Special Issue:
Advancements in Animal Health and Production in Low and Middle-Income Countries

Molecular Investigation of Virulence Factors among *Escherichia coli* Isolated from Diarrheal Infections in Neonatal Calves

Noor Ali Abdulzahra*, Rawaa Adday Ali

Microbiology Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq.

Abstract | The cattle business incurs significant financial losses due to reduced productivity and increased veterinary expenses resulting from Escherichia coli, a common bacterial pathogen that causes diarrhea in calves. Therefore, the purpose of this work was to use the *16S rRNA* gene, the VITEK2 system, biochemical assays, and microbiological methods to molecularly detect certain virulence factors of E. coli isolated from intestinal infections in calves. Between October 2024 and February 2025, a total of 75 rectal swab samples were taken from diarrheal calves from various farms in the Babylon district. Of these, 60 (80%) were male and 15 (20%) were female, and the calves ranged in age from one day to three months. Of the 75 samples, 70 (93.3%) exhibited positive bacterial growth, and 5 (6.6%) showed negative bacterial growth. Of the positive bacterial growth, 20 (28.5%) were Gram-positive bacteria, while the remaining 50 (71.4%) were Gram-negative bacteria. Escherichia coli was detected in 30/70 (42.8%) of bacterial isolates from both sexes, primarily in individuals aged 1–30 days. Out of 30/70 (42.8%) E. coli isolates, 30/30 (100%) had the 16S rRNA gene, as confirmed by the study's molecular data, which verified the bacterium's identity at the molecular level. Although other NCD risk factors may influence disease outcome, our data indicate that several adhesins and toxin genes were identified, suggesting that E. coli from calves' feces exhibited extraintestinal and diarrheagenic profiles. The findings indicated a correlation between diarrhea and the prevalence of E. coli overall. Regardless of the type of diarrheal infection, the F5 (K5) gene was the most commonly found virulence factor in E. coli, detected in 20/30 (66.6%) of samples in the 1–7-day age range, according to PCR data. The heat-labile enterotoxins LT-II and LT-I genes showed weakly represented involvement of 2/30 (6.67%) and 1/30 (3.3%), respectively, whereas the results showed 15/30 (50%) for F17. Heat-stable enterotoxins ST-a and ST-b were not found.

Keywords | Bacteria, *E. coli*, Neonatal, Calves, Diarrhea, Virulence genes

Received | August 09, 2025; **Accepted** | September 12, 2025; **Published** | September 17, 2025
***Correspondence** | Noor Ali Abdulzahra, Microbiology Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq; **Email:** noor.ali@vet.uoqasim.edu.iq
Citation | Abdulzahra NA, Ali RA (2025). Molecular investigation of virulence factors among *Escherichia coli* isolated from diarrheal infections in neonatal calves. J. Anim. Health Prod. 13(s1): 517-522.
DOI | https://dx.doi.org/10.17582/journal.jahp/2025/13.s1.517.522
ISSN (Online) | 2308-2801

INTRODUCTION

Calf scours, also known as neonatal calf diarrhea (NCD), is a common illness that affects newborn calves. Calf scours can happen anywhere from the first few hours of life to six weeks, depending on the cause. A variety of infectious factors contribute to the development of NCDs. According to Aditya *et al.* (2023), rotavirus, coronavirus, bacteria (*E. coli K99*, *Clostridium perfringens* type C, and *Salmonella* spp.), and parasites (*Cryptosporidia* and *coccidia*) can exist alone or in combination. Furthermore, scours outbreaks can frequently be caused by a combination of

factors, including inadequate nutrition of the pregnant dam, especially during the last third of gestation, neglect of the newborn calf, including insufficient quantity and/or quality of colostrum, exposure to harsh environments, overcrowding, and poor sanitation in the calving area, or a combination of these factors (Rathor *et al*., 2021).

