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Molecular identification of the most frequent pathotypes of *Escherichia coli* in calves with diarrhoea in the Cajamarca region of Peru

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Abstract

Background: Colibacillosis caused by *Escherichia coli* causes significant economic losses in the livestock sector worldwide and is one of the calves' leading causes of diarrhea.

Aim: This study aimed to identify the most frequent *E. coli* molecularly pathotypes in calves with diarrhea in six provinces of the Cajamarca region in the northern highlands of Peru.

Methods: Twenty-eight herds of dairy cattle under a semi-intensive rearing system were evaluated; 95 samples were isolated from calves with diarrhea up to the first month of life, 62 males and 33 females, during the rainy season.

Results: The presence of virulence genes of *E. coli* strains was more prevalent in males; the *astA* (89.47%), *st* (83.15%), and *f5* (57.89%) genes were more expressed, and the *lt* (17.89%) and *stx2* (1.05%) genes were less expressed. The *eae* gene (21.05%) was more present in females.

Conclusion: When *E. coli* strains express virulence genes *astA*, *st*, and *f5* and their atypical double, triple, and quadruple combination between different observed pathotypes, they give rise to the formation of several pathotypes by the horizontal transfer of virulence genes, which can cause colibacillosis processes in more virulent calves, which is one of the most important causes of diarrhea in calves in the region of Cajamarca, compromising the sanitary viability in the herds.

Keywords: Calves, Colibacillosis, *E. coli*, Pathotypes.

Introduction

The most critical and severe disease that attacks new born animals is colibacillosis, caused by several infectious agents (Lee *et al.*, 2019), the primary etiological agent is *Escherichia coli* (*E. coli*) which causes enteritis in calves and causes significant health problems in calves (Tutija *et al.*, 2022) and causes significant economic losses in the livestock industry worldwide *E. coli* bacillosis represents one of the most important causes of morbidity and mortality in calves. Also, it causes a decrease in the growth of the animal and an increase in treatment and control costs (Algammal *et al.*, 2020).

There are several strains, among them enterotoxigenic *E. coli* (ETEC) which is considered to be an essential cause of diarrhoeal disease in humans, calves, pigs, and poultry (Bernal-Reynaga *et al.*, 2013). Enterohaemorrhagic

E. coli (EHEC) is the most frequent strain in apparently healthy calves with diarrhea, with a high implication in the aetiopathogenesis of calf diarrhea, being a reservoir of *E. coli* in calves and potentially pathogenic to humans (Aref *et al.*, 2018). Meanwhile, enteroaggregative *E. coli* (EAEC) is associated with childhood malnutrition, regardless of the presence of diarrhea (Havt *et al.*, 2017). However, diarrhoeagenic *E. coli* is the leading cause of gastroenteritis in children in developing countries and is associated with high levels of antimicrobial resistance, each strain possesses different virulence genes, including *eae*, *tir*, and *bfpA* for enteropathogenic pathotype (EPEC) and *ap AA* for EAEC. Two different toxins have been described in EHEC, including Stx1/Stx2 (Shiga-like toxin) and *ehxA* (enterohaemolysin), while an invasion plasmid (vir regulon) has been described in EIEC. There

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are two enterotoxins, thermostable (ST) and labile-stable (LT), by ETEC (Cabal *et al.*, 2016). Antibiotic resistance is currently one of the greatest threats to health, food security, and development worldwide, accelerated by the misuse and abuse of different drugs in both humans and animals, which prolongs hospital stays and increases medical costs and mortality. Resistance to ampicillin (92.5%), tetracycline (76.6%) and trimethoprim/sulfamethoxazole (70.1%), 51.92% to sulfamethoprim, 26.92% to neomycin, and 9.61% to enrofloxacin has been reported (Cabrera-González *et al.*, 2023).

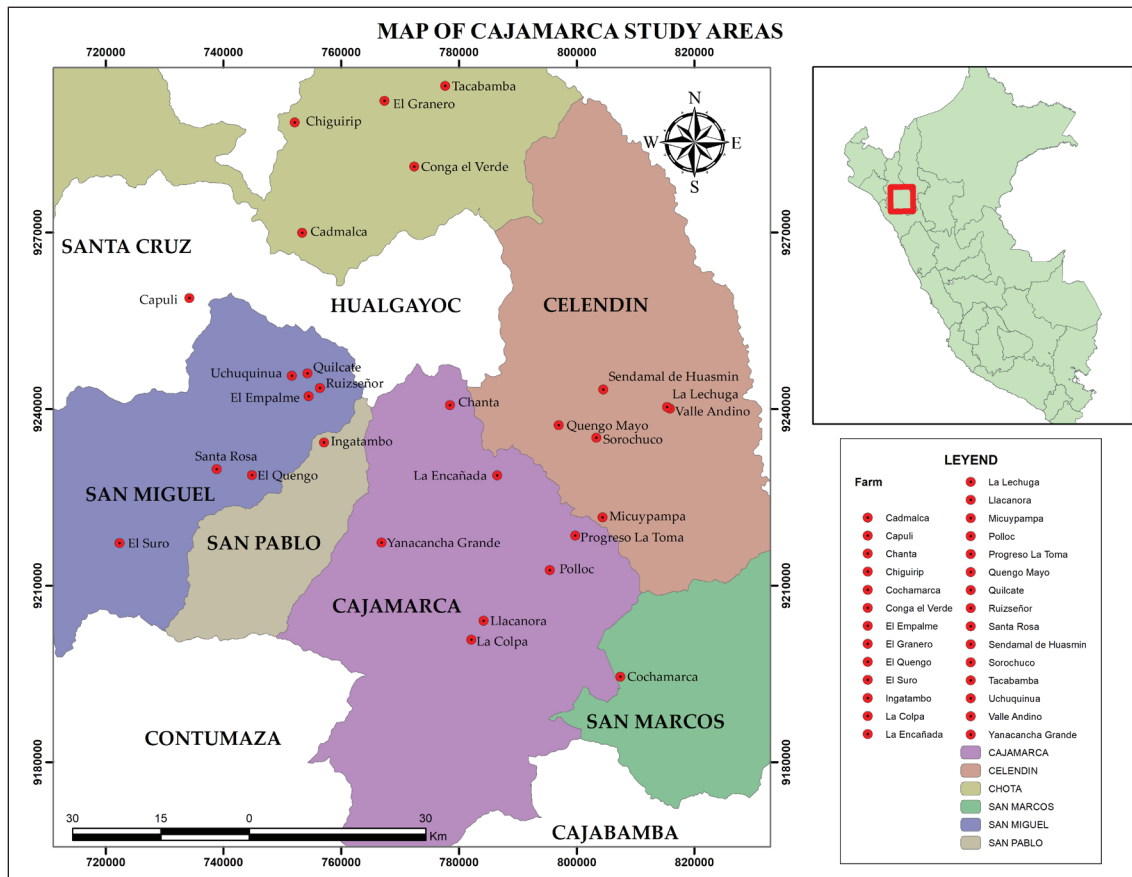
According to the 2012 agricultural census, Peru's livestock population is 5,156,000 head of cattle. Of this population, 78% is located in the highlands, 11% in the coast, and 10% in the jungle. Livestock activity is complementary to agriculture, forming integral production systems mainly oriented to self-consumption and artisanal production of dairy products, producing 15% of the country's milk production. Cajamarca is one of the leading milk-producing regions, producing 1 million l of milk per day (more than 360 million l per year), 40% of which goes to processing plants and a significant percentage to large milk companies such as Gloria and Nestlé, one of the most critical activities

