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VETERINARY MICROBIOLOGY - RESEARCH PAPER

Detection and analysis of Shiga toxin producing and enteropathogenic *Escherichia coli* in cattle from Tierra del Fuego, Argentina

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Abstract

Shiga toxin producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) are pathovars that affect mainly infants' health. Cattle are the main reservoir of STEC. Uremic hemolytic syndrome and diarrheas can be found at high rates in Tierra del Fuego (TDF). This study aimed to establish the prevalence of STEC and EPEC in cattle at slaughterhouses in TDF and to analyze the isolated strains. Out of 194 samples from two slaughterhouses, STEC prevalence was 15%, and EPEC prevalence was 5%. Twenty-seven STEC strains and one EPEC were isolated. The most prevalent STEC serotypes were O185:H19 (7), O185:H7 (6), and O178:H19 (5). There were no STEC *eae* + strains (AE-STEC) or serogroup O157 detected in this study. The prevalent genotype was *stx*2c (10/27) followed by *stx*1a/*stx*2hb (4/27). Fourteen percent of the strains presented at least one *stx* non-typeable subtype (4/27). Shiga toxin production was detected in 25/27 STEC strains. The prevalent module for the Locus of Adhesion and Autoaggregation (LAA) island was module III (7/27). EPEC strain was categorized as atypical and with the ability to cause A/E lesion. The *ehx*A gene was present in 16/28 strains, 12 of which were capable of producing hemolysis. No hybrid strains were detected in this work. Antimicrobial susceptibility tests showed that all strains were resistant to ampicillin and 20/28 were resistant to aminoglycosides. No statistical differences could be seen in the detection of STEC or EPEC either by slaughterhouse location or by production system (extensive grass or feedlot). The rate of STEC detection was lower than the one reported for the rest of Argentina. STEC/EPEC relation was 3 to 1. This is the first study on cattle from TDF as reservoir for strains that are potentially pathogenic to humans.

Keywords Cattle · Shiga toxin producing *Escherichia coli* · Enteropathogenic *Escherichia coli* · Hemolytic uremic syndrome · Tierra del Fuego · Argentina

Introduction

Shiga toxin producing *Escherichia coli* (STEC) and Enteropathogenic *E. coli* (EPEC) are microorganisms known for causing severe gastroenteritis in infants [1]. STEC can cause a wide range of clinical disease patterns, including watery diarrhea, acute bloody diarrhea, and hemolytic uremic syndrome (HUS), which can lead to death by producing at least one of two cytotoxins called Shiga toxins (Stx), which are capable of inhibiting protein synthesis [2]. There are two types of Stx (Stx1 and Stx2), as well as several different subtypes [3, 4]. Non-typeable subtypes (NT) have also

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been reported [5]. There are 4 Stx1 subtypes (a, c–e) and 12 Stx2 subtypes (a–l) so far reported [5, 6]. EPEC causes diarrhea, affecting mainly children under 1 year of age, with high morbidity and mortality in developing countries [7]. It produces a lesion in the intestine epithelia known as "attaching and effacing" (A/E), produced by factors encoded by genes located in a pathogenicity island (PAI) called Locus of Enterocyte Effacement (LEE). The information needed to synthesize the protein intimin and a type 3 secretion system, along with different effector proteins, lies within LEE [8]. EPEC strains can be considered typical (tEPEC) or atypical (aEPEC) based on the presence of a type IV fimbriae called bundle forming pili (BFP). Atypical strains have been found in different animal species, and even some of these aEPEC are related to human gastroenteritis [7].

Cattle are the main reservoir for STEC and can eliminate the agent present in their intestinal microbiota through feces [9, 10]. Regarding EPEC, serogroups implicated in human diseases have been isolated from cattle suggesting that these animals could represent a reservoir for the pathogen [11, 12]. STEC causes a zoonotic disease, which can affect humans by oral ingestion of fecal-contaminated products [10]. The serotype O157:H7 is the most prevalent serotype and is responsible for the most severe disease patterns. However, more than 400 serotypes of *E. coli* can produce Stx [13].