The most significant bacterial cause of newborn calf diarrhea (NCD) is *Escherichia coli*. *Enterotoxigenic E. coli* (*ETEC*), *Shiga toxigenic E. coli* (*STEC*), *Enteropathogenic E. coli* (*EPEC*), and *Enterohemorrhagic E. coli* (*EHEC*) are among the strains of *E. coli* that cause noncommunicable diseases. One of the primary causes of NCDs is *ETEC* strains, such as K99 (F5), F41, the F17 family, and STa, which cause diarrhea through the action of their toxins and fimbriae. Additionally, the pathogenic potential of *ETEC* strains is correlated with the age of the calves. Newborn animals under one week old are more susceptible to *ETEC* strains (Algamma *et al*., 2020; Mohsen *et al*., 2024).

Both heat-labile (*LT*) and heat-stable (ST). Stable (*STa* and *STb*) enterotoxins can colonize bacteria in the small intestines thanks to the attachment of bacteria to epithelial surfaces mediated by fimbrial adhesins (*F5*, *F17*, and *F41*). The toxins stimulate the secretion of fluids and electrolytes from intestinal epithelial cells, which ultimately leads to diarrhea (Kolenda *et al*., 2015).

The aim of this study was to use the *16S rRNA* gene, the VITEK2 system, biochemical assays, and microbiological methods to detect certain virulence factors of *E. coli* isolated from intestinal infections in calves.

MATERIALS AND METHODS

Specimens collecting and processing

A total of 75 rectal swabs were taken from diarrheal calves on various farms in the Babylon Governorate between October 2024 and February 2025. A sterile swab from the locations of diarrheal infections was then promptly submitted to the College of Veterinary Medicine's Advanced Microbiology Laboratory at Al-Qasim Green University in Iraq. After being sterilized at 121 ˚C for 15 minutes and incubated at 37 ˚C for 24 hours, the samples were cultivated on Eosin Methylene Blue, Blood Agar, MacConkey Agar, Brain Heart Broth, Nutrient Agar, and S.S. Agar. The pH was set to 7. The petri dish was incubated at 37˚C overnight. Additionally, no growth was observed after two days of incubation. Following the isolation of bacterial samples, the morphology and Gram reaction were assessed by microscopic examination using Gram staining. Biochemical tests such as catalase and Oxidase assessments were then used to identify the bacteria (Forbes *et al*., 2007; McFadden, 2000; Abed *et al*., 2024). According to the manufacturer's instructions, the VITEK2 system (bioMérieux, Paris, France) is used to confirm the identity of D-GNB cards.

DNA extraction and PCR assay

Using a commercial total genomic DNA extraction kit (the G-spin™ Genomic DNA Extraction Kit (Intron, Korea)), the total genomic DNA of 30 isolates of *E. coli* was extracted following an overnight liquid growth of this pathogen (Mosa *et al*., 2022). The extraction was performed according to the manufacturer's instructions. Using deep-freezing equipment, the nucleic acid was maintained at -20˚C. The PCR technique was then used to test and identify every gene listed in Table 1. After staining the gel with 0.5 µg/ml RedSafe™ nucleic acid dye, electrophoresis was performed at 90 V for 45 minutes using the gel document system (Cleaver, United Kingdom) to examine and separate the migration of PCR products on a 0.7% agarose gel (iNtRoN, Biotech Inc., Korea). Utilizing a UV transilluminator set at 320 nm, DNA bands were visualized.

Table 1: Conditions and sequence of primers.

Species	Gene	Primer name	5'-3'	Annealing (°C)	PCR product	Accession number	Reference
E. coli	*F5*	F5-1F	CCAGGCCCCGCAGTAATGACTGC	58 (°C)/30 sec	278 bp	M35282.1	Picco *et al*., 2015
		F5-1R	CCACCATTAGAGGAGGCGCGG				
	F17	F17-2F	GGGCTGACAGAGGAGGTGGGC	58 (°C)/30 sec	411 bp	L14319.1	Picco *et al*., 2015
		F17-2R	CCCGGGCACACTTCATCACGG				
	Sta	Sta-1F	ATTTTTATTTTCTGTATTGCTTT	58 (°C)/30 sec	176 bp	MF955844.1	Picco *et al*., 2015
		Sta-1R	GGATTACAACACAGTTCACAGCAT				
	STb	STb-2F	ATGCCATTTCTCTTGTCATC	58 (°C)/30 sec	175 bp	OQ615950.1	Picco *et al*., 2015
		STb-2R	GGGCGCCAAAGTACGTCTC				
	LT-I	LTI-1F	CCGAATTCGTGTATATAGTGC	58 (°C)/30 sec	708 bp	MN414477.1	Picco *et al*., 2015
		LTI-1R	GGGCAGACATATGTATGGGA				
	LT-II	LTII-2F	ACGGCGTTATCCTCTCTCTC	58 (°C)/30 sec	274 bp	AF242418.1	Rodas *et al*., 2009
		LTII-2R	TGGTCGCGTGACAGTATGTG				