in the Cajamarca region being the production of milk and its derivatives. The objective was to molecularly identify the most frequent *E. coli* pathotypes in calves with diarrhea.

Materials and Methods

Sample collection

Fecal samples were collected from 28 Holstein and Brown Swiss cattle dairy herds with grazing systems in the Cajamarca region's six provinces. Ninety-five samples were isolated from calves with diarrhea up to one month of age, 62 males and 33 females. The selection of sampled animals was based on the presence of diarrhea and the total number of calvings, considering that the herds where samples were collected do not use sexed semen. Fecal samples were collected during the rainy season, which is divided into two seasons: rainy and dry, with 15 February to 15 March being the season with the highest frequency of heavy and sudden rains; dry corresponds to autumn-winter between May and September (September 2021 - March 2022) (Fig. 1). Each herd consisted of 20 animals, of which 5 were calves. The number of samples taken was the total number of calves born in the herds of both sexes that presented diarrhea. The calves were handled painlessly



by trained personnel (veterinarians) with the owner's express permission. Fecal samples were obtained directly from the rectum using sterile polyethylene bags of first use, collecting approximately 3 g of feces, which were identified and transferred in a technoport box containing cooling ice gels (ThermoSafe—USA) to the laboratory of Biotechnology in Animal Health of the Agricultural Experimental Station Baños del Inca for the isolation and bacteriological identification of *E. coli*.

Isolation of *E. coli*

Using a bacteriological loop, 300 ml (µl) aliquot of feces was seeded on MacConkey agar (Becton, Dickinson, and Company/Loveton Circle Sparks, MD 21152, USA). The aliquot was then incubated (Memmert/ICO-50/ Schwabach/Germany) in aerobiosis at 37°C for 24 hours; colonies with morphological growth development typical of *E. coli* were identified.

DNA extraction from isolated *E. coli* colonies

Two selected colonies of typically colored (pinkish) *E. coli* colonies obtained from each fecal sample were seeded on MacConkey agar and deposited on powdered, liquid microbial growth medium (2xYT) (Sigma, REF Y2377) at 37°C for 18 hours for development and multiplication in an incubator (CO incubator2 ICO50-Memmert/Germany); without CO activation, operating as a standard incubator, subsequently, samples were evaluated in a spectrophotometer (PCR MAX Lambda 64272, Bibby Scientific Ltd, UK), using optical density and concentration pre-set in the spectrophotometer, the colony forming units were analyzed at 600 nm. To obtain *E. coli* genomic DNA, the Wizard® Genomic DNA purification kit (Promega, REF. A1120), following

the manufacturer's instructions, the purified DNA was stored in 1,500 ml (µl) polypropylene microcentrifuge tubes (Eppendorf™); finally, the samples were identified and stored under refrigeration (Samsung refrigerator, RT35K5930S8/PE, Samsung /Mexico) at four°C, for subsequent use in the polymerase chain reaction (PCR) analysis processes.

Molecular identification of *E. coli*

PCR technique was used, using primers (F5'-TCA GCGCGAAGTCTTTCTTTCTTTTATAACC-3', R5'-CGTCGGTAATCACCATTCCC-3'), to amplify the uidA gene (248 bp). The thermal profile was: denaturation 94°C/2', 25 cycles of denaturation 94°C/30", hybridisation 55°C/30", extension 72°C/45"; final extension 72°C/2'. DNA fragments amplified to identify *E. coli* strains were separated by molecular weight by electrophoresis (1.5% agarose). Fragment analysis was performed by gel staining with Sybr Green (Thermo Fisher) and observed on a UV transilluminator at 302 nm Labnet U1001 Taiwan. Amplification of the uidA gene was used as it is specific for *E. coli* detection.

Determination of the virulence gene profile

For the amplification of virulence genes (f5, st, lt, eae, stx1, stx2, hlyA, and astA), 2 PCR-multiplex (eae, hlyA, stx1, and astA), (lt, st, and f5) and a regular PCR (stx 2), with different primer pairs for each gene and with a different thermal profile (Tables 1 and 2); the procedure was performed with a thermal cycler (PCRmax Alpha-UK), the amplified DNA fragments were separated by molecular weight (bp) by 1.5% electrophoresis, the analysis was performed by agarose gel staining with Syber Green (Thermo Fisher) and observed in a 302

Table 1. Primer sequence for the identification of the virulence genetic profile of *E. coli* (Awad et al., 2020).