Some STEC strains harbor LEE and can produce the A/E lesion. Fakih et al. [14] denominated these strains as AE-STEC. Patients who suffer HUS caused by AE-STEC strains can develop immune responsiveness to LEE-coded factors [15]. Yet in spite of the fact that AE-STEC is linked to human diseases in up to 80% of cases, LEE is not essential for successful infection (Rivas et al., 2016) [16]. Strains that do not have the LEE PAI are known as LEE negatives [17]. Montero et al. [18] reported the existence of another PAI that may contribute to intestinal colonization called Locus of Adhesion and Autoaggregation (LAA), associated with LEE negative strains. LAA can be present as a complete or incomplete structure, with four modules described that can be analyzed by gene markers as *hes*, *iha*, *les*P, *pag*C, *tps*A, *tps*B, and *agn*4 [18].

E. coli can also share genetic markers from different pathovars, generating hybrid strains like the Stx-producing EAEC O104:H4 strain, which has the ability to produce more complex clinical patterns [16, 19].

HUS is an endemic disease in Argentina, with reported cases increasing during the summer [20, 21]. The higher rates of HUS are located in the southern regions of the country, particularly in Chubut and Tierra del Fuego (TDF). TDF reports also high rates of acute diarrhea [22].

The province of Tierra del Fuego, Antártida e Islas del Atlántico Sur, is an island located in the southernmost part of Argentina and has different climate conditions than the rest of the country. Argentina's legislation restricts the movement of cattle to TDF, so meat production on the island is mainly locally produced. Therefore, *E. coli* strains in TDF may have characteristics only locally found. Also, it is interesting to assess antimicrobial resistance, given the emergence of global antimicrobial resistance, and the importance of cattle industries in Argentina. Prack McCormick et al. [23] found that in *E. coli*, multidrug resistance is associated with both animal species and animal production, being more prevalent in intensive productions. Information regarding antimicrobial resistance in beef cattle is still missing.

It is important to stress that there are no previous studies of STEC and EPEC in cattle in TDF. The correlation between STEC's prevalence and other *E. coli* pathovars in reservoirs and sources of infections has not been yet studied.

The aim of this study is to determine the prevalence of STEC (STEC LEE-negative / AE-STEC) and EPEC in cattle at slaughterhouses in TDF, as well as to evaluate the virulence profile of the strains and analyze the correlation between these pathovars.

Methods

Samples

We carried out a cross-sectional epidemiological study in June 2016 in order to determine the prevalence of STEC (STEC LEE-negative/AE-STEC) and EPEC in cattle from TDF.

The sample size was 126 animals from each of the two slaughterhouses, one located in Ushuaia and the other in Rio Grande. We considered a standard normal distribution ($Z = 1.96$), precision at 5%, 95% confidence level, and an estimated 9% of STEC and EPEC prevalence based on data [24]. The cattle were swabbed at the rectum, and each sample was sent to the lab maintained in Stuart Transport Media. The study was approved by the Institutional Committee on Animal Care and Use of Experimental Animals (CICUAL; Nº 2017/8) at the Facultad de Ciencias Veterinarias, Universidad de Buenos Aires.

Detection and isolation of strains

In order to detect non-O157 EPEC and STEC strains, each swab sample was enriched in 5 mL trypticase soy broth (TSB), incubated at 37 °C for 6 h, and then streaked onto Mac Conkey agar (MAC) for further incubation at 37 °C another 18 h.

STEC O157 detection was carried out by enriching each sample in 5 mL of TSB plus tellurite cefixime and incubated at 37 °C for 6 h. Then, we screened the serogroup by immunochromatography test (Reveal® *E. coli* O157) following the supplier's instructions. The positive samples were then

subjected to Immunomagnetic separation and streaked onto sorbitol Mac Conkey agar (SMAC) [25, 26].