RESULTS

A total of 75 rectal swab samples were collected from diarrheic calves across various farms in Babylon Governorate. Out of these, 70 samples (93.3%) exhibited positive bacterial growth, while 5 samples (6.66%) were negative. Among the positive cultures, 50 isolates (71.4%) were identified as Gram-negative bacteria, while 20 isolates (28.5%) were Gram-positive.

The amount of *E. coli* present was higher in the calves with an infection rate. It was detected in 30/70 (42.8%) bacterial isolates of the fecal samples based on culture characteristics, biochemical tests, and confirmation by the VITEK2 system, with age groups (1-30 days) for both sexes. Molecular data from this study revealed that out of 70 *E. coli* isolates, *16S rRNA* gene sequencing (Figure 1) confirmed the identity of the bacterium at the molecular level in 30/70 (42.8%) isolates.

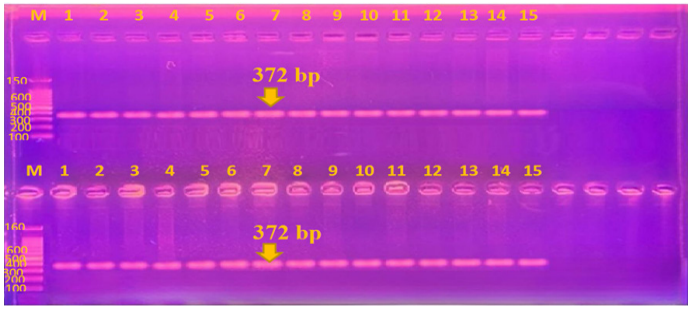

Figure 1: The PCR product of 16S rRNA gene in 1–2% agarose gels electrophoresis for 30 *E. coli*.

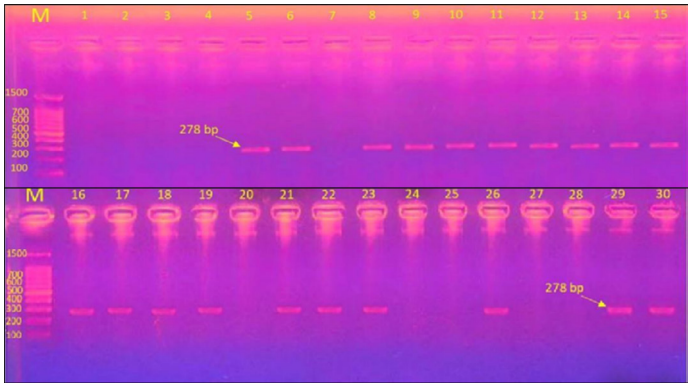

Figure 2: The PCR product of the F5 gene in 1–2% agarose gels electrophoresis for 30 *isolates of E. coli*.

PCR data revealed a high rate of the F5 (k55) gene involved 20/30(66.6) isolates of *E. coli* neonatal calves in the age group (1-7 days), which had a higher infection rate, as shown in Figure 2. Results observed 15/30 (50%) for the F17 gene, as shown in Figure 3. In contrast, the heat-labile enterotoxins LT-II and LT-I genes revealed a low rate of involvement, with 2/30 (6.67%) and 1/30 (3.3%), respectively, as shown in Figure 4 and 5. There were no detectable heat-stable enterotoxins *ST-a* and *ST-b*. Current results observed a close correlation between the prevalence rates of ETEC target virulence genes in *E. coli* and the age of infected and healthy calves, there were *F5* (*k55*) *gene* high in diarrheic neonates (Table 2) (<1 week old); low in healthy calves strongly age-dependent, and *F17* gene common in both diarrheic and healthy calves, broader age range, significance for diarrhea often depends on co-expressed toxins.