Pathotype	Generation	Primer sequence	PCR amplification (bp)
EPEC	MAR (eae)	F: TCAATGCAGTTCCGTTATCAGTT R: GTAAAGTCCGTTACCCCCCCCCCCCCCAACCTG	482 bp
	Stx1 (stx1),	F: CGATGTTACGGTTTGTGTGTGTTACTGTGTGTGACAGC R: AATGCCACGCTTCCCAGAATTG	244 bp
STEC	Stx2 (stx2)	F: CCATGACAACGGACGGACAGCAGTT R: CCTGTCAACTGAGCAGCAGCAGCAGCACTTTTGT	779 bp
	HlyA (hlyA)	F: AGCTGCAAGTGCAGGGGGTCTG R: TACGGTTATGCTGCAAGTTCAC	569 bp
	LT (lt)	F: GGGCACACACAGATTATATATATACCGTGC R: CGGTTCTCTATATTATTATTATCCCTGTT	450 bp
ETEC	STREET	F: ATTTTTTTTMTTCTTGTATRTCTT R: CACCCCGGTACARGCAGGATT	190 bp
	Fimbria f5 (k99)	F: TATTATATATCTTAGGTGGTGGTGGTGGTATGG R: GGTATATCCTTTAGCAGCAGCAGCAGTATTTATTC	314 bp
EAEC	ESTE1 (astA)	F: TGCCATCATCATCAACACACACACAGTATATATATCCG R: ACGGCTTTTGTAGTCCCTTCCTTCCAT	102 bp

Table 2. Components and thermal amplification profile of virulence genes of *E. coli* (Awad et al., 2020).

Virulence genes	Mix components: PCR/volume (µl)	Thermal profiling—PCR
eae, hlyA, stx ₁ and astA	5 µl of Master Mix	Initial denaturation 94°C, 5 minutes/1 cycle
	5 µl of template and 0,5 µl for each F and R primer (4 µl total)	35 cycles Denaturation 94°C, 30 seconds Alignment 62°C, 30 seconds Extension 72°C, 1 minute Final extension 72°C, 5 minutes / 1 cycle
	11 µl of ultrapure water	
stx ₂	5 µl of Master Mix	Initial denaturation 95°C, 3 minute / 1 cycle
	5 µl of template and 1 µl for each F and R primer (2 µl in total)	35 cycles Denaturation 95°C, 20 second Alignment 58°C, 40 second Extension 72°C, 90 second Final extension 72°C, 5 minute / 1 cycle
	13 µl of ultrapure water	
lt, st and f5	5 µl of Master Mix	Initial denaturation 95°C, 5 minutes / 1 cycle
	5 µl of template and 0,5 µl for each F and R primer (3 µl in total)	40 cycles Denaturation 95°C, 45 seconds Alignment 50°C, 1 minute
	12 µl of ultrapure water	Extension 72°C, 90 seconds Final extension 72°C, 5 minutes / 1 cycle

nm UV transilluminator—Labnet U1001 Taiwan. For the identification of virulence genes, the virulence genes were analyzed with the GelAnalyzer 23.1.

Statistical analysis

Results were analyzed using Graph Pad Prism 9.3.1 software (Prism Software, Irvine, CA, USA). The normality of the data was determined by Kolmogorov-Smirnoff analysis of variance (ANOVA) ($p \leq 0.05$), followed by Tukey’s post-hoc test for evaluations between virulence genes related to the identification of *E. coli* pathotypes. The information obtained was considered statistically significant at $p \leq 0.05$.

Ethical approval

Rectal fecal samples were obtained from animals without anesthesia or tranquilizers by certified and well trained Veterinarians at the Faculty of Veterinary Sciences of the National University of Cajamarca, Peru (Clinica Veterinaria Ministerio de Agricultura) in propylene bags following established protocols. Fecal samples were referred to the Biotechnology in Animal Health laboratory for coproparasitological analysis after obtaining the farmers’ authorization.

Results

Ninety-five fecal samples from calves with diarrhea were selected by characteristic growth on MacConkey agar and molecularly by PCR targeting amplification of the uid A gene (Fig. 2).

Single and multiplex PCR techniques targeting the amplification of the virulence genes eae, hlyA, stx1, astA (Fig. 3), stx 2 (Fig. 4), lt, st, f5 (Fig. 5) were optimized to identify pathogenic pathotypes of *E. coli* present in calf diarrhea samples.

The results showed that the presence of virulence genes of *E. coli* strains isolated from diarrhea did not show significant statistical differences between sexes; the most prevalent genes were astA (89.47%) (males, $n = 56/95$; females, $n = 29/95$).

Next, the st gene was expressed in lower proportion (83.15%) (males, $n = 51/95$; females, 28/95), f 5 (57.89%) (males, $n = 34/95$; females, $n = 21/95$).

It could also be observed that the genes lt (17.89%) (males, $n = 14/64$; females, 3/64), stx 2 (1.05%) (males, $n = 1/95$; females, $n = 0/95$) were expressed in lower proportion. Interestingly, the virulence gene eae (21.05%) was more prevalent in females ($n = 14/95$) than in males ($n = 6/95$). The hly A gene did not amplify in the processed samples (Fig. 6).

In the virulence gene identification profile of the *E. coli* isolates, the presence of 3 pathotypes that do not express genes of other pathotypes was observed: the EAEC (4.21%, $n = 4/95$), which mainly expressed the astA gene more frequently in males ($n = 3/4$) than in females ($n = 1/4$).

On the other hand, other pathotypes were only expressed in males as ETEC (3.15%), males 3/95, females 0/95 expressing the lt, st, and f5 genes; and shigatoxigenic (STEC) (1.05%) expressing the stx 1 gene mainly in males ($n = 1/95$), the EPEC was not expressed in the samples (Fig. 7).

Hybrid pathotypes of *E. coli* expressing a combination of virulence genes between pathotypes were also present. Double pathotypes formed by EAEC/ETEC strains (44.21%) expressing common genes such as astA, lt, st, and f5 were more prevalent in males ($n = 35/95$) than in females ($n = 7/95$).