We obtained a loop from the confluence culture zone in order to extract DNA and conducted multiplex PCR for STEC markers *stx*1, *stx*2, and *rfb*O157 [27] and single PCR for EPEC marker *eae* [28]. Primers for detection of genetic markers are referred in Table S1.

We then analyzed up to 50 colonies by PCR to find those markers identified in the confluence culture zones [25, 26].

For an AE-STEC strain to be classified as such, it has to carry both *eae* and at least one of the *stx*. According to the results, the samples were thus classified as negative, positive by screening, and positive by isolation. We characterized the isolates phenotypically (morphology, Gram stain, and motility) and confirmed them as *E. coli* through biochemical tests [29].

STEC and EPEC characterization

Genotypical characterization

In STEC strains, we analyzed the Stx subtypes: Stx1 subtypes (*stx*1a, *stx*1c, *stx*1d) and Stx2 subtypes (*stx*2a, *stx*2b, *stx*2c, *stx*2d, *stx*2e, *stx*2g), for identification by conventional PCR amplification [4]. Non-typeable strains were characterized by RFLP-PCR [3]. In addition, we evaluated the presence of *eae* gene. In STEC LEE-negative strains, we searched for the presence of the four modules (I-IV) of the LAA Island [18]. In EPEC isolates, we searched for the gene *bfp*A [30].

In all strains, we analyzed by PCR the presence of an enterohaemolysin (*ehxA*) and STEC autoagglutinating adhesin (*saa*) [31]. The presence of hybrid strains was determined searching for genetic markers of other *E. coli* pathovars: *aai*C [32] and *aag*R [33] for enteroaggregative *E. coli* strains (EAEC); *elt* and *est*A for enterotoxigenic *E. coli* strains (ETEC) [34]; *daa*E for diffuse aggregation *E. coli* strains (DAEC) [35]; and *inv*E for enteroinvasive *E. coli* strains (EIEC) [34]. Primers for detection of genetic markers are referred in Table S1.

Phenotypic characterization

The production of enterohaemolysin was tested in a TS-based agar using washed blood and non-washed blood ovine red blood cells [36].

To identify the production of Stx, we used the *SHIGA TOXIN QUIK CHEK,* which is a rapid membrane enzyme immunoassay test. For that purpose, we followed the supplier's recommendations.

EPEC strains were tested for adherence pattern to HeLa cells [37, 38]. We incubated the HeLa cells with 20 µL of an overnight culture of the strains (3- and 6-h assays). Then, the cultures were washed with PBS, fixated with methanol, stained with Giemsa, and finally analyzed under the microscope. We also examined the ability of the strains to cause A/E lesion using fluorescent actin stain (FAS). For this assay, HeLa cells and strains cultures were mixed, incubated for 3 h, and then fixated with formaldehyde 3%. The fixed cells were treated with 0.1% Triton X-100, washed with PBS, and then stained with fluorescein isothiocyanate-phalloidin (5 µg/mL). We kept the cells in a dark and humid environment for 30 min; we washed the cells again with PBS and analyzed the cells under the fluorescence microscope. The existence of fluorescent areas in adhesion zones was considered a positive FAS test [39].

Finally, all isolates were serotyped by agglutination assays with rabbit antisera: 187 sera were used against somatic antigens (O), and 53 sera were used against flagellar antigens (H) at the Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM) [40, 41].

Antimicrobial susceptibility testing

Antimicrobial susceptibility was conducted in all STEC and EPEC isolated strains, against the antibiotics enlisted by the reference center INEI-ANLIS: amikacin, ampicillin, ciprofloxacin, colistin, chloramphenicol, gentamicin, norfloxacin, nalidixic acid, tetracycline, streptomycin, and trimethoprim-sulfamethoxazole [36]. We used the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol according to CLSI recommendations [42]. To evaluate colistin resistance, we used the recommended method COLISTIN AGAR SPOT [43]. In the strains resistant to ampicillin, we evaluated the presence of broad-spectrum beta-lactamase (BLEE, AmpC, and KPC), performing the Kirby-Bauer test with strategic colocation of the following discs alongside boronic acid: amoxicillin clavulanic, cefotaxime, cefotaxime clavulanic, ceftazidime, meropenem, and imipenem [44].