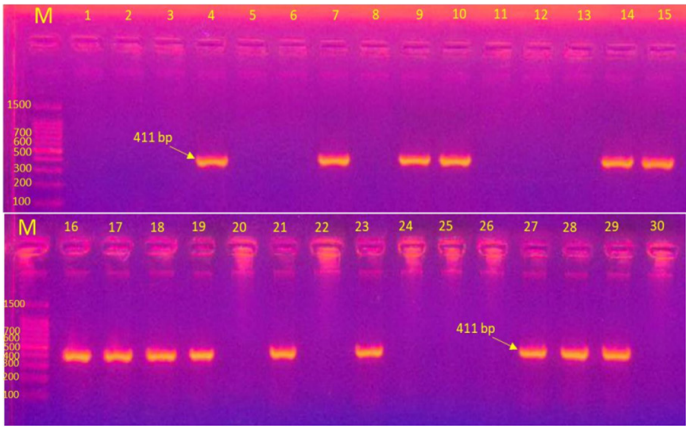

Figure 3: The PCR product of the F17 gene in 1–2% agarose gels electrophoresis for 30 *E. coli* isolates.

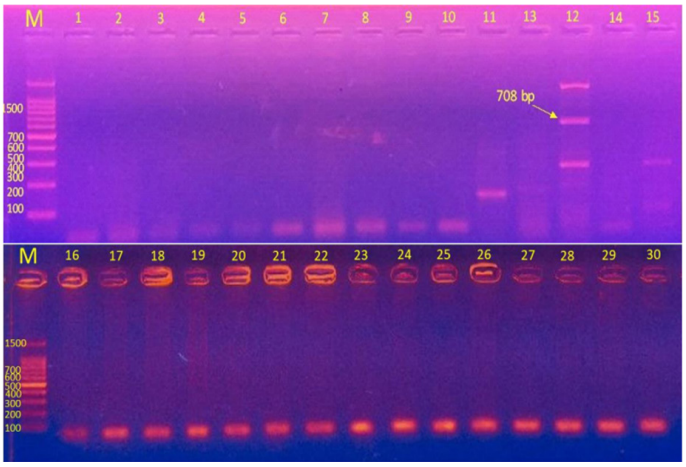

Figure 4: The PCR product of the LT-l gene in 1–2% agarose gels electrophoresis for 30 *E. coli* isolates.

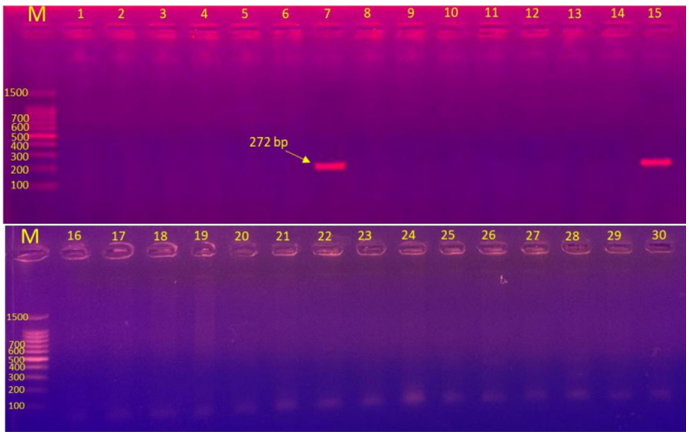

Figure 5: The PCR product of the LT-ll gene in 1–2% agarose gels electrophoresis.

Table 2: Summary of prevalence ranges and associations for target ETEC virulence genes in *E. coli* from calves.