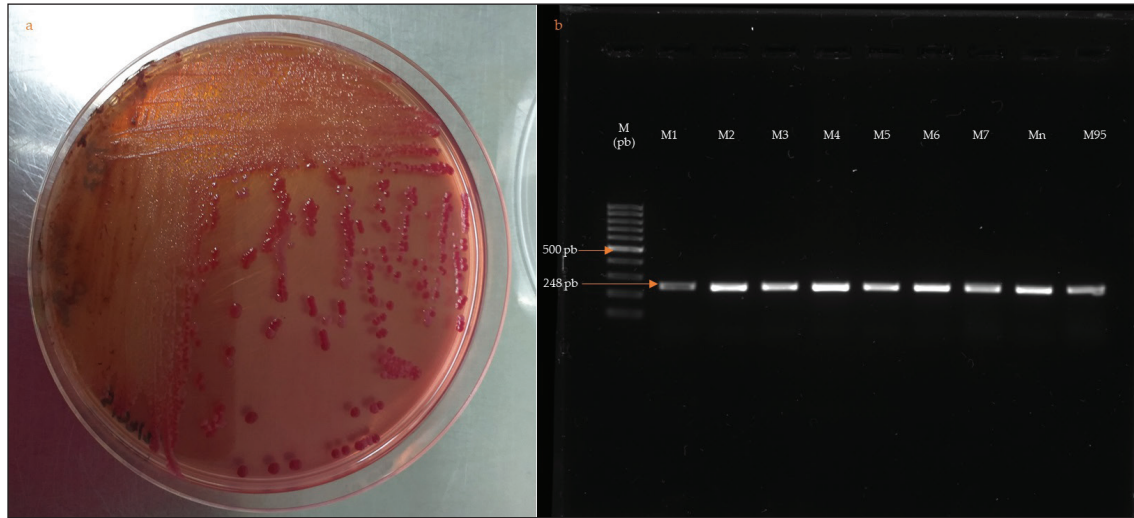


Fig. 2. (a): Growth of colonies on MacConkey II Sorbitol. (b): Amplification of the uidA gene (lane 2, lane 3, lane 95, [248 bp]) in 1.5% agarose, with 100 bp molecular marker.

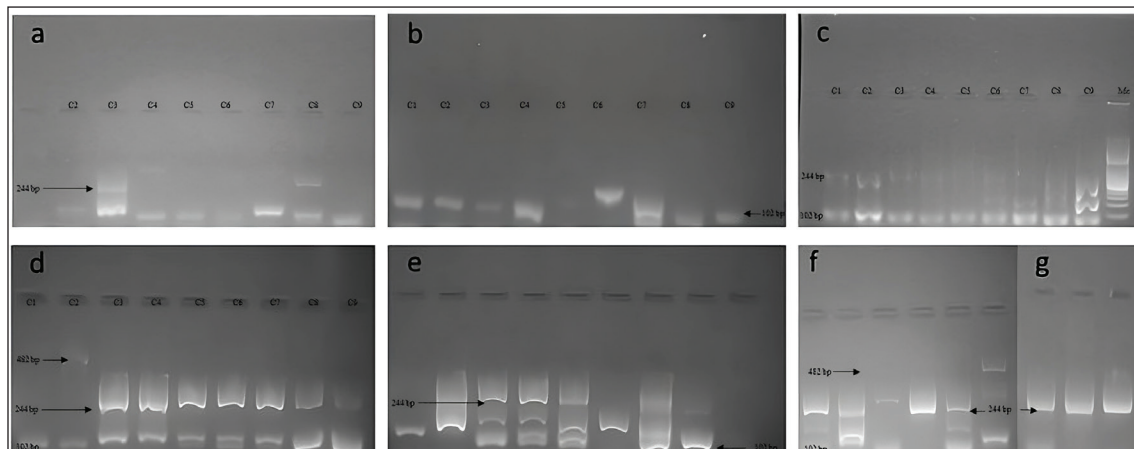


Fig. 3. Single and multiplex PCR techniques targeting the amplification of virulence genes. (a): lanes 3 and 8 positive for the stx1 gene (244 bp), lanes 2 to 9 positive for the astA gene (102 bp). (b): lanes 1 to 9 positive for the astA gene except lane 5 (102 bp). (c): lanes 1,2 and 9 positive for the stx1 gene (244 bp), lanes 1 to 9 positive for the astA gene (102 bp). (d): lanes 1 to 9 positive for the astA gene (102 bp); lanes 3, 4, 5, 6, 7, and 8 positive for the stx1 gene (244 bp); lanes 2 positive for the eae gene (482 bp). (e): lanes 3, 4, 5, and 8 positive for the stx1 gene (244 bp), lanes 1 to 8 positive to the astA gene (102 bp). (f): lanes 2,5 and 6 positive to the astA gene (102 bp); lanes 1, 2, 4, and 5 positive for the stx1 gene (244 bp); lane 6 positive for the eae gene (482 bp); g: lanes 1, 2, and 3 positive to the eae gene (244 bp).

Another pathotype combination was STEC/EAEC (3.15%), expressing Stx1, Stx2, hlyA, and astA genes, more prevalent in males ($n = 2/95$) than in females ($n = 1/95$); the pathotype combination EPEC/EAEC (1.05%) and EPEC/ETEC (2.10%) were expressed in males ($n = 1/95$) and females ($n = 2/95$), respectively (Fig. 8).

Likewise, *E. coli* strains were observed to share virulence genes corresponding to several pathotypes, with genes common to three and four different

pathotypes. The profiles were 21.05% of the STEC/EAEC/ETEC combination, sharing the genes stx1, stx2, astA, lt, st, and f5, expressed more in males ($n = 12/95$) than in females ($n = 8/95$).

