood samples by hemoculture. The study isolated only the SepEC and ETEC groups from samples. All SepEC isolates were determined to carry type 1 pilus responsible for adhesion. In addition, it was determined that 9/10 of the SepEC group carried the colicin V gene responsible for pathogenicity. Also, all *E. coli* isolated from calves aged 3–15 d were found to be resistant to antibiotics. In conclusion, primary enteritis is caused by rotavirus *Cryptosporidium* spp. and ETEC. However, it was determined that SepEC group *E. coli* causing septicemia showed different antigenic and genetic features than *E. coli* in the intestinal tract. The virulence factors of the SepEC group may vary due to genomic plasticity, and their antigenic structures should be more closely examined and added to vaccine test studies.

Key words: *Escherichia coli*; septicemia; calf diarrhea; antimicrobial resistance

RESUMEN

La diarrea en los terneros puede ser causada por bacterias, virus y parásitos. Entre las bacterias, *Escherichia coli* se considera responsable de la aparición de diarrea entérica y septicemia en estos animales, afecciones que requieren atención inmediata. El objetivo del estudio fue denificar el patotipo causante de diarrea y septicemia entérica y factores asociados. Se analizó muestras de sangre y heces de 10 terneros de 3–15 d de edad que presentaron diarrea. Los análisis de las heces determinaron *E. coli* K99 en un 2/10, rotavirus el 4/10, rotavirus y *Cryptosporidium* spp. el otro 4/10. El hemocultivo registro presencia de *E. coli* en el 10/10 de las muestras. Todos los aislamientos de SepEC portarón pilus tipo 1 responsable de la adhesión, un 9/10 porto el gen de la colicina V responsable de la patogenicidad. Además, se encontró que todas las *E. coli* aisladas de terneros de 3 a 15 d de edad eran resistentes a los antibióticos. SepEC causante de septicemia mostró características antigénicas y genéticas diferentes a las de *E. coli* en el tracto intestinal. En conclusión, la enteritis primaria es causada por rotavirus, *Cryptosporidium* y ETEC. Se pensó que los factores de virulencia del grupo SepEC pueden variar debido a la plasticidad genómica y sus estructuras antigénicas deberían examinarse más de cerca y agregarse a los estudios de prueba de vacunas.

Palabras clave: *Escherichia coli*; septicemia; diarrea terneros; resistencia antimicrobiana

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INTRODUCTION

Losses due to calf diarrhea are among the largest economic losses for dairy and meat producers [1]. It has been reported that 57 and 53% of fatal diseases in calves under one month of age are diarrhea [2], leading to dehydration, depression, sepsis, and death [3]. Calves born with agammaglobulinemia due to failure of passive transfer immunity (FTPI) cause primary intestinal infection due to bacteremia and a systemic inflammatory response that causes septicemia [4]. Antimicrobial treatment should be applied in cases of diarrhea that present with septicemia, fever, and coma [3] to avoid animals' death due to lack of antimicrobial therapy [5].

Multiple enteric pathogens such as viruses, bacteria, and protozoa are part of the etiology of diarrhea in calves [6], the most common being bovine rotavirus (BRV), bovine coronavirus (BCoV), bovine viral diarrhea virus (BVDV), *Salmonella enterica*, *Escherichia coli*, *Clostridium perfringens*, and *Cryptosporidium* spp. [7]; *E. coli* infections are examined under two pathotypes, intestinal pathogenic *E. coli* (InPEC) and extra-intestinal pathogenic *E. coli* (ExPEC). *E. coli* diarrheas (InPEC) are examined under six patho groups; enterotoxigenic *E. coli* (ETEC), enterohemorrhagic/shiga toxin-producing *E. coli* (STEC/EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and in extra-intestinal *E. coli* infections (ExPEC) [8].

Of these bacteria, ETEC is the most common cause of diarrhea in calves. ETEC adheres to epithelial cells with fimbriae antigen (commonly known as F5) and secretes heat-labile toxin (LT) and heat-stable toxin (ST) [9, 10]. Because the pH is less than 6.5, the epithelial cells of the distal part of the small intestine are most suitable for colonization by ETEC, causing villous atrophy and damage to the lamina propria [11].

In ExPEC pathotype O6, O8, O11, O15, O20, O25, O27, O78, O128, O148, O149, O159, O173 somatic antigens are dominant [12]. Extra-intestinal infections are those causing septicemia, urinary tract infections, and neonatal meningitis produced by (ExPEC) such as (SepEC) septicemia associated *E. coli*, (UPEC) uropathogenic *E. coli* and (NMEC) neonatal meningitis *E. coli*. It has been reported that this group of pathogens has various virulence factors (such as toxins, polysaccharide capsule, adhesin, invasin, iron acquisition factors, and lipopolysaccharides) and genomic plasticity [13]. SepEC bacteria adhere to the mucosal surface, colonizing it with adhesins. Following intestinal infection, the mucosal surface is destroyed [14]. SepEC resists the antibacterial activity of the calf's innate immune system. Thanks to the siderophores, the bacteria can multiply in iron-limited tissues and then pass to the circulatory system and all internal organs [15].

This study aimed to determine the relationship between bacterial, viral, or parasitic agents that cause enteric infections. It was also aimed to reveal the relationship between phenotypic/genotypic characteristics between SepEC in the bloodstream and *E. coli* strains in gut microbiota.

MATERIALS AND METHODS

Collection of samples

Newborn calves were monitored on the farm with a capacity of 3,500 dairy cows in Konya-Turkiye, which presented symptoms of diarrhea and sepsis during the cold and wet seasons of December and January. Blood and feces samples were collected, following the methodology proposed by Fecteau [16], classifying diarrheal feces according to color and mucus. At least 10 mL of blood was taken directly from the jugular vein until it reached the line specified on the aerobic blood culture bottle (BACT/ALERT® FA PLUS BMX410851). Ten g of feces from the rectums of calves with diarrhea were collected into fecal collection containers (10022-168, VWR) containing peptone water. The samples were delivered to the laboratory for analysis on the same day via cold chain.

Evaluation of stool samples with rapid lateral flow test

The field lateral flow test named BoviD-5 Ag (RG13-02, Bionote, USA) was used according to the kit instructions to determine the agent causing diarrhea from diarrheal stool samples. A sample was taken from the diarrheal stool with a swap. The swap containing the feces was transferred to the sample tube containing the assay diluent and was homogenized until the feces was separated from the swap. It was waited for 30 s for the sediments to settle. And the supernatant was taken, and 4 drops were added to each well for diagnose (bacterial; *E. coli* K99, viral; rotavirus and coronavirus, protozoal; *Giardia* spp. and *Cryptosporidium* spp.). It was waited for 10 min for the test and control lines to take shape.

Microbiological examination of blood samples (blood culture) and agent isolation-identification

The blood culture bottle was incubated at 37°C 50 rpm in a shaking incubator (MIR-254-PE, Panasonic) [17]. Blood cultures were passaged at 24, 48, 72 h intervals onto blood agar (NCM0075A, LabM), selective media MacConkey agar (MC) (70143, Sigma Aldrich), and Eosin Methylene Blue agar (EMB) (70186, Sigma Aldrich) and petri dish with media incubated 16-24 h at 37 °C and 5% CO_2. At the end of incubation, lactose-positive *E. coli* colonies were observed as metallic green colonies on EMB agar and pink-colored colonies on MC agar.

Microbiologic examination of stool samples and isolation and identification of agents

For *E. coli* isolation, each stool sample transported to the laboratory, one drop of feces was passaged on MC and EMB agar, which are selective media for *E. coli* isolation, and incubated at 37°C for 16-24 h. At the end of incubation, lactose-positive *E. coli* colonies formed metallic green-colored colonies on EMB agar and pink-colored colonies on MC agar [18]. For Salmonella isolation, 1 g of stool was passaged in 9 mL peptone water (CM1049, Lab M) for 16-24 h at 37°C for pre-enrichment. Then, 1 mL of the peptone enriched sample was transferred to new tube containing 9 mL of Rappaport Vassiliadis Salmonella Enrichment Broth (HP007, LabM), which is a Salmonella selective enrichment medium, and incubated at 42°C for 24 h. Then, a loopful of the enriched medium was passaged to XLT-4 selective agar (CM1061, Thermo Scientific) and incubated at 37°C for 48 h and observed whether black-colored Salmonella colonies would form [19].