Virulence gene/ Combination	Prevalence range in diarrheic calves (%)	Prevalence range in healthy calves (%)	Key regions/studies for notable prevalence	Notes (e.g., age association, significance)
F5 (K99)	0.6 - 50 (Meta-analysis: 12.9)	0 - 2.3 (Meta-analysis: 2.3)	India (high), Iran (high in ETEC), Meta-analysis (moderate)	Strongly associated with diarrhea in calves <1 week old. Prevalence may be declining over time.
F17	4.8 - 41 (Meta-analysis: 31.6)	0 - 43.3 (Meta-analysis: 27.1)	Uruguay, Korea, Argentina (high); Meta-analysis (high)	Common in both diarrheic and healthy calves; broader age range than F5. The significance of diarrhea depends on the co-expressed toxin variants.
LT-I	0 - 28 (as % of total ETEC, e.g., Iran); (Meta-analysis: 3.8 overall)	0 - 2.8 (Meta-analysis: 2.8)	Iran, Egypt (regionally high in ETEC)	Broadline prevalence is not consistently associated with diarrhea in broad comparative data.
LT-II	8.3 - 19.9	Limited comparative data	Brazil (notably prevalent)	Appears to be more consistently reported in South American studies.
STa	0.3 - 25 (Meta-analysis: 7.0-7.9)	0.3 - 0.6 (Meta-analysis: 0.3 - 0.6)	Meta-analysis (moderate, significant association with diarrhea); Iran (high in ETEC)	Significant association with diarrhea, especially with F5. Prevalence may be increasing over time.
STb	Generally low/not detected; one report of 23.5% in a specific combo (Egypt)	Limited comparative data	Egypt	Primarily a porcine toxin; minor role in typical calf ETEC.
F5 + STa	e.g., 14.1% of total isolates in one Iranian study were F5+F41+STa	Rare	Widely reported as typical ETEC	Highly indicative of ETEC diarrhea in young calves.
F17 + STa/ LT	Variable (e.g., F17+STa 7.7% in Argentina)	Variable	Argentina	Suggestive of ETEC if toxin is present.
LT + F5/ F41	Variable (e.g., up to 100% of ETEC in one Iranian study were LT+F5)	Limited data	Iran (regionally high LT+F5)	Indicates specific ETEC lineages.

DISCUSSION

The high morbidity and mortality rates among young calves, as well as the economic effects of the condition, make neonatal calf diarrhea (NCD) a significant concern in livestock production. One of the most prevalent infections linked to diarrhea in calves, *Escherichia coli,* has been shown to cause significant morbidity and fatality rates globally. However, surprisingly little research has been done to assess the connection between calf diarrhea and *E. coli* Hur *et al.* (2013). *Escherichia coli* was identified in areas with a higher infection rate in the current study. The prevalence of *E. coli* 30/70 (42.8%) among bacterial isolates was analyzed by age group (1–30 days) for both sexes, and the possibility of associations between *E. coli* and diarrhea, as well as age groups, was investigated. The isolation results obtained in this study were comparable to those reported in numerous previous studies (Ull, 2022; Gamsjäger *et al.*, 2021). Furthermore, this study provides significant new insights into *E. coli* as a causal agent and its connection to the pathophysiology of calves' diarrhea, with findings that align with those of earlier studies (Klein-Jöbstl *et al.*, 2019; Croxen *et al.*, 2013).

The current findings for the particular identification of virulence genes showed a high rate of 20/30 (66.6%) for *F5* (*K99*) and 15/30 (50%) for *F17*. These high rates could represent a significant contribution to infection. *K99* or *F5*, facilitating bacterial adherence to intestinal epithelial cells, fimbrial adhesion contributes to the colonization of the small intestine epithelial cells of neonatal calves. In addition to reporting that the majority of bovine *ETEC* produce *K99* fimbriae (Pourtaghi *et al.*, 2013), they verified the strong association between enterotoxigenicity and the presence of the *K99* antigen.