The EPEC/EAEC/ETEC combination was present in 6.31%, the most prevalent genes eae, astA, st, f 5, with equal values in males ($n = 3/95$) and females ($n = 3/95$). On the other hand, strains sharing genes from four pathotypes were identified with 7.36% of EPEC/STEC/EAEC/ETEC, with common genes of eae, stx1,

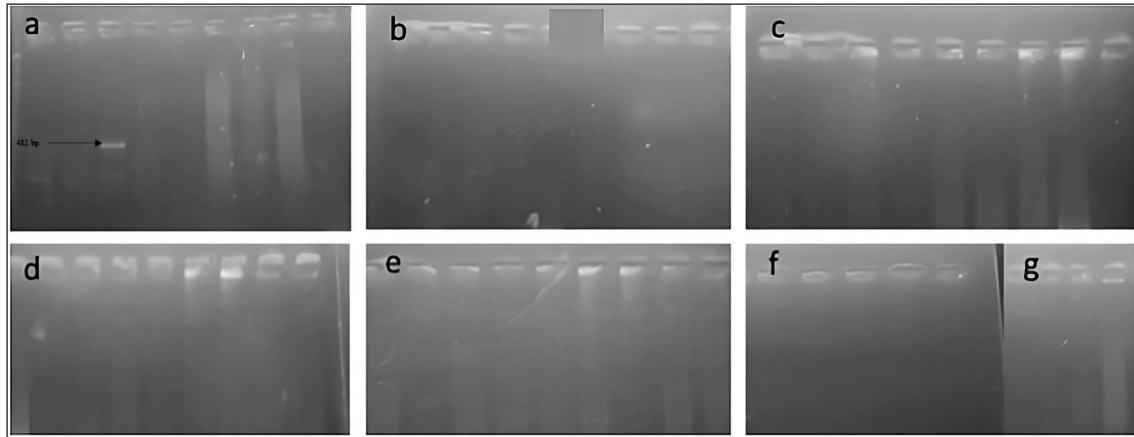


Fig. 4. Single and multiplex PCR techniques targeting the amplification of virulence genes. (a): Lane 3 stx 2 gene positive (799 bp). (b, c, d, e, f, g): all lanes negative.

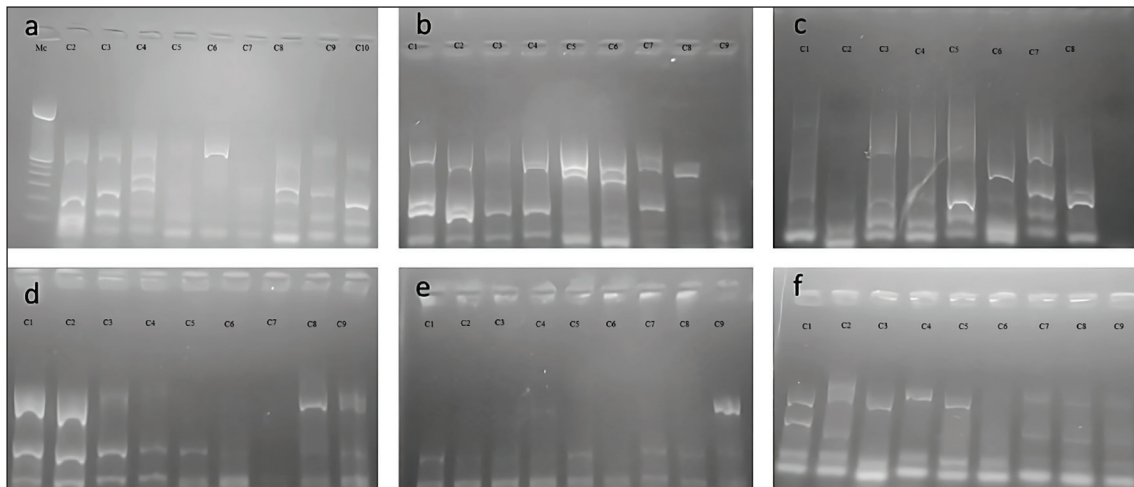


Fig. 5. Single and multiplex PCR techniques targeting the amplification of virulence genes. (a): Lanes 1, 2, 3, 5, and 8 positive to Lt gene (450 bp), lanes 1 to 9 positive to st gene (190 bp), lanes 3, 7, and 8 positive to f5 gene (314 bp). (b): lanes 1, 2, 4, 5, 6, 7, and 8 positive to Lt gene (450 bp), lanes 1 to 9 minus 8 positive to st gene (190 bp), lanes 1 to 7 positive to f5 gene (314 bp). (c): lanes 1, 3, 4, 5, 7, and 8 positive to Lt gene (450 bp), lanes 1 to 9 minus 8 positive to st gene (190 bp), lanes 1 to 7 positive to f5 gene (314 bp); lane 6 positive for Lt gene (450 bp), the rest negative, lanes 1 to 8 positive for st gene (190 bp), lanes 1, 3, 4, 5, 7, and 8 positive for f5 gene (314 bp) (d): lanes 1, 2, 8, and 9 positive to the Lt gene (450 bp), lanes 1 to 9 minus 7 positive to the st gene (190 bp), lanes 1 to 9 minus 1 and 7 positive to the f5 gene (314 bp). (e): lane 9 positive to Lt gene (450 bp), lanes 1 to 9 positive to st gene (190 bp), lanes 1 to 9 minus 6 positive to f5 gene (314 bp). (f): lanes 1 to 9 positive to st gene (190 bp), lanes 1 to 5 positive to Lt gene (450 bp), lane 1 positive to f5 (314 bp). Prevalence of amplified virulence genes of *E. coli* strains according to sex.

astA, st, and f5; this virulence profile is mainly found in females ($n = 6/95$) compared to males ($n = 1/95$) (Fig. 9).

In the statistical analysis, a significant difference ($p < 0.0001$) could be found for pathotypes and virulence genes of *E. coli* isolates from calf samples with diarrhea (Fig. 10).