Statistical analysis

Statistical analysis of the obtained data was carried out using the test of differences between proportions (InfoStat 2016e), considering the following variables: production system, farm, and slaughterhouse location.

Results

From a total of 194 samples, 124 were obtained in Rio Grande (RG) and 70 in Ushuaia (U). The samples in RG were taken at 3 different farms, 2 of them with extensive grass cattle and the other one feedlot. The samples in U were obtained from a single extensive grass breeding facility.

STEC prevalence at the screening stage was 15% (30/194), while EPEC was detected in 5% of the samples (10/194). No sample revealed the genetic markers for both pathovars at the same time. We isolated 27 LEE-negative STEC strains and one EPEC strain from the positive samples. AE-STEC strains were not detected. All the isolated strains were identified as *E.coli*.

Regarding the type of *stx*, 59.2% of STEC strains carried *stx*2 alone (16/27), 7.4% carried *stx*1 (2/27), and 33.3% of STEC strains carried both *stx*1/*stx*2 (9/27). Considering subtypes, the most prevalent was *stx*2c (10/27), followed by *stx*1a/*stx*2hb (4/27), and 15% of the strains (4/27) carried at least one non-typeable *stx* (*stx*NT). A total of 25/27 of the isolated STEC strains were able to produce Stx. None of the strains carried the gene *saa*; 15/27 carried the gene *ehx*A, 12 of which were able to produce the expected hemolysis. In search for the LAA island, we found that 11/27 strains where positive for at least one module, and module III was prevalent (7/27). None of the strains carried module IV, and no strain carried the 4 modules of the LAA PAI at the same time. Results are referred in Table 1.

The isolated EPEC strain was *bfp*A negative; therefore, we classified it as atypical EPEC (aEPEC). In regard to additional virulence factors, we characterized the strain as *saa*—*ehx*A +, without the capability to produce hemolysis (Table 1). HeLa essays did not reveal a defined adhesion pattern. However, the latter did not affect the strain's capability to produce A/E lesion.

No hybrid strains were detected in this study.

The 27 isolated STEC strains belonged to 12 different serotypes, the most prevalent O185:H19 (26%), O185:H7 (22,2%), and O178:H19 (18,5%). The remaining 9 serotypes (33,3%) were O1:H21, O6:H34, O113:H21, O130:H11, O130:H-, O171:H2, O174:H28, O179:H8, and O187:H7, with one strain for each serotype. The EPEC strain belonged to the O152:H25 serotype.

Antimicrobial susceptibility tests revealed resistance to ampicillin in all the analyzed strains. The assays did not confirm the presence of broad-spectrum beta-lactamases. Some strains showed resistance to streptomycin and amikacin; however, none of them were resistant to more than two families of antibiotics (Table S2).

No significant differences were observed ($p > 0.05$) between variables (production system, farm, and slaughterhouse location). STEC/EPEC detection showed a ratio 1 to 3 considering screening positive samples.

Discussion

We studied the prevalence of STEC (LEE-negative and AE-STEC) and EPEC in TDF 's cattle. We analyzed 194 rectal swabs samples from cattle in slaughterhouses. STEC prevalence at the screening stage was 15%, with all the strains being STEC LEE-negative. This result is lower than the prevalence reported for cattle in the rest of the country, which ranges between 22 and 67% [9, 45–47].

EPEC prevalence was 5%, similar to the one reported by Pizarro et al. [46] in Mendoza. As far as we know, there are no other studies regarding EPEC's prevalence in the country.

In concordance with Masana et al. [48], STEC detection showed no significant statistical differences regarding production system (feedlot and grass cattle). On the other hand, Padola et al. [45] and Tanaro et al. [49] revealed in their studies higher rates of detection of STEC in feedlot samples.