The current findings concurred with those of other studies by Öztürk *et al.* (2024) and Ryu *et al.* (2020), which demonstrated that both *F5* and *F17* are the most prevalent and important *ETEC* adhesins and have the strongest correlation with the presence of diarrhea. Some *ETEC* have multiple types of fimbriae, and *F5* can be isolated

from a single diarrheal calf these findings aligned with those of the current investigation (Cengiz *et al*., 2020). Calves affected by this condition are typically between 1 and 7 days old, with the majority of occurrences occurring in calves younger than 5 days old. According to Kolenda *et al*. (2015), calves are particularly vulnerable to F17 and F5 ETEC within the first 48 hours of their lives, after which they begin to develop resistance to the organism. *ETEC F5* (formerly *K99*) and *F17* fimbriae, as well as *ST* and *LT* toxins, are substantially linked to newborn calf diarrhea (NCD). One of the leading causes of NCDs has been identified as *ETEC*. The *F17* family is the most prevalent virulence factor (24%), according to studies by Umpierrez *et al*. (2016), which disagree with our current findings. Only one virulence factor, *F17*, was detected in 21 calves (18.2%) the incidence rate.

LT-II and *LT-II*, two more genes related to heat-labile enterotoxins, were found to be weakly represented in the current study at 2/30 (6.67%) and 1/30 (3.3%), respectively. A heat-sensitive enterotoxin, referred to as *LT* (*LT-II*, *LT-I*), is produced by *E. coli* and may become inactive when exposed to heat (Josune et al., 2025). It results in watery diarrhea in enterotoxigenic *E. coli* (*ETEC*) infections. Additionally, our findings align with those of multiple investigations by Picco *et al*. (2015) and Umpiérrez *et al*. (2016), which demonstrated a notable decrease in the genes encoding heat-labile *ETEC* toxins.

E. coli produces heat-stable enterotoxins A (STa) and B (STb) strains that induce diarrhea in neonatal calves. To assist ETEC colonize the small intestine, evade the immune system, and induce an inflammatory reaction, *STa* and *STb* work in tandem with *K99* fimbriae pathophysiology of enterotoxigenic *Escherichia coli* (*ETEC*), which causes neonatal animals to have severe diarrhea (Sheikh *et al*., 2022; Mahmood *et al*., 2020). The current results did not reveal the presence of heat-stable enterotoxins *ST-a* and *ST-b*. However, previous research revealed that the sole enterotoxin gene found was st, and neither lt nor stb genes were found in any of the analyzed strains. These results are consistent with those of Nagy and Fekete (2005), who found that human and porcine *ETEC* strains are the leading producers of the LT and STb enterotoxins. In contrast, porcine and bovine *ETEC* strains generate the *STa* enterotoxin.

In neonatal calves, *STa* is more important, but in post-weaning animals, *STb* is more virulent. *ETEC* strains generate heat-stable enterotoxins (*STa*, *STb*) and heat-labile enterotoxins (*LT*), which cause diarrhea by depleting electrolytes and water. The density of *STa* receptors has been found to vary along the vertical axis of the small intestine (villus/crypt unit) in most mammals. They discovered that the enterocytes in the proximal villus region of the intestinal tract have the highest amount of *STa* receptors (Krause *et al*., 1990). *ETEC* strains that produce heat-stable toxin (ST), either alone or in combination with heat-labile toxin (LT), appear to cause the most severe illness, according to epidemiological data. Immunity is not anticipated to be involved in protecting newborn calves from this diarrheal illness because *STa* has a tiny molecular mass (2 kDa) and is not immunogenic (Turner *et al*., 2006).

CONCLUSIONS

This study confirms that *Escherichia coli*, particularly strains harboring *F5* and *F17* virulence genes, plays a significant role in neonatal calf diarrhea. Molecular detection highlighted a high prevalence of *F5* among calves under one week old. Effective monitoring and control strategies are crucial for reducing ETEC-related infections and associated economic losses.

ACKNOWLEDGEMENT

Authors want to express gratitude to all those who assisted throughout this project.

NOVELTY STATEMENT

This study offers a novel approach to understanding Escherichia coli infections by integrating molecular detection of key virulence genes (F5, F17, LT1, LT2, Sta and STb) with host immune response profiling, specifically IL-10 and TNF-α cytokines. The identification of F5 and F17 as the most prevalent virulence factors highlights their potential diagnostic and epidemiological importance. Unlike prior studies that often examine either bacterial genetics or host immunity in isolation, this research combines both aspects, providing a comprehensive view of the host-pathogen interaction and offering insights that may improve targeted diagnostics and disease management strategies in veterinary settings.