Biochemical Identification of enteric Gram-negative bacteria

All bacterial isolated that grew on MC agar or were detected as Gram-negative bacilli by Gram staining were biochemically confirmed with a tipped tube test. The agents were evaluated in terms of lactose-glucose-H_2S in the first tube, mannitol movement in the second tube, and urea-indole in the third tube, and the bacteria were identified according to the report by Lassen, 1975 [20].

Serotyping of isolated *Escherichia coli*

The strains were stored at -80 °C with the appropriate code number. Since the ETEC group expresses fimbriae antigen, all isolated *E. coli* were passaged on MINCA agar (potassium dihydrogen phosphate 1.36 g·L^{-1}, disodium hydrogen phosphate 8.05 g·L^{-1}, casamino acids 1.0 g·L^{-1}, glucose 1.0 g·L^{-1}, agar 15.0 g·L^{-1} trace salt solution 1 mL).

The latex test detected *E. coli* isolated on MINCA agar with F5 (K99) antiserum (51173, SSI Diagnostica). For detecting somatic antigens, *E. coli* to be passaged on MINCA Agar, antiserum Pool1 EPEC/VTEC/STEC O26, O103, O111 EAEC, O145, O157 (44292, SSI Diagnostica København, DK), antiserum Pool2 EPEC O55, O119, O125ac, O127, O128ab (44293, SSI Diagnostica København, DK) were confirmed by latex testing with antiserum Pool3 EPEC O86, O114, O121, O126, O142 (77713, SSI Diagnostica København, DK).

Also, these pool antigens could detect EAEC serotypes such as O127 in Pool2 and O86 in Pool3. In addition, the isolated strains were heat inactivated in an autoclave (NC100, Nuve) at 121°C under 1 ATM pressure for 75 min, SAT antigens (~108·mL^{-1}) were prepared, and serotyping was performed with E. coli O1 STEC, O2 STEC, O8 ETEC, O9, O33, O38, O78 ETEC/EAEC and O101 ETEC monoclonal antisera (SSI Diagnostica København, DK) according to lace formation by SAT method in microplate [21]. The strains were serotyped with a total of 23 different somatic antigens. For selective isolation of VTEC, STEC, EHEC Group, *E. coli* O157:H7 was identified by passaging on sorbitol MacConkey agar *E. coli* O157:H7 MUG Agar (44782, Merck) and confirmed by latex test with antiserum pool1 (44292, SSI Diagnostica). The strains were also passaged on blood agar containing 5% sheep blood to detect the presence of hemolysin enzymes. Nonmotile, lactose-negative colonies grown on EMB and MC agar were typed as EIEC. They were also molecularly confirmed by polymerase chain reaction (PCR). *E. coli* that did not fit the other typing classes were molecularly typed with aggregative features and confirmed with Pool2 and Pool3 antisera and named EAEC. *E. coli* O9 K99 ATCC 31616, *E. coli* O157 ATCC 43895, and non-pathogenic *E. coli* ATCC 25922 were positive controls.

Determination of antibiotic susceptibility of isolates

The antibiotic resistance of the isolates was determined by the disk diffusion method. *E. coli* isolates were incubated at 37°C on tryptic soy broth (1054590500, Sigma Aldrich) for 12 h. After being adjusted to McFarland (DEN, Biosan) 0.5 standard turbidity, they were passaged onto 100 mL of Mueller Hinton agar (70191, Sigma Aldrich), spread with a drigalski and incubated at 37°C for 12 h [22].

Zone diameters were recorded in mm. Antibiotic resistances were determined according to Clinical and Laboratory Standards Institute (CLSI) 2022 data. As a result of antimicrobial testing, it is defined as resistant (R) if the zone cap is smaller than the CLSI breaking point ranges, intermediate (I) if it is in the range, and sensitive (S) if it is larger or equal. If the bacterial isolate was sensitive to 3 of the antibiotic groups, it was categorized as multidrug-resistant (MDR); if it was sensitive to two or only one antimicrobial group, it was categorized as extensively drug-resistant (XDR); and if it was resistant to all of them, it was categorized as pan drug (PDR) [23].

An analysis was made to determine the most preferred antimicrobial agents in enteric therapy in calves and their resistance to imipenem, which is preferred in human use. These antibiotics list: P: Penicillin, AMC: Amoxacillin, CFP: Cefoperazone, CRO: Ceftriaxone, TE: Tetracycline, IPM: Imipenem, SXT: Trimethoprim/sulfamethoxazole, CN: Gentamicin, ENR: Enrofloxacin, E: Erythromycin. The clinical breakpoint was based on the resistance data of CLSI veterinary isolates. For quality control strains, *E. coli* ATCC 25922 were tested with the isolates.

DNA isolation from stool samples

According to the kit instructions, deoxyribonucleic acid (DNA) was isolated from diarrhea stools using the QIAamp DNA Stool Mini Kit (51604, Qiagen). The quality and quantity of DNA isolates were measured with a spectrophotometer (Nanodrop 2000, Thermo Scientific).

Identification of bacteria with molecular methods

DNA was isolated from the pre-identified agents according to the Wizard® Genomic DNA Purification Kit protocol. The concentrations of the isolated DNA were determined by spectrophotometer (Nanodrop 2000, Thermo Scientific). Set 1, set 2, and set 3 multiplex primers were designed according to the study of Lee [24], and set 4 multiplex primers were designed according to the study of Oh [25] and Vandekerchove [26]. The primer pairs specified in TABLE I in the project were used.

For the PCR mixture, five µL Master mix (5x), 0.1 µL forward primer (10 pmol/ µL, 0.1 µL reverse primer (10 pmol·µL^{-1}), two µl DNA (100 ng·µL^{-1}) 17.9 µL sterile nuclease-free water were added for a total volume of 25 µL. The thermal cycle (T100, Bio-Rad) was repeated 34 times with a pre-denaturation step at 94°C for 10 min, followed by 94°C denaturation for 1 min, 60°C binding for 1 min, 72°C extension for 1 min, and final extension at 72°C for 10 min. A 1% agarose gel was prepared for electrophoresis (maxicell-minicell, EC Apparatus Corporation) of PCR products. Ethidium bromide was added to the gel to a final concentration of 0.5 µg·mL^{-1}. Gel wells were loaded with five µL each of PCR products and 100 bp DNA ladder. The results were visualized by a gel imaging device (212 Pro, Gel-Logic).

RESULTS AND DISCUSSION

In the herd from which samples were collected, rotavirus was detected in 4/10 feces with diarrhea in newborn calves. In the other 4/10, rotavirus and *Cryptosporidium* spp. were detected as mixed. *E. coli* K99 was detected in only 2/10 (TABLE II). In all animals, the fever was around 40°C, the hair was fluffy, and the mucous membranes were dehydrated. Only animal number 4 with rotavirus diarrhea had a fever of 41°C (FIG. 1). It was determined that the age range of animals infected with rotavirus and *Cryptosporidium* spp. was between 10-15 d. Animals with *E. coli* K99 diarrhea were aged 3-4 d, and this finding is compatible with other studies [27, 28]. *Salmonella* spp. and *Giardia* spp. were not detected in fecal samples with diarrhea. A study conducted with 300 calves with diarrhea in the region reported that 40% of calves in the 15-29-day age group had rotavirus infection, and 12.9% had rotavirus + *Cryptosporidium* spp. infection [29]. In the prevalence studies of the agents causing diarrhea in calves in Türkiye, *E. coli* was detected at a rate of 9.4% -27.45% [30, 31, 32, 33, 34].