In our study, we detected mainly strains *stx*2 +, similarly to the ones found in other studies from Argentina [9, 46, 50, 51]. Of the 27 STEC strains isolated in this work, 25 (92%) presented the gene *stx*2, and 11 strains carried the gene *stx*1 (40%). The most prevalent subtype for Stx2 was *stx*2c (11/25), whereas 10 isolated strains could not be sub-typified using conventional PCR [4]. Of the latter 10 strains, 7 were identified using PCR- RFLP as *stx*2hb [3], and the remaining 3 strains could not be subtyped (*stx*2NT). These 3 *stx*2NT were obtained as follows: 2 from grass cattle from Ushuaia and 1 from the feedlot in Rio Grande. The most prevalent subtype for Stx1 was *stx*1a (9/11). One of the Stx1 could not be identified with the methods described [3, 4] (Table 1).

Considering the genetic profiles, *stx*2c was the most prevalent gene (10/27), followed by *stx*1a/*stx*2hb (4/27), and 15% of the strains (4/27) carried at least one non-typeable Stx (Table 1). Other studies in mainland Argentina also found non-typeable (NT) strains yet in a lesser proportion [49–53]. These results may suggest that STEC strains from TDF's cattle have characteristics of their own. In order to further investigate NT strains, it will be necessary to perform their sequencing. We found that 25/27 STEC strains were able to produce Stx using *SHIGA TOXIN QUIK CHEK*. A total of 3/25 of the Stx producing strains carried NT toxins ($24fRG_{III}$, $24fRG_{II}$, $U41_V$) (Table 1). Considering that the sensitivity of the test may vary according to the Stx subtype, the characteristics of the strain, and the amount of Stx produced, the absence of Stx production in the remaining 2 strains should be confirmed through toxicity assays in Vero cells cultures.

In this work, we did not find AE-STEC. Orden et al. [24] neither found AE-STEC strains in healthy cattle in Spain, being the most prevalent profile *stx*2 + *eae*-. This is also true in previous reports from other studies [9, 46, 54, 55].

Etcheverría and Padola [56] analyzed STEC strains from different sources (minced beef, cattle, and environment), and they did find AE-STEC, yet in a lower proportion than STEC LEE-negative. Other studies in mainland Argentina revealed a higher degree of detection of AE-STEC O157 [48, 53, 57] and non-O157 AE-STEC [50, 51]. The differences in the

Table 1 Virulence profiles and serotypes of the isolated STEC and EPEC strains from cattle in TDF

Serotype	*stx*	Stx production	*eae*	*bfp*A	*saa*	LAA mod 1	LAA mod 2	LAA mod 3	LAA mod 4	*ehx*A	Hemolysis	No. of isolations	Identification	Production system and location
O1:H21	*stx*2c	-	-	ND	-	-	-	-	-	-	-	1	123fRGII	A
O6:H34	*stx*1c *stx*2c	+	-	ND	-	-	-	-	-	-	-	1	123fRGIII	A
O113:H21	*stx*2c	+	-	ND	-	-	-	-	-	+	-	1	U17	C
O130:H11	*stx*1a *stx*2a	+	-	ND	-	+	-	-	-	+	+	1	44cRG	B
O130:H-	*stx*1a *stx*2a	+	-	ND	-	+	-	-	-	+	+	1	51cRG	B
O152:H25	-	-	+	-	-	-	-	-	-	+	-	1	93fRG	A
O171:H2	*stx*2NT	+	-	ND	-	-	-	-	-	-	-	1	U41V	C
O174:H28	*stx*2a	+	-	ND	-	-	+	+	-	+	+	1	56cRG	B
O178:H19	*stx*2c	+	-	ND	-	-	-	-	-	-	-	1	100fRG	A
O178:H19	*stx*2c	+	-	ND	-	-	-	+	-	-	-	2	5fRG; 11fRG	A
O178:H19	*stx*2c	+	-	ND	-	+	+	+	-	-	-	1	16fRG	A
O178:H19	*stx*1a *stx*2NT	+	-	ND	-	-	-	-	-	+	+	1	24fRGII	A
O179:H8	*stx*2a	+	-	ND	-	-	-	-	-	+	+	1	74fRG	A
O185:H7	*stx*2c	+	-	ND	-	+	+	-	-	+	-	1	U27	C
O185:H7	*stx*2c	+	-	ND	-	-	-	-	-	+	-	1	66fGRI	A
O185:H7	*stx*2c	+	-	ND	-	-	+	-	-	-	-	1	U13	C
O185:H7	*stx*2c	+	-	ND	-	+	-	+	-	-	-	1	8fRG	A
O185:H7	*stx*2hb	+	-	ND	-	-	-	+	-	-	-	1	63fRG	A
O185:H7	*stx*2hb	+	-	ND	-	-	-	-	-	-	-	1	64fRG	A
O185:H19	*stx*1a	+	-	ND	-	-	-	-	-	+	+	2	66fRGIII; 85fRG1Vd	A
O185:H19	*stx*1a *stx*2hb	+	-	ND	-	-	-	-	-	+	+	3	85fRG1Xa; 114fRGIV; 120fRG	A
O185:H19	*stx*1a *stx*2hb	+	-	ND	-	-	-	+	-	+	+	1	114fRGIII	A
O185:H19	*stx*1NT *stx*2hb	+	-	ND	-	-	-	-	-	+	+	1	24fRGIII	A
O187:H7	*stx*2NT	-	-	ND	-	-	-	-	-	-	-	1	U41I	C

References**:** ND, not determined; -, absence of gene marker; +, presence of gene marker; A, feedlot Río Grande; B, extensive grass Río Grande; C, extensive grass Ushuaia. Genes markers: *stx* (Shiga toxin); *eae* (intimine); *bfp* (bundle forming pili); *saa* (STEC autoagglutinating adhesin); LAA (modules 1 to 4 locus of adhesion and autoaggregation); *ehx*A (enterohaemolysin)

results could be related to different sampling methods and detection protocols.

It is important to highlight that nowadays, the diagnostic pathways are better and include the search for non-O157 serogroups [58]. In 2008, Coombes et al. [59] reported a 60% increase in clinical HUS worldwide related to non-O157 serogroups, while the HUS cases related to O157 strains increased by only 13%. In 2015, Byrne et al. [60] carried out a study in England that revealed that non-O157 strains were related to highest rates of HUS hospitalization than O157 strains. Moreover, Valilis et al. [61] analyzed the pathogenic role of non-O157 strains, concluding that they are more related to acute diarrhea in humans than O157 strains.

The LEE-negative strains isolated in our work belonged to non-O157 serogroups: O1:H21; O6:H34; O113:H21; O130:H11; O130:H-; O171:H2; O174:H28; O178:H19; O179:H8; O185:H7; O185:H19; and O187:H7 (Table 1). Serotypes O113:H21, O130:H11, O174:H28, O178:H19, and O185:H7 have been previously detected in Argentina [50, 51, 56, 62]. Etcheverría and Padola [56] detected O8:H19, O26:H11, O91:H21, O113:H21, O117:H7, O130:H11, O145:H-, O157:H7, O171:H2, and O178:H19 as prevalent serotypes, some of them equally present in different sources.

The O174 serogroup has been detected in various sources (cattle, rodents, food, humans), and it has been related to HUS in Argentina [50, 52]. It was also described by Masana et al. [50] as one of the non-O157 serogroups responsible for 30% of clinical HUS in Argentina.

Previous reports in Argentina detected STEC O130:H11 and STEC O178:H19 as prevalent serotypes in dairy and feedlot cattle, abattoirs, and local markets [50, 62–64]. Both serotypes have also been associated to HUS in Argentina [63]. STEC O178:H19 has also been related to HUS worldwide [65–69]. A comparison between strains of STEC O178:H19 from different sources confirmed the absence of the *eae* gene. Its absence has no impact on STEC O178.H19 pathogenesis [70].

Qin et al. [71] isolated the O6:H34 serotype from clinical pediatric HUS cases