AUTHOR CONTRIBUTION

Both authors worked equally in the data collected, data analysis, writing the paper, and conceived and designed the analysis.

Generative AI or AI-assisted Technology Statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abed DA, Hamzah KJ, Abdul-Kareem S, Mehrzad J (2024). Bacteriological and molecular study of some urinary tract infections bacteria in human and cows in Babylon Province. Pak. Vet. J., 44(4).

Aditya A, Tabashsum Z, Martinez ZA, Tung, C.W., Suh, G., Nguyen, P., Biswas, D. (2023). Diarrheagenic Escherichia coli and their antibiotic resistance patterns in dairy farms and their microbial ecosystems. J. Food Prot., 86(3): 100051. https://doi.org/10.1016/j.jfp.2023.100051

Algammal AM, El-Kholy AW, Riad EM, Mohamed HE, Elhaig MM, Yousef SAA, Hozzein, W.N., Ghobashy, M.O.I. (2020). Genes encoding the virulence and the antimicrobial resistance in enterotoxigenic and shiga-toxigenic *E. coli* isolated from diarrheic calves. Toxins, 12(6): 383. https://doi.org/10.3390/toxins12060383

Cengiz S, Adıgüzel MC (2020). Determination of virulence factors and antimicrobial resistance of *E. coli* isolated from calf diarrhea, part of eastern Turkey. Ankara Üniv. Vet. Fakültesi Dergisi, 67(4): 365–371. https://doi.org/10.33988/auvfd.640990

Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB (2013). Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin. Microbiol. Rev., 26(4): 822–880. https://doi.org/10.1128/CMR.00022-13

Forbes BA, Sahm DF, Weissfeld AS, Brawn IA (2007). Bailey and Scott's Diagnostic Microbiology. 12th ed. St. Louis, MO: Mosby.

Gamsjäger L, Haines D, Pajor E, Lévy M, Windeyer M (2021). Impact of volume, immunoglobulin G concentration, and feeding method of colostrum product on neonatal nursing behavior and transfer of passive immunity in beef calves. Animal, 15(1): 100265. https://doi.org/10.1016/j.animal.2021.100345

Hur J, Jeon BW, Kim YJ, Oh IG, Lee JH (2013). *Escherichia coli* isolates from calf diarrhea in Korea and their virulent genetic characteristics. J. Vet. Med. Sci., 75(4): 519–522. https://doi.org/10.1292/jvms.12-0378

Josune Salvador-Erro, Yadira Pastor, Carlos Gamazo. 2025. Targeting Enterotoxins: Advancing Vaccine Development for Enterotoxigenic Escherichia coli ETEC. Toxins 2025, 17(2), 71.

Klein-Jöbstl D, Quijada NM, Dzieciol M, Feldbacher B, Wagner M, Drillich M, Schmitz-Esser S, Mann E. (2019). Microbiota of newborn calves and their mothers reveals possible transfer routes for newborn calves' gastrointestinal microbiota. PLoS One, 14(4): e0214877. https://doi.org/10.1371/journal.pone.0220554

Kolenda R, Burdukiewicz M, Schierack P (2015). A systematic review and meta-analysis of the epidemiology of pathogenic *Escherichia coli* of calves and the role of calves as reservoirs for human pathogenic E. coli. Front. Cell. Infect. Microbiol., 5: 23. https://doi.org/10.3389/fcimb.2015.00023

Krause WJ, Freeman RH, Forte LR (1990). Autoradiographic demonstration of specific binding sites for *E. coli* enterotoxin in various epithelia of the North American opossum. Cell Tissue Res., 260(3): 387–394. https://doi.org/10.1007/BF00318641

MacFaddin JF (2000). Biochemical tests for identification of medical bacteria. 3rd ed. Philadelphia, PA: Lippincott Williams and Wilkins.

Mahmood AK, Hamzah KJ, Dirwal AR, Salh AH (2020). Isolation of *Escherichia coli* from skin wounds in cow. Plant Arch., 20(1): 3108-3110.