Discussion

Escherichia coli is established as the primary health obstacle in dairy herds (Mohammed *et al.*, 2019). Some data, such as breeding system, calving, colostrum consumption of neonates, and perinatal antibiotic treatments to cows in milk production, could not be used due to the absence of records of producers in

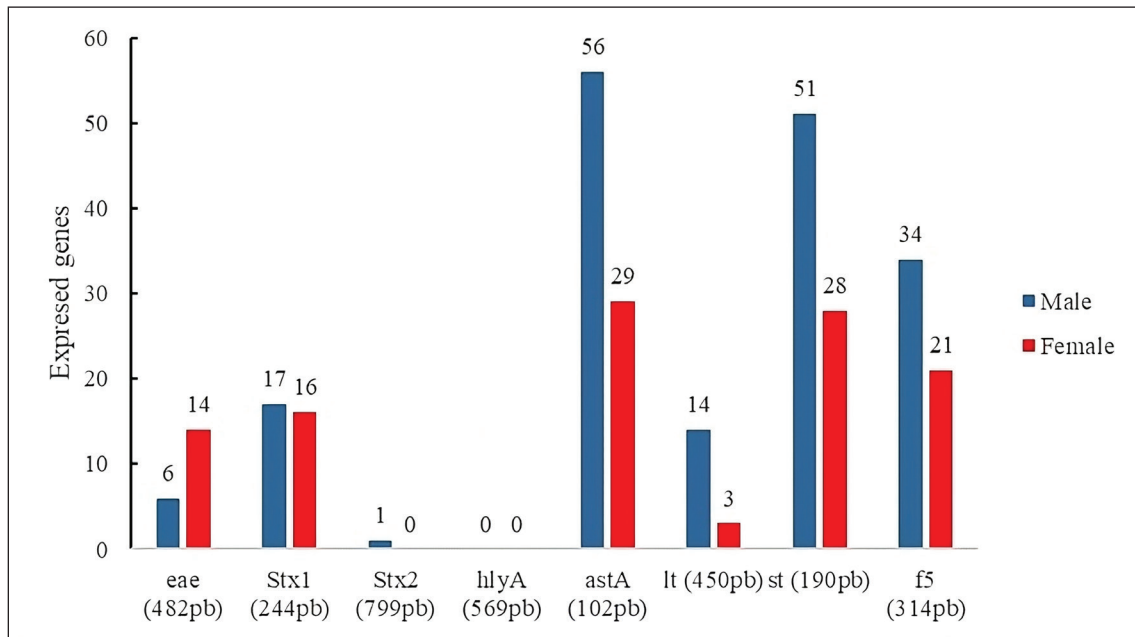


Fig. 6. Hly A gene did not amplify in the processed samples.

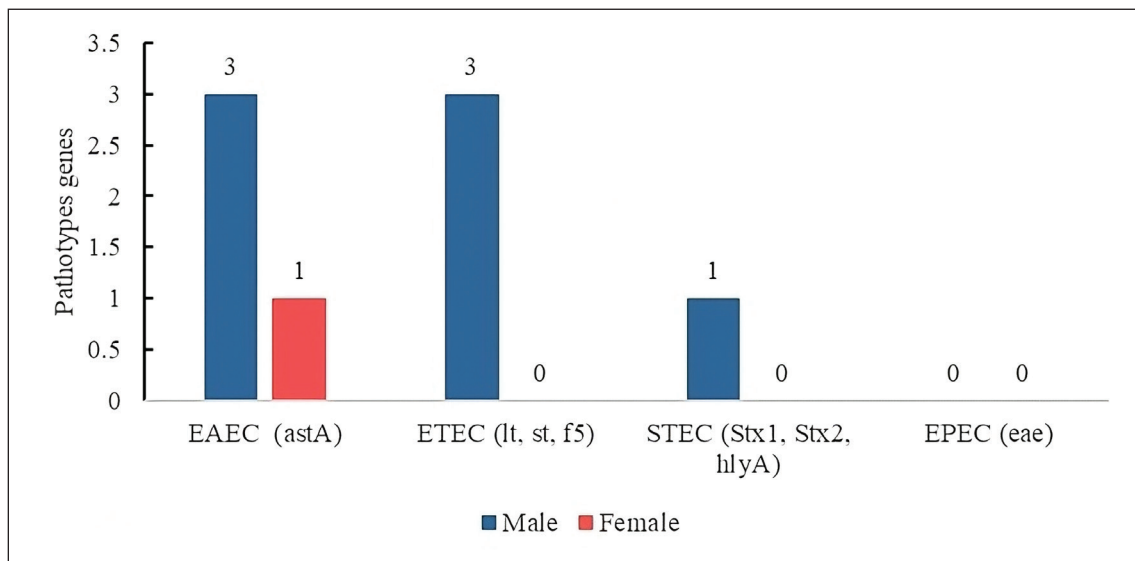


Fig. 7. Strains of pure pathotypes of *E. coli* present in calf diarrhea.

the Cajamarca region; it is also suggested to include other research factors to reduce the possible risk causes associated with the presentation of *E. coli* diarrhea in calves such as genetic characteristics of animals, dietary supplementation with probiotics, environmental factors (Bi *et al.*, 2017), the study was conducted in a pasture-based rearing system without any technical criteria for the prevention of gastroenteric diseases; therefore, it should be interpreted with caution as the sampling was based on animals with the presence of diarrhea.

The presence of the *astA* gene (89.47%) detected in *E. coli* strains as the most prevalent, which encodes a thermostable toxin, was reported by other researchers and is very frequent in many pathogenic pathotypes (EAEC, ETEC, STEC, and EHEC) (Zajacova *et al.*, 2012); this gene has higher pathogenicity and virulence than other associated genes that cause severe diarrhea in calves, compromising the health viability of herds and the economics of producers (Awad *et al.*, 2020). The presence of the *st* (83.15%) and *f5* (57.89%)