Since studies of calf diarrhea generally focus on diarrheal agents (bacteria, virus, or protozoa), the bacteria causing septicemia are overlooked. However, diarrhea and septicemia calves' prevalence are approximately 9.26%-31% [5, 35, 36]. In this study, no pathogens other than *E. coli* were isolated from blood cultures. All the isolated strains were confirmed to be *E. coli* with differential media MC and EMB media. In addition, the results were supported by the triple tube method. All strains did not show hemolysis on blood agar, and *E. coli*

TABLE I
Primer sets used in the study

Bacteria	Gene	Primer	PCR Product (bp)	Multiplex Set
E. coli ETEC	F4	F– GCCTGGATGACTGGTGATTT / R– TCTGACCGTTTGCAATACCC	709	Set 1
E. coli ETEC	F5	F– TTGGGCAGGCTGCTATTAGT / R– TAGCACCACCAGACCCATTT	222	Set 1
E. coli ETEC	F6	F– GCGTGCATCGAAATGAGTT / R– GGTGGTTCCGATGTATGCTT	589	Set 1
E. coli VTEC	F18	F– CTTTCACATTGCGTGTGGAG / R– ATTCGACGCCTTAACCTCCT	444	Set 1
E. coli ETEC	F41	F–GGAGCGGGTCATATTGGTAA / R– CTGCAGAAACACCAGATCCA	941	Set 1
E. coli ETEC	STa	F– GAAACAACATGACGGGAGGT / R– GCACAGGCAGGATTACAACA	229	Set 2
E. coli ETEC	STb	F– CCTACAACGGGTGATTGACA / R– CCGTCTTGCGTTAGGACATT	480	Set 2
E. coli ETEC	LT	F– GGTTTCTGCGTTAGGTGGAA / R– GGGACTTCGACCTGAAATGT	605	Set 2
E. coli SHEC	Stx2e	F– TGGTGTCAGAGTGGGGAGAA / R– TACCTTTAGCACAATCCGCC	351	Set 2
E. coli EHEC	EAST1	F– CCATCAACACAGTATATCCGA / R– GGTCGCGAGTGACGGCTTTGT	111	Set 2
E. coli EHEC	*fimA*	F– TGGTGGGACCGTTCACTTTA / R– AAGGTCGCATCCGCATTAG	443	Set 3
E. coli EHEC	*fimH*	F– ATGAAACGAGTTATTACCCTGTTTG / R– TTATTGATAAACAAAAGTCACGCC	903	Set 3
E. coli EPEC	AIDAI	F– TGGTGGGAAAACCACTGCTA / R– TAGCCGCCATCACTAACCAG	771	Set 3
E. coli EPEC	pAA	F– CCATAAAGACAGCTTCAGTGAAAA / R– GTATTACTGGTACCACCACCATCA	162	Set 3
E. coli EAEC	*aggR*	F– TTAAAATAAGTCAARAATTGTTTTGGTGTTA / R–ATTATAAAAATTAACAATATCAGAATACATCAGTACAC	715	Set 4
E. coli EIEC	*ipaH*	F– CCTTTTCCGCGTTCCTTGA / R– CAGCAGCAACAGCGAAAGAC	104	Set 4
E. coli SepEC	*cvaC*	F– TTTCGACACCCCGGTAAAGG / R– TGTCAGTCTGGTTTACGGGC	242	Set 4

TABLE II
Physiological conditions and Bovid–5ag test results visualization of the calves sampled.

Calf No	Age (days)	Fever	Dehydration	Feces	Bovid–5ag Test Result
1	11	39.9	+++	Mucoid yellow diarrhea	Rotavirus + *Cryptosporidium* spp.
2	15	39.7	+++	Mucoid diarrhea	Rotavirus + *Cryptosporidium* spp.
3	12	40.2	+++	Mucoid yellow diarrhea	Rotavirus + *Cryptosporidium* spp.
4	11	41	+++	Mucoid diarrhea	Rotavirus
5	14	39.8	+++	Mucoid diarrhea	Rotavirus
6	15	40	+++	Mucoid yellow diarrhea	Rotavirus + *Cryptosporidium* spp.
7	4	39.5	+++	Mucoid yellow diarrhea	*E. coli* K99
8	3	39.6	+++	Mucoid yellow diarrhea	*E. coli* K99
9	10	39.2	+++	Mucoid diarrhea	Rotavirus
10	9	39.6	+++	Mucoid diarrhea	Rotavirus

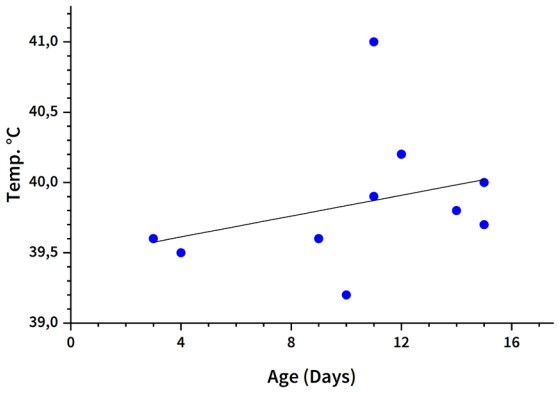

FIGURE 1. Age and temperature distribution in infected calves *P*<0.05

strains showed S colony characteristics. It was determined that the *E. coli* strains isolated from feces (numbered 4, 5, 7, and 8) showed white color growth on sorbitol MC agar, which is characteristic of *E. coli* O157 (EHEC) (TABLE III). This feature was not detected in any *E. coli* strains isolated from blood. In another study, researchers found that 7% of calves with bacteremia symptoms showed growth from blood cultures, and 20% of the positive blood cultures were identified as *E. coli* [35]. However, in another study on this subject, researchers emphasized that 80% of septicemia was caused by *E. coli* [37]. These researchers reported that in addition to *E. coli*, *Salmonella* spp., *Enterobacter aerogenes*, *Campylobacter fetus*, *Klebsiella* spp., *Streptococcus dysgalactiae*, and *Trueperella pyogenes* were isolated from blood cultures of septicemic calves [35, 37].

E. coli strains are serologically classified according to the antigenic differences of the 173 O (somatic) and 56 H (flagellar) antigens, based on the typing scheme according to the rule laid down by Kauffmann in 1947 [38]. *E. coli* isolated from blood 6/10 (4, 5, 6, 8 and 9, 10) carry OK3 (O86, O114, O121, O126, O142) group somatic antigens. In calves 1 and 3, fecal and blood–isolated *E. coli* carry the O33 (2/10) somatic antigen. Animal number 3 also carries the *E. coli* K99 fimbriae antigen isolated from feces. *E. coli* isolated from the blood of the same calf

TABLE III
Biochemical properties and colony structure of *E. coli* isolated from blood culture and fecal samples in differential medium

Strains	Colony Morphology	Sorbitol MC	MC	EMB	Blood Agar	Hemolysis
ATCC 31616	S	–	+	+	+	–
ATCC 43895	S	+	+	+	+	–
ATCC 25922	S	–	+	+	+	–
1D*	S	–	+	+	+	–
1K*	S	–	+	+	+	–
2D	S	–	+	+	+	–
2K	S	–	+	+	+	–
3D	S	–	+	+	+	–
3K	S	–	+	+	+	–
4D	S	+	+	+	+	–
4K	S	–	+	+	+	–
5D	S	+	+	+	+	–
5K	S	–	+	+	+	–
6D	S	–	+	+	+	–
6K	S	–	+	+	+	–
7D	S	+	+	+	+	–
7K	S	–	+	+	+	–
8D	S	+	+	+	+	–
8K	S	–	+	+	+	–
9D	S	–	+	+	+	–
9K	S	–	+	+	+	–
10D	S	–	+	+	+	–
10K	S	–	+	+	+	–

*D: *E. coli* isolated from fecal origin, *K: *E. coli* isolated from blood origin

does not have this feature. *E. coli* isolated from the blood of calf number 2 carries the somatic antigen O78 (1/10). *E. coli* isolated from the blood of calf number 7 has an O8 (1/10) antigen feature. It was determined that *E. coli* strains isolated from the feces of calves 7 and 8 calves contained K99 fimbriae antigen. Thirty percent of the strains were negative for somatic antigen and capsular antigen. The strains isolated from the feces and blood of calf number 1 showed different somatic antigen characteristics (TABLE IV). In another study, it was determined that ExPEC strains isolated from Pigs carried somatic antigens O161, O8, O11, O138, O101 and O26 [39]. Although the O somatic antigen is generally used for typing ETEC strains [40], it has been tried to determine which somatic antigen the SepEC strains have.