Mohsen AK, Jassim SE, Alaridhi TT, Al-Erjan AM, Lahhob QR, Mudhafar M (2024). Revolutionizing infectious disease management in animals through advanced phage therapy: A comprehensive strategy for the eradication of multidrug-resistant bacterial pathogens. J. Anim. Health Prod., 12(s1): 157-167. https://doi.org/10.17582/journal.jahp/2024/12.s1.157.167

Mosa AH, Hamzah KJ, Aljabory HA (2022). First study on the molecular prevalence of caprine arthritis encephalitis virus in goats in Babylon, Iraq. Vet. World, 15(4): 1129. https://doi.org/10.14202/vetworld.2022.1129-1133

Nagy B, Fekete P (2005). Enterotoxigenic *Escherichia coli* in veterinary medicine. Int. J. Med. Microbiol., 295(6–7): 443–454. https://doi.org/10.1016/j.ijmm.2005.07.003

Öztürk D, Kale M, Şahan Yapıcıer Ö, Saltık HS, Atlı K, Yaman S (2024). Diarrhea in neonatal calves bacterial and viral factors. Acta Sci. Vet., 52(1): 140125. https://doi.org/10.22456/1679-9216.140125

Picco NY, Alustiza FE, Bellingeri RV, Grosso MC, Motta CE, Larriestra AJ, Vissio C, Tiranti KI, Terzolo HR, Moreira AR, Vivas AB (2015). Molecular screening of pathogenic *Escherichia coli* strains isolated from dairy neonatal calves in Cordoba province. Rev. Argent. Microbiol., 47(1): 4–11. https://doi.org/10.1016/j.ram.2015.01.006

Pourtaghi H, Dahpahlavan V, Momtaz H (2013). Virulence genes in *Escherichia coli* isolated from calves with diarrhoea in Iran. Comp. Clin. Pathol., 22(3): 513–515. https://doi.org/10.1007/s00580-012-1442-5

Rathor AS, Bhati T, Singh AP (2021). *In vitro* antibacterial activity of extracts of Dalbergia sissoo and Aegle marmelos against enterotoxigenic *Escherichia coli* from calves. J. Phytopharmacol., 10(5): 289–293. https://doi.org/10.31254/phyto.2021.10503

Ryu J, Kim S, Park J, Choi K (2020). Characterization of virulence genes in *Escherichia coli* strains isolated from pre-weaned calves in the Republic of Korea. Acta Vet. Scand., 62(1): 13. https://doi.org/10.1186/s13028-020-00543-1

Salvador-Erro J, Pastor Y, Gamazo C (2025). Targeting enterotoxins: Advancing vaccine development for enterotoxigenic *Escherichia coli* (ETEC). Toxins, 17(2): 71. https://doi.org/10.3390/toxins17020071

Sheikh A, Tumala B, Vickers TJ, Martin JC, Rosa BA, Sabui S, Basu S, Simões RD, Mitreva M, Storer C, Tyksen, E., Head, R. D., Beatty, W., Said, H. M., Fleckenstein, J. M (2022). Enterotoxigenic *Escherichia coli* heat-labile toxin drives enteropathic changes in small intestinal epithelia. Nat. Commun., 13: 6886. https://doi.org/10.1038/s41467-022-34687-7

Turner SM, Chaudhuri RR, Jiang ZD, DuPont H, Gyles C, Penn CW, Pallen MJ, Henderson IR (2006). Phylogenetic comparisons reveal multiple acquisitions of the toxin genes by enterotoxigenic *Escherichia coli* strains of different evolutionary lineages. J. Clin. Microbiol., 44(12): 4528–4536. https://doi.org/10.1128/JCM.01474-06

Ull T (2022). Bacterial causes of intestinal disease in dairy calves: Acceptable control measures. Vet. Clin. N. Am. Food Anim. Pract., 38(1): 107–119. https://doi.org/10.1016/j.cvfa.2021.11.008

Umpiérrez A, Acquistapace S, Fernández S, Oliver M, Acuña P, Reolón E, Zunino P (2016). Prevalence of *Escherichia coli* adhesion-related genes in neonatal calf diarrhea in Uruguay. J. Infect. Dev. Count., 10(5): 472–477. https://doi.org/10.3855/jidc.7102