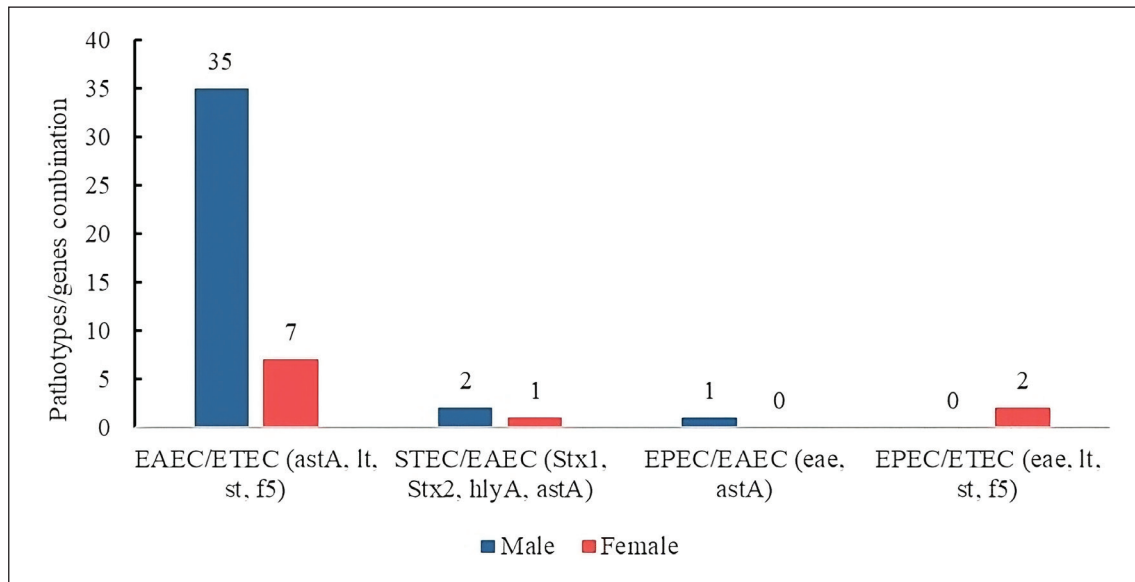


Fig. 8. *E. coli* hybrid strains showing virulence genes of two pathotypes.

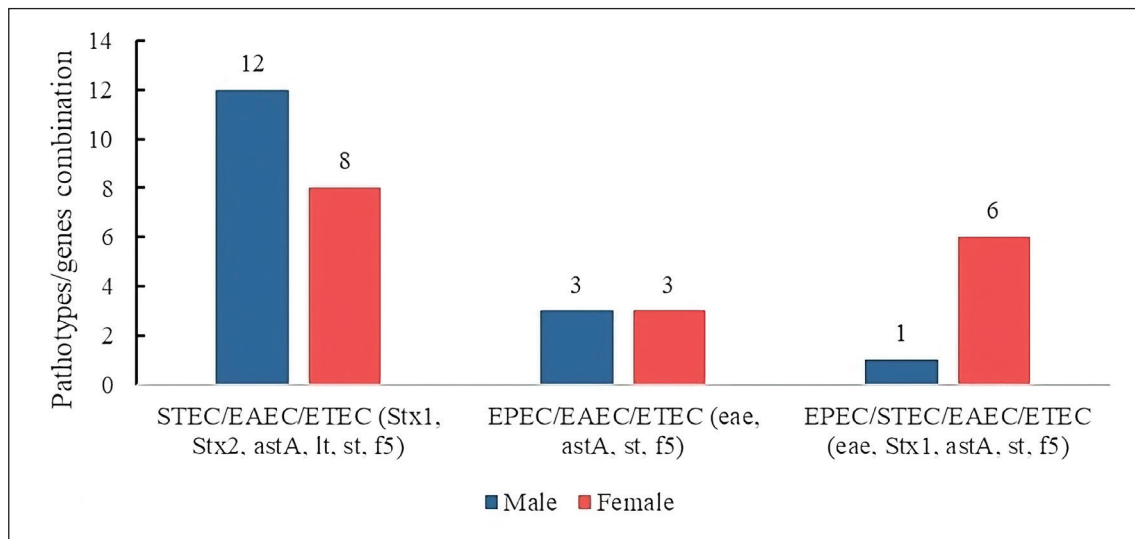


Fig. 9. *E. coli* hybrid strains expressing virulence genes of three and four pathotypes.

genes are a cause for concern in Cajamarca livestock because the f5-fimbria gene is used for colonization and establishment in small intestine cells at the surface protein level; it also constitutes the initial phase of colibacillosis and is associated with thermostable st and thermolabile lt enterotoxins (17.89%) that increase its pathogenicity (Awad *et al.*, 2020), similarly, it could be observed that the f5 gene was found in a high number of calves, contrary to what other authors claim with a prevalence of 5.3% (Shams *et al.*, 2012), this may be because, in the breeding area, vaccines are not used for

the prevention of colibacillosis in calves (Ryu *et al.*, 2020).

It was also observed that Shiga toxin virulence genes Stx 2 (1.05%) were minimally present and manifested in males ($n = 1/95$) compared to females. On the other hand, the presence of the hly A gene (0%) was not identified, which is of concern because ruminants are considered potential reservoirs (Kagambèga *et al.*, 2012), which can contaminate water food and increase the potential risk of infection by exposure (Sethulekshmi *et al.*, 2018).