Isolated *E. coli* strains were tested with penicillin, B-lactam combination, 3rd generation cephalosporin, carbapenem, aminoglycoside, macrolide, tetracycline, quinolones, folate antagonists according to CLSI 2022 data. Of the 20 *E. coli* strains (ten from blood and ten from feces) isolated, three fecal and eight blood cultures isolated were MDR, and seven fecal and five blood cultures isolated were XDR. None of the *E. coli* isolates are PDR.

E. coli strains isolated from both the feces and blood of animals 1, 7, and 8 were found to be XDR. *E. coli* strains of animals 2 and 6 were found as MDR. Differences were observed in antibiotic susceptibility of *E. coli* isolated from the blood and feces of animals 3, 4, 5, 9, and 10. While *E. coli* (SepEC) isolated from the blood of animals 3 and 4 were XDR, *E. coli* strains isolated from the same animals' feces were detected as MDR. In animals 5, 9, and 10, *E. coli* isolated from their feces was detected as XDR, while *E. coli* isolated from the blood of the same animals was detected as MDR.

TABLE IV
Evaluation of isolated *E. coli* strains in terms of somatic and fimbriae antigen presence

	OK1	OK2	OK3	K99	O1	O2	O8	O9	O33	O38	O78	O101
ATCC 31616	–	–	–	+	–	–	–	+	–	–	–	–
ATCC 43895	+	–	–	–	–	–	–	–	–	–	–	–
ATCC 25922	–	–	–	–	–	–	–	–	–	–	–	–
1D*	–	–	–	–	–	–	–	–	+	–	–	–
1K*	–	–	–	–	–	–	–	–	+	–	–	–
2D	–	–	–	–	–	–	–	–	–	–	–	–
2K	–	–	–	–	–	–	–	–	–	–	+	–
3D	–	–	–	+	–	–	–	–	+	–	–	–
3K	–	–	–	–	–	–	–	–	+	–	–	–
4D	–	–	–	–	–	–	–	–	–	–	–	–
4K	–	–	+	–	–	–	–	–	–	–	–	–
5D	–	–	–	–	–	–	–	–	–	–	–	–
5K	–	–	+	–	–	–	–	–	–	–	–	–
6D	–	–	–	–	–	–	–	–	–	–	–	–
6K	–	–	+	–	–	–	–	–	–	–	–	–
7D	–	–	–	+	–	–	–	–	–	–	–	–
7K	–	–	–	–	–	–	+	–	–	–	–	–
8D	–	–	–	+	–	–	–	–	–	–	–	–
8K	–	–	+	–	–	–	–	–	–	–	–	–
9D	–	–	–	–	–	–	–	–	–	–	–	–
9K	–	–	+	–	–	–	–	–	–	–	–	–
10D	–	–	–	–	–	–	–	–	–	–	–	–
10K	–	–	+	–	–	–	–	–	–	–	–	–

*D: *E. coli* isolated from fecal origin, *K: *E. coli* isolated from blood origin

All *E. coli* strains isolated in the study were found to have resistance properties to antimicrobial agents (TABLE V). All isolates were susceptible only to imipenem, which is the carbapenem group. It was determined that the only antimicrobial agent that could be recommended for treatment was 3rd generation cephalosporins. In general, the SepEC group is more resistant than the InPEC group. The bacteria sharing the same flora acquire such different resistance mechanisms, which is explained by the fact that the SepEC group has gene plasticity. Most of the isolates obtained are XDR, and the remainder are MDR. In another study, it was reported that MDR and XDR strains were isolated from *E. coli* isolated from calf diarrhea [41]. It is clear that antibiotics are needed to fight bacteria; therefore, antibiotic resistance is a critical issue in line with the concept of one health [42]. The use of most antibiotics for the treatment of *E. coli* diarrhea in calves shows the development of resistance due to malpractices since the effective therapeutic concentration is not reached in the intestine. In addition, there are calves with a maximum of 15 d of age in the study group.

DNA samples isolated from bacteria and stool were evaluated by Set 1 multiplex PCR for the gene encoding F4 (715 bp), F5 (222 bp), F6 (589 bp), F18 (441 bp), F41 (941 bp) fimbriae antigens. Only the positive control with *E. coli* O9 K99 somatic characteristics, ATCC 31616, was genetically positive for the F5 (K99) fimbriae antigen 222 bp gene region (FIG. 2). All *E. coli* isolated from fecal DNA, blood, and stool–causing diarrhea and septicemia were similarly negative for ETEC gene characteristics.

TABLE V
Antibiotic resistance results of isolated *E. coli* strains according to CLSI 2022

	P–10	AMC–20/10	CFP–75	CRO–30	TE–30	IPM–10	SXT–25	CN–10	ENR–5	E–15
1D*	R	R	S	S	R	S	R	R	R	R
1K*	R	R	R	S	R	S	R	R	R	R
2D	R	R	S	I	R	S	R	R	R	R
2K	R	R	S	S	R	S	S	S	S	R
3D	R	R	S	S	R	S	R	S	R	S
3K	R	R	R	R	R	S	R	R	R	R
4D	R	R	S	S	R	S	R	S	R	R
4K	R	R	S	S	R	S	R	R	R	R
5D	R	R	R	R	R	S	R	R	R	S
5K	R	R	S	S	R	S	R	R	I	S
6D	R	R	R	S	R	S	R	S	R	S
6K	R	R	R	R	R	S	R	S	R	S
7D	R	R	R	R	R	S	R	R	R	R
7K	R	R	R	R	R	S	R	R	R	R
8D	R	R	R	R	R	S	R	R	R	S
8K	R	R	R	R	R	S	R	R	R	R
9D	R	R	S	R	R	S	R	R	R	R
9K	R	R	S	S	R	S	R	R	I	R
10D	R	R	R	S	R	S	R	R	R	R
10K	R	R	S	S	R	S	R	R	R	S

P: Penicillin, AMC: Amoxacillin, CFP: Cefoperazone, CRO: Ceftriaxone, TE: Tetracycline, IPM: Imipenem, SXT: Trimethoprim/sulfamethoxazole, CN: Gentamicin, ENR: Enrofloxacin, E: Erythromycin.*D: *E. coli* isolated from fecal origin, *K: *E. coli* isolated from blood origin

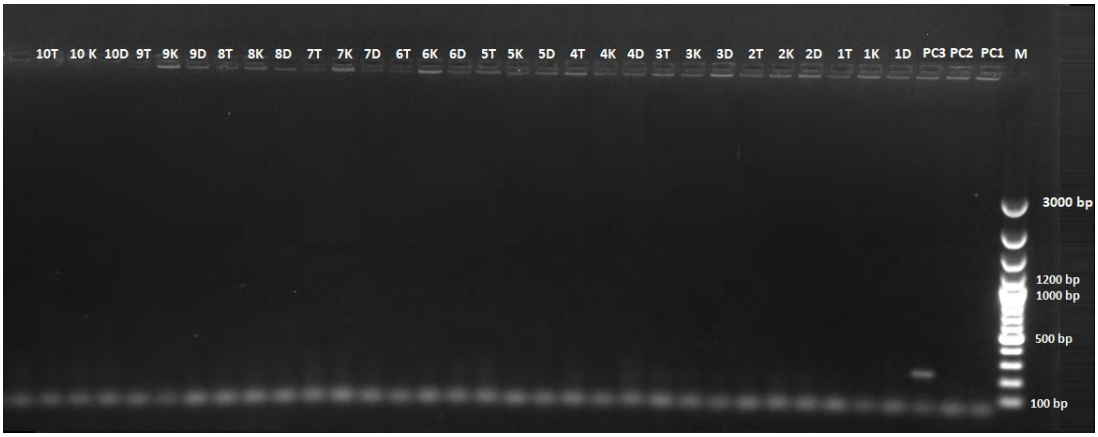

FIGURE 2. Multiplex PCR result with Set 1 primers for detection gene of fimbriae antigens; PC1–ATCC 25922, PC2–ATCC 43895, PC3–ATCC 31616. T: Whole DNA isolated from feces, D: *E. coli* DNA isolated from feces, K: *E. coli* DNA isolated from blood

The DNA samples were evaluated by Set 2 multiplex PCR for enterotoxin-encoding genes such as STa (229 bp), STb (480 bp), LT (605 bp), Stx2e (351 bp) and EAST1 (111 bp). Only the positive control, ATCC 43895 *E. coli* O157, was positive for the 351 bp gene region encoding the Shiga toxin named Stx2e (FIG. 3). *E. coli* O157 EHEC strain exists as a reservoir of cattle, so products of animal origin are known to cause human food infection. In addition, studies indicate that EHEC strains can infect calves in less than 36 h, even though they have asymptomatic reservoirs in adult cattle [43]. Although some bacteria (fecal samples of calves 4, 5, 7 and 8) show EHEC features biochemically, it has been determined that they do not have these features serotypically and genetically. In addition, it was determined that SepEC colonies isolated from the blood of these animals did not form white colonies on Sorbitol MC agar. It was thought that they did not have O157 somatic antigen because they did not acquire a pathogenic form in which this feature could develop due to being a reservoir.

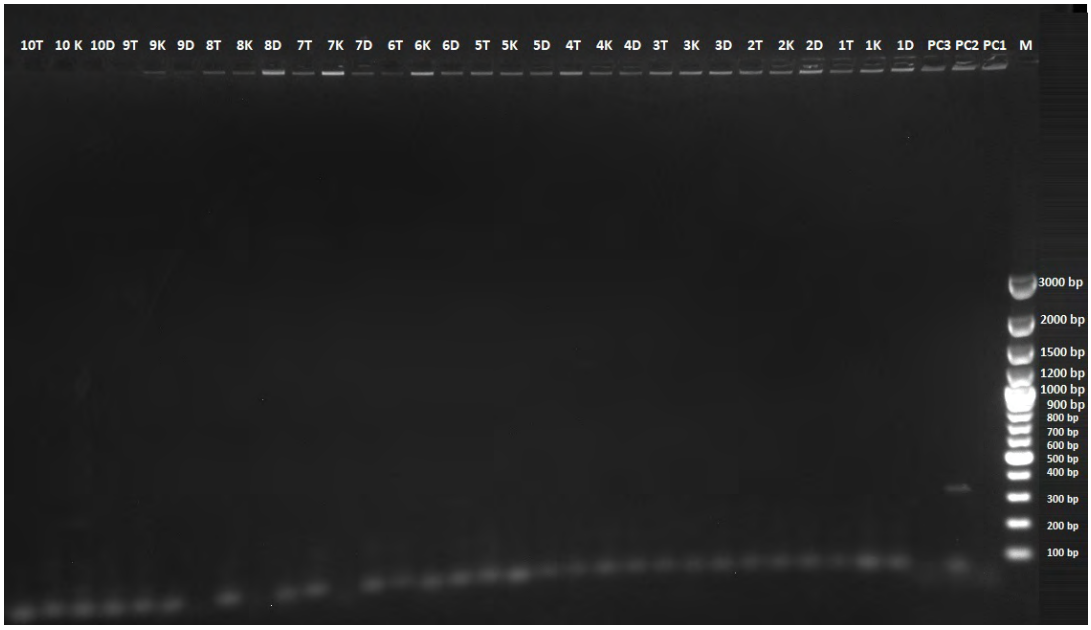

FIGURE 3. PCR result with Set 2 primers for the detection of enterotoxin gene; PC1–ATCC 25922, PC2–ATCC 43895 *E. coli* O157, PC3–ATCC 31616. T: Whole DNA isolated from feces, D: *E. coli* DNA isolated from feces, K: *E. coli* DNA isolated from blood

Molecularly, for the detection of genes responsible for adhesin with Set 3 primers, type 1 pilus (*fimA* 443 bp and *fimH* 903 bp), the adhesin gene AIDA-I (771 bp) related to diffuse adhesion, and pAA (162 bp) gene regions for A/E lesions were evaluated. Samples with positive *fimA* and *fimH* gene regions simultaneously were evaluated as type 1 pilus positive. The *fimA* and *fimH* genes, which encode type 1 pilus antigen, were found to be positive at the same time in all agents causing septicemia (SepEC). The positive control, *E. coli* O9 K99 ATCC 31616 (PC1) type 1 pilus was found positive. Non-pathogenic *E. coli* ATCC 25922 (PC2) was found positive only for the *fimA* gene. For this reason, it was evaluated as negative for type 1 pilus antigen (FIG. 4).

ATCC 43895 (PC3) with *E. coli* O157 was found to be positive in terms of type 1 pilus and pAA gene region causing A/E lesion (FIG. 4). ExPEC is known to have a wide variety of virulence factors; Adhesins such as Type 1 fimbriae, P fimbriae, S fimbriae, invasin such as Ibe ABC, iron uptake antigens such as aerobactin, intracellular survival antigen such as outer membrane protein and colonization factors such as cvaC colicinV and cytotoxins [12, 44]. In a previous study with calves with septicemia, the virulence genes of SEPEC isolates were examined, and aerobactin was found in 88%, and the gene responsible for 80% of fimbriae-associated adhesion [37]. In the study, type 1 fimbriae set 3 multiplex PCR, responsible for its adhesion, and cvaC gene, responsible for SEPEC colonization, were studied in set 4 multiplex PCR. It was determined that 10/10 of the SepEC strains examined in this study carried type 1 fimbriae, and 9/10 had the cvaC gene.

Set 4 multiplex PCR prepared for the last two pathotypes causing intestinal infections (InPEC), EAEC (aggR 715 bp gene region), EIEC (ipaH 104 bp), and for the detection of extra-intestinal infections ExPEC, SepEC pathotype, colicin V gene region cvaC 242 bp for all samples tested. All samples and control DNAs were molecularly negative for EAEC and EIEC. Positive controls and all samples were positive for the colicin gene region. Only all fecal DNA obtained from sample number 10 and *E. coli* DNA isolated from feces and blood culture were negative for the colicin V (cvaC) gene region (FIG. 5). EAEC and EIEC strains were not isolated in the study. These pathotypes have often been associated with human infections.

In other studies, the ratio of EAEC (0.9%) and EIEC (0%), EPEC (2.9%), and ETEC (1.9%) was isolated from the feces of 113 newborn calves [45]. It has been reported that the significant source of EIEC infection for humans is an infection of fecal-oral origin, with chronic diarrhea generally seen in underdeveloped Countries. No animal reservoir has been reported before [46]. The Colicin V gene is among the virulent factors in the ExPEC bacterial group. Another finding in

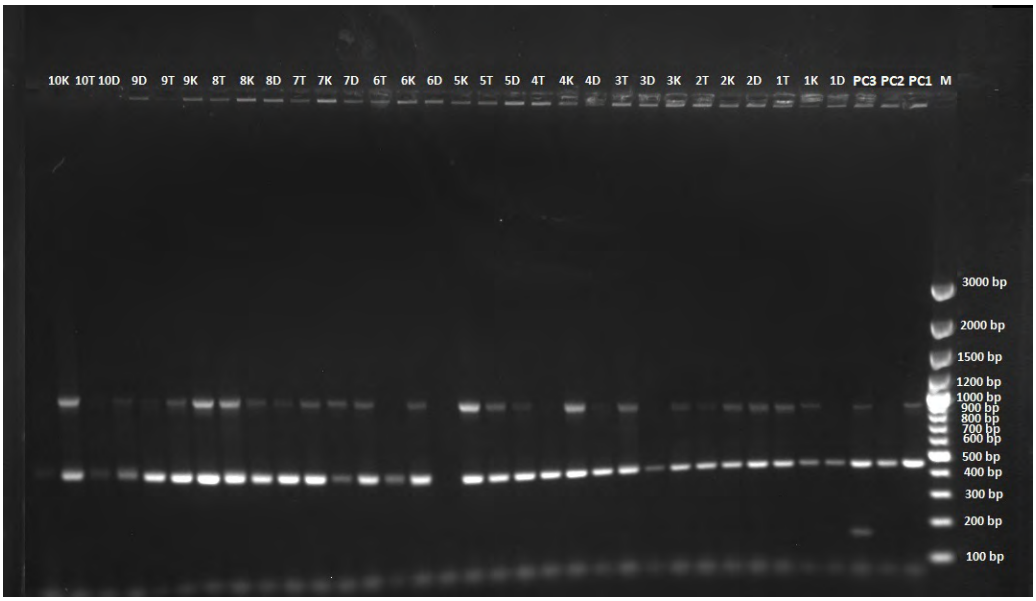

FIGURE 4. Set 3 multiplex PCR results used for the detection of gene regions responsible for adhesion, such as type 1 pilus, AIDA–I and pAA; PC1–ATCC 31616, PC2–ATCC 25922, PC3–ATCC 43895. T: Whole DNA isolated from feces, D: *E. coli* DNA isolated from feces, K: *E. coli* DNA isolated from blood.

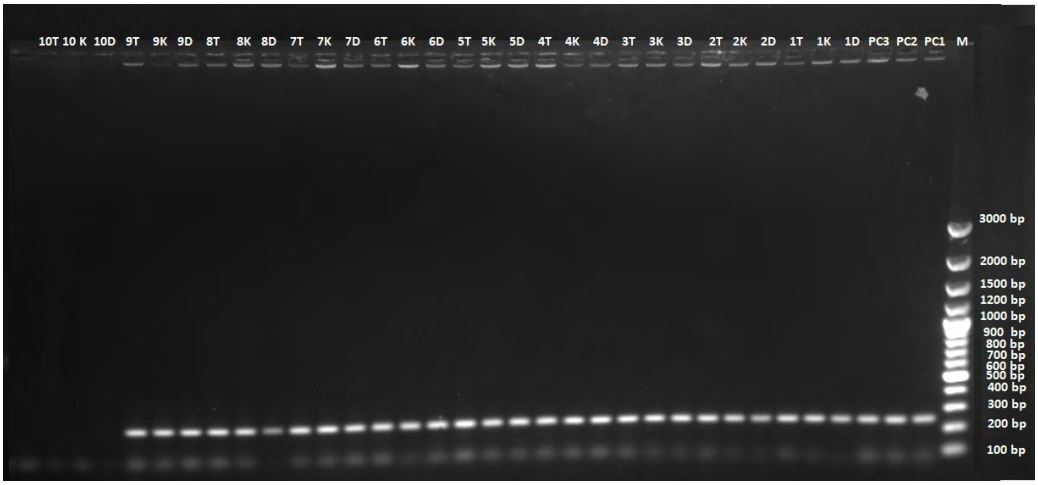

FIGURE 5. Set 4 multiplex PCR result for detection of aggR (EAEC), ipaH (EIEC) and cvpaC (SepEC) gene regions. T: Whole DNA isolated from feces, D: *E. coli* DNA isolated from feces, K: *E. coli* DNA isolated from blood

the study is the presence of plasmid carrying the CvaC gene, which is the Colicin V gene, in the SepEC strains. Samples were positive for this gene CvaC, except samples from animal number 10. It is usually produced at a time of stress encountered by the bacterium. It is thought to be secreted into the intestinal tract to colonize the surface, reducing bacterial competition efficiently. Colicin has also been reported to have a toxic effect on eukaryotic cells, and its primary virulence is formed this way [47]. This gene region has been found in human SepEC and UPEC cases. It is also known that another ExPEC group, the Avian Pathogen *E. coli* (APEC) group bacteria, carries these virulence features.

In the study, no isolates with EPEC, EHEC, EAEC, and EIEC morphological biochemical serotype or genetic characteristics could be detected. Only two ETEC group isolates were obtained from InPEC *E. coli* diarrhea. According to the study results, ETEC, rotavirus, and *Cryptosporidium* spp. cause infection in the intestinal tract, which causes diarrhea symptoms, and SepEC causes septicemia.

In the diarrheal feces screened with the bovid–5ag test, animal numbers 7 and 8 were positive for *E. coli* F5 and were found to carry F5 fimbriae antigen by serotyping test. However, it was determined that the same isolates did not genetically carry the F5 gene. In addition, it was determined that SepEC isolates that cause septicemia in the blood did not carry the K99 antigen in serotyping. Another current concept in *E. coli* infections is the presence of hybrid or heteropathogenic *E. coli* strains. Heteropathogenic strains such as EPEC/ETEC, ExPEC/STEC, and ExPEC/EPEC have been reported

to cause more severe infections [48]. It has also been reported that these strains are prone to genetic change [49]. While there was an ETEC group carrying K99 in feces in this study, the absence of K99 fimbriae in SepEC isolates isolated from blood shows that the ETEC/ExPEC group may be a heteropathogenic infection derivative. ExPEC strains carry different combinations of virulence factors such as P fimbriae, S/F1C fimbriae subunits, Dr-antigen-binding adhesins, aerobactin receptor, and group 2 capsule synthesis, and colicin V [50].

The most important condition of ExPEC infections is colonization of the intestinal wall. In the study, SepEC group microorganisms, due to the pathogenesis and deterioration in peristaltic movements caused by infections caused by ETEC, rotavirus, and *Cryptosporidium* spp. in the intestinal tract, provide colonization by providing adhesion with type 1 pilus. It was also determined that SepEC isolates may carry O8, O33, O78 and OK3 (O86, O114, O121, O126, O142) group somatic antigens. Notably, 6/10 of SepEC isolates carry the OK3 group somatic antigen. Except for animal number 8, the common feature of this group is that the primary infection is caused by rotavirus. It is thought that the SepEC group isolates present in the flora as a result of the damage caused by ETEC, rotavirus / *Cryptosporidium* spp. infection in enterocytes performs pathogenicity with the aforementioned somatic antigens (adhesin). This view is also supported by the set 3 multiplex PCR and rapid lateral flow test results. The positive presence of type 1 fimbriae and Colicin V gene regions in DNA isolated from whole feces indicates that SepEC agents are opportunistic in the flora. However, bacteria isolated from feces exhibit morphologically different characteristics from SepEC isolates. This condition may be related to the change in the antigenic structure of the bacteria as they cross the intestinal mucosa.

CONCLUSIONS AND IMPLICATIONS

While all calf diarrhea studies or field treatments focus on InPEC diarrhea, such as ETEC and EPEC, the opportunistic pathogen SepEC bacteria are overlooked. As stated in the working hypothesis, it was determined that rotavirus and *Cryptosporidium* spp. was primary agent for diarrhea. While ETEC group *E. coli* caused less damage to the intestinal mucosa, and it was observed that the pathogenesis was made by the opportunistic pathogen SepEC group. This result shows that the generalization about the same agent in calf diarrhea causing enteric infection with septicemia should be abandoned. Rapid lateral flow tests for calf diarrhea should be used for early diagnosis and successful treatment in farm field conditions. Interestingly, although the SepEC originates from the intestinal flora, it has been observed to have different morphological, antibiotic resistance, serotype, and genetic features from InPEC. *E. coli* vaccine studies, generally done in cattle, focus on ETEC and EPEC groups.

In addition to these factors, it has been determined that the antigenic structures responsible for type 1 pilus adhesion of the SepEC group, which causes severe destruction when it infects the organism, should be examined more closely and that these antigens should be included in preventive vaccine studies. In the study, the antibiotic resistance of bacteria isolated from calves at a maximum of 15 d of age. This result shows that medical need to take severe precautions against antibiotic resistance.

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Ethics approval

The study protocol was approved by Ethical Committee of Faculty of Veterinary Medicine, Selcuk University, Türkiye (Approval no:2022/24).

Conflict of interest

The authors declare that they have no conflict of interest.

BIBLIOGRAPHIC REFERENCES

[1] Lorenz I, Fagan J, More SJ. Calf health from birth to weaning. II. Management of diarrhoea in pre-weaned calves. Ir. Vet. J. [Internet]. 2011; 64(9):1-6. doi: https://doi.org/d3vqkr

[2] Hur TY, Jung YH, Choe CY, Cho YI, Kang SJ, Lee HJ, Ki KS, Baek KS, Suh GH. The dairy calf mortality: the causes of calf death during ten years at a large dairy farm in Korea. Korean J. Vet. Res. [Internet]. 2013; 53(2):103-8. doi: https://doi.org/gmxmmn

[3] Constable PD. Antimicrobial use in the treatment of calf diarrhea. J. Vet. Intern. Med. [Internet]. 2004; 18(1):8-17. doi: https://doi.org/fwt57r

[4] Pardon B, Alliët J, Boone R, Roelandt S, Valgaeren B, Deprez P. Prediction of respiratory disease and diarrhea in veal calves based on immunoglobulin levels and the serostatus for respiratory pathogens measured at arrival. Prev. Vet. Med. [Internet]. 2015; 120(2):169-76. doi: https://doi.org/f7gbcw

[5] Fecteau G, Van Metre DC, Pare J, Smith BP, Higgins R, Holmberg CA, Jang S, Guterbock W. Bacteriological culture of blood from critically ill neonatal calves. Can. Vet. J. 1997; 38(2):95-100. Cited in PUBMED; PMID 9028592

[6] Bartels CJ, Holzhauer M, Jorritsma R, Swart WA, Lam TJ. Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. Prev. Vet. Med. [Internet]. 2010; 93(2-3):162-169. doi: https://doi.org/ch7mpc

[7] Cho YI, Yoon KJ. An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. J. Vet. Sci. [Internet]. 2014; 15(1):1-17. doi: https://doi.org/f5wkwh

[8] Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. Nat. Rev. Microbiol. [Internet]. 2004;2(2):123-140. doi: https://doi.org/bb2pfn

[9] Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. [Internet]. 1998;11(1):142-201. doi: https://doi.org/gm8zp8

[10] Foster D, Smith GW. Pathophysiology of diarrhea in calves. Vet. Clin. N. Am. - Food Anim. Pract. [Internet]. 2009; 25(1):13-36. doi: https://doi.org/cpxrvm

[11] Francis D, Allen S, White R. Influence of bovine intestinal fluid on the expression of K99 pili by *Escherichia coli*. Am. J. Vet. Res. 1989; 50(6):822-826. Cited in PUBMED; PMID 2569853

[12] Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, Choroszy-Krol I. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut Pathog. [Internet]. 2019; 11(10):1-16. doi: https://doi.org/gg5pns

[13] Köhler CD, Dobrindt U. What defines extraintestinal pathogenic *Escherichia coli?* Int. J. Med. Microbiol. [Internet]. 2011; 301(8):642–647. doi: https://doi.org/dwpgdb

[14] Bertin Y, Martin C, Girardeau JP, Pohl P, Contrepois M. Association of genes encoding P fimbriae, CS31A antigen and EAST 1 toxin among CNF1–producing *Escherichia coli* strains from cattle with septicemia and diarrhea. FEMS Microbiol. Lett. [Internet]. 1998; 162(2):235–239. doi: https://doi.org/d8dgvm

[15] Watts RE, Totsika M, Challinor VL, Mabbett AN, Ulett GC, De Voss JJ, Schembri MA. Contribution of siderophore systems to growth and urinary tract colonization of asymptomatic bacteriuria *Escherichia coli*. Infect. Immun. [Internet]. 2012; 80(1):333–344. doi: https://doi.org/cqfdf2

[16] Fecteau G, Smith BP, George LW. Septicemia and meningitis in the newborn calf. Vet. Clin. N. Am. – Food Anim. Pract. [Internet]. 2009; 25(1):195–208. doi: https://doi.org/c3hn3h

[17] Minassian AM, Newnham R, Kalimeris E, Bejon P, Atkins BL, Bowler IC. Use of an automated blood culture system (BD BACTEC™) for diagnosis of prosthetic joint infections: easy and fast. BMC Infect. Dis. [Internet]. 2014; 14:1–7. doi: https://doi.org/gb3z5j

[18] Moxley R. Enterobacteriaceae: *Escherichia.* In: McVey DS, Kennedy M, Chengappa MM, editors. Veterinary Microbiology. 3rd ed. Ames, Iowa, USA: John Wiley & Sons; 2013; p 62–74.

[19] Hadimli HH, Pinarkara Y, Sakmanoğlu A, Sayin Z, Erganiş O, Uslu A, Al–Shattrawi HJ. Serotypes of Salmonella isolated from feces of cattle, buffalo, and camel and sensitivities to antibiotics in Turkey. Turkish J. Vet. Anim. Sci. [Internet]. 2017; 41(2):193–198. doi: https://doi.org/k5hk

[20] Lassen J. Rapid identification of Gram-negative rods using a three-tube method combined with a dichotomic key. Acta Pathol. Microbiol. Scand. B. [Internet]. 1975; 83(6):525–533. doi: https://doi.org/ccgj2r

[21] Scheutz F, Cheasty T, Woodward D, Smith HR. Designation of O174 and O175 to temporary O groups OX3 and OX7, and six new *E. coli* O groups that include Verocytotoxin-producing E. coli (VTEC): O176, O177, O178, O179, O180 and O181. APMIS. 2004; 112(9):569–84. doi: https://doi.org/cgtmh2

[22] McFarland J. The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. J. Am. Med. Assoc. [Internet]. 1907; 49(14):1176–1178. doi: https://doi.org/csdkhx

[23] CLSI. Performance standards for antimicrobial susceptibility testing. 32nd. ed. CLSI supplement M100. Clinical and Laboratory Standard Institute; 2022; 402 p.

[24] Lee SI, Kang SG, Kang ML, Yoo HS. Development of multiplex polymerase chain reaction assays for detecting enterotoxigenic *Escherichia coli* and their application to field isolates from piglets with diarrhea. J. Vet. Diagn. Investig. [Internet]. 2008; 20(4):492–496. doi: https://doi.org/bhw3p9

[25] Oh KH, Kim SB, Park MS, Cho SH. Development of a one–step PCR assay with nine primer pairs for the detection of five diarrheagenic *Escherichia coli* types. J. Microbiol. Biotechnol. [Internet]. 2014; 24(6):862–868. doi: https://doi.org/k5hm

[26] Vandekerchove D, Vandemaele F, Adriaensen C, Zaleska M, Hernalsteens JP, De Baets L, Pasmans F. Virulence–associated traits in avian *Escherichia coli*: comparison between isolates from colibacillosis–affected and clinically healthy layer flocks. Vet. Microbiol. [Internet]. 2005; 108(1–2):75–87. doi: https://doi.org/ft63sz

[27] Aydin F, Umur Ş, Gökçe G, Genç O, Güler MA. [The Isolation and Identification of Bacteria and Parasites from Diarrhoeic Calves in Kars District]. Kafkas Univ. Vet. Fak. Derg. [Internet]. 2001 [cite 20 Jun 2023]; 7(1):7–14. Turkish. Available in: https://bit.ly/3unUPSw.

[28] Brunauer M, Roch FF, Conrady B. Prevalence of worldwide neonatal calf diarrhoea caused by bovine rotavirus in combination with Bovine Coronavirus, *Escherichia coli* K99 and *Cryptosporidium* spp.: A Meta–Analysis. Anim. [Internet]. 2021; 11(4):1014. doi: https://doi.org/gnqsw3

[29] Işik–Uslu N, Derinbay–Ekici O, Avci O. ELISA–based Point Prevalence of enteropathogens in diarrheic calves in Central Anatolia Region of Turkey. Rev. Cient. Fac. Cien. Vet. Univ. Zulia. [Internet]. 2023; 33(2):1–6. doi: https://doi.org/k5hn

[30] Içen H, Arserim NB, Işik N, Özkan C, Kaya A. Prevalence of Four Enteropathogens with Immunochromatographic Rapid Test in the Feces of Diarrheic Calves in East and Southeast of Turkey. Pak. Vet. J. [Internet]. 2013 [cited 20 Jun 2023]; 33(4):496–499. Available in: https://bit.ly/3R8utvm.

[31] Cengiz S, Adiguzel MC. Determination of virulence factors and antimicrobial resistance of *E. coli* isolated from calf diarrhea, part of eastern Turkey. Ankara Univ. Vet. Fak. Derg. [Internet]. 2020; 67(4):365–371. doi: https://doi.org/kdr6

[32] Küliğ C, Coşkun A. [Prevalence of *E. coli*, Cryptosporidium, *Clostridium perfringens*, Rotavirus and Coronavirus in Neonatal Calves with Diarrhea in Sivas]. Turk. Vet. J. [Internet] 2019 [cited 21 Jun 2023]; 1(2):69–73. Turkish. Available in: https://bit.ly/3GbBBSt.

[33] Güler L, Gündüz K, Ok Ü . Virulence factors and antimicrobial susceptibility of *Escherichia coli* isolated from calves in Turkey. Zoon. Publ. Health. [Internet]. 2008; 55(5):249–257. doi: https://doi.org/dwhbkb

[34] Altuğ N, Yüksek N, Özkan C, Keleş İ, Başbuğan Y, Ağaoğlu ZT, Kaya A, Akgül Y. [Rapid Etiological Diagnosis of Neonatal Calf Diarrhoea with Immunochromatographic Test Kits]. YYU Vet. Fak. Derg. [Internet] 2013 [cited 15 Jun 2023]; 24(3):123–128. Turkish. Available in: https://bit.ly/3M0Rbqx.

[35] Garcia J, Pempek J, Hengy M, Hinds A, Diaz–Campos D, Habing G. Prevalence and predictors of bacteremia in dairy calves with diarrhea. J. Dairy Sci. [Internet]. 2022; 105(1):807–817. doi: https://doi.org/k5hp

[36] Lofstedt J, Dohoo IR, Duizer G. Model to predict septicemia in diarrheic calves. J. Vet. Intern. Med. [Internet]. 1999; 13(2):81–88. doi: https://doi.org/fnqg4w

[37] Uriarte ELL, Pasayo RAG, Masso M, Paez LC, Moncla MD, Donis N, Malena R, Méndez A, Morrell E, Giannitti F, Armendano JI, Faverin C, Centrón D, Parreño V, Odeón AC, Quiroga MP, Moreira AR. Molecular characterization of multidrug–resistant

Escherichia coli of the phylogroups A and C in dairy calves with meningitis and septicemia. Microb. Pathog. [Internet]. 2022; 163:105378. doi: https://doi.org/k5hq

[38] Kauffmann F. The serology of the coli group. J. Immunol. [Internet]. 1947; 57(1):71–100. doi: https://doi.org/k5hr

[39] Tan C, Tang X, Zhang X, Ding Y, Zhao Z, Wu B, Cai X, Liu Z, He Q, Chen H. Serotypes and virulence genes of extraintestinal pathogenic *Escherichia coli* isolates from diseased pigs in China. Vet. J. [Internet]. 2012; 192(3):483–488. doi: https://doi.org/bwns5h

[40] Debroy C, Roberts E, Fratamico PM. Detection of O antigens in *Escherichia coli*. Anim. Health Res. Rev. [Internet]. 2011; 12(2):169–185. doi: https://doi.org/cw8q6w

[41] Feuerstein A, Scuda N, Klose C, Hoffmann A, Melchner A, Boll K, Rettinger A, Fell S, Straubinger RK, Riehm JM. Antimicrobial resistance, serologic and molecular characterization of *E. coli* isolated from calves with severe or fatal enteritis in Bavaria, Germany. Antibiot. [Internet]. 2021; 11(1):23. doi: https://doi.org/k5hs

[42] World Health Organization. Antimicrobial resistance: global report on surveillance. [Internet]. Geneva: WHO. 2014 [cited 18 Apr 2023]. 232 p. Available in: https://bit.ly/3R0VrWV.

[43] Lange ME, Uwiera RR, Inglis GD. Enteric *Escherichia coli* O157: H7 in Cattle, and the Use of Mice as a Model to Elucidate Key Aspects of the Host–Pathogen–Microbiota Interaction: A Review. Front. Vet. Sci. [Internet]. 2022; 9:937866. doi: https://doi.org/gsndw5

[44] Sora VM, Meroni G, Martino PA, Soggiu A, Bonizzi L, Zecconi A . Extraintestinal pathogenic *Escherichia coli*: Virulence factors and antibiotic resistance. Pathog. [Internet]. 2021; 10(11):1355. doi: https://doi.org/k5jh

[45] Khawaskar DP, Sinha DK, Lalrinzuala MV, Athira V, Kumar M, Chhakchhuak L, Thomas P. Pathotyping and antimicrobial susceptibility testing of *Escherichia coli* isolates from neonatal calves. Vet. Res. Commun. [Internet]. 2022; 46(2):353–362. doi: https://doi.org/k5jj

[46] Pasqua M, Michelacci V, Di Martino ML, Tozzoli R, Grossi M, Colonna B, Morabito S, Prosseda G. The intriguing evolutionary journey of enteroinvasive *E. coli* (EIEC) toward pathogenicity. Front. Microbiol. [Internet]. 2017; 8:2390. doi: https://doi.org/gcrbhw

[47] Šmajs D, Micenková L, Šmarda J, Vrba M, Ševčíková A, Vališová Z, Woznicová V. Bacteriocin synthesis in uropathogenic and commensal *Escherichia coli*: colicin E1 is a potential virulence factor. BMC Microbiol. [Internet]. 2010; 10(1):1–10. doi: https://doi.org/c3gtfm

[48] Santos ACDM, Santos FF, Silva RM, Gomes TAT. Diversity of hybrid–and hetero–pathogenic *Escherichia coli* and their potential implication in more severe diseases. Front. Cell. Infect. Microbiol. [Internet]. 2020; 10:339. doi: https://doi.org/k5jk

[49] Pokharel P, Dhakal S, Dozois CM. The diversity of *Escherichia coli* pathotypes and vaccination strategies against this versatile bacterial pathogen. Microorgan. [Internet]. 2023; 11(2):344. doi: https://doi.org/k5jm

[50] Johnson JR, Murray AC, Gajewski A, Sullivan M, Snippes P, Kuskowski MA, Smith KE. Isolation and molecular characterization of nalidixic acid–resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob. Agents Chemother. [Internet]. 2003; 47(7):2161–2168. doi: https://doi.org/fjxvjv