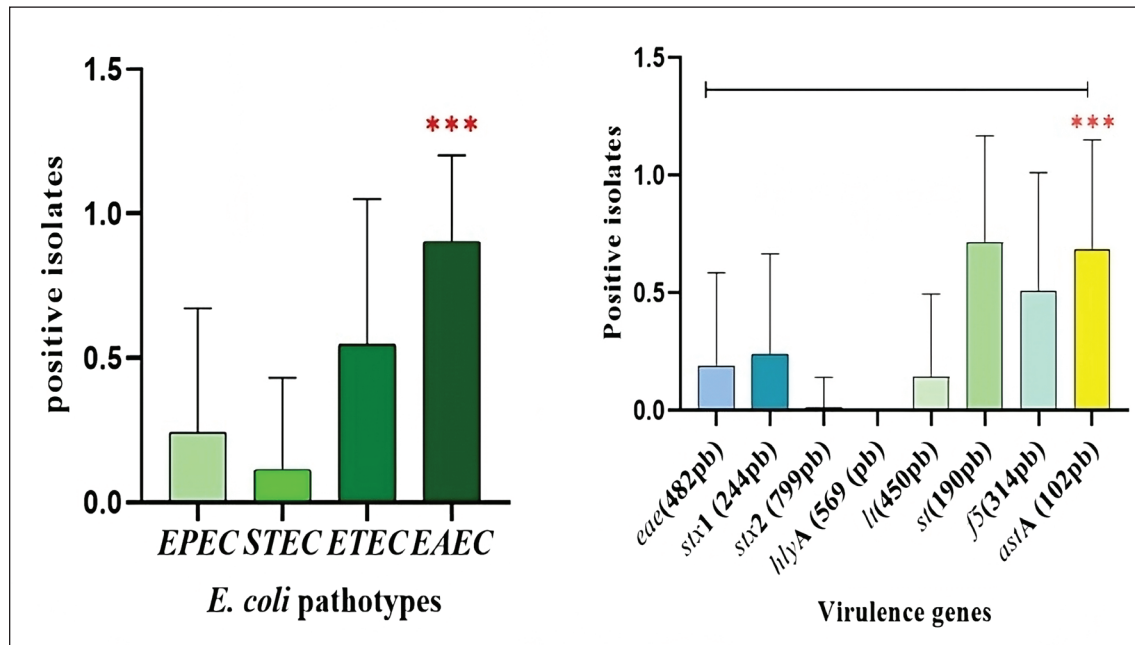


Fig. 10. (a): Pathotypes identified, (b): virulence genes amplified. Connector lines above the bars indicate the presence of statistical difference ($p < 0.0001$).

The presence of virulence genes encoding pathotypes of *E. coli* was observed in isolates of pure pathotypes, which do not share genes expressing other pathotypes. Thus, isolates of enteroaggregative *E. coli*—EAEC (6.25%) were isolated, which correlates with other research reporting calf samples with diarrhea from 2% (Belete *et al.*, 2022) to a detection rate of 39% (Coura *et al.*, 2019). In addition, they are common pathotypes that cause mild to moderate infections in animals (Pakbin *et al.*, 2021); there is concern about the risks it could cause to the population through contamination of milk, water consumption, and food contaminated with feces (Jeamsripong *et al.*, 2019). The virulence factors observed in isolation in these pathotypes were 87.5% of the *astA* gene EAEC, which is higher than the rate found in calves of 15.6%, 32.6% (Yuste *et al.*, 2006), possibly due to differences in the type of rearing, the calf diarrhea prevention measures used by farmers, the species, the breed and the altitudinal floor where the cattle are raised.

Another important pathotype that did not present virulence genes from other pathotypes was STEC 1.05%, identifying the virulence gene *stx 1*, which is zoonotic and can contaminate feed with animal manure during the handling process (Sapountzis *et al.*, 2020). In addition, relatively low prevalences of the virulence gene *stx 1* (9.3%), expressed in pre-weaned calves with diarrhea and 0.3% *stx* (Ryu *et al.*, 2020), coinciding with our results where *stx* was expressed more than *stx*, with this virulence gene being observed more in animals with diarrhea (Fakih *et al.*, 2017), which is considered a potential

problem that these strains adapt to the environment and transmit these resistance characteristics to various bacteria found in the environment (Metlay *et al.*, 2006).

Hybrid strains of *E. coli* that exhibit virulence factors of various pathotypes are more virulent and essential, so understanding their pathogenicity and their association cause disease in humans and animals is very challenging (Lee *et al.*, 2023); these strains have been reported worldwide and are associated with public health problems (Santos *et al.*, 2020), these virulence factors are contained in phages and plasmids that allow horizontal gene transfer leading to the generation of hybrid pathotypes (Jesser and Levy, 2020), observe strain profiles of *E. coli* strains consisting of two pathotypes EAEC/ETEC; CEEA/EPEC; EPEC/ETEC; STEC/ETEC; three pathotypes EAEC/ETEC,/STEC; EAEC/ETEC/EPEC and four pathotypes EPEC/STEC/ETEC/ETEC/ETEC/EAEC; isolated from feces of calves with diarrhea.

Among the most critical hybrid pathotypes of *E. coli*, we have the STEC/ETEC association, which represents a potential threat due to its higher virulence compared to other pathotypes and can be associated with causing diarrhea and hemolytic uraemic syndrome in humans (Bai *et al.*, 2019), representing a potential threat due to its ability to be transmitted to dairy farmers in the Cajamarca region.

Another significant hybrid pathotype of *E. coli* observed was the EPEC/STEC combination, which is also zoonotic for causing diarrhea and mortality in children, suggesting that this combination is highly

virulent because it contains the *eae* and *est* genes encoding binding lesions, effacement, and thermostable enterotoxins of *E. coli* (Lee et al., 2023) which could increase the potential threat of bovine reservoirs of *E. coli* in the transmission of these pathogenic strains to producers. On the other hand, combinations of three and four pathotypes of *E. coli* where the presence of the *astA*, *eae*, and *f5* genes was more prevalent, suggesting that this combination may confer greater virulence to these strains because the *eae* gene encodes intimin, which causes lesions in the gut (Trabulsi et al., 2002) and confers adhesins (fimbriae or pili) caused by virulence factor *f5* (Dubreuil, 2017) and the *astA* gene, present in most of the pathotypes analyzed, can cause outbreaks of diarrhea (Yatsuyanagi et al., 2003), in addition to the fact that in the Cajamarca region there is resistance to the drugs used, it was reported that 96.15% of the *E. coli* strains were resistant to tetracycline. The virulence gene *astA* may confer more excellent resistance to the strains identified. Therefore, it is necessary to know and identify the main pathotypes and virulence genes of *E. coli*, establish control and monitoring programs, use antibiotics appropriately, and avoid contact of feces with food, especially during manual milking and dairy food processing.

Conclusion

The most frequent pathotypes of *E. coli* in calves with diarrhea were EAEC and ETEC, which caused disease pathogenesis in calves, and the most prevalent virulence genes were *astA*, *st*, and *f5*.

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Authors' contributions

Conceptualization, methodology, and writing of the original manuscript, MCG Project management and fund acquisition CQP Overall revision of the manuscript, formal analysis HC English revision and editing, formal analysis WYAG, FAT Standardisation and validation of laboratory protocols, research ATS Laboratory Sample processing DRV Conceptualisation and Methodology MCR All authors have read the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Data availability

Data supporting the findings of this study are available from the corresponding author, MCG and MCR upon reasonable request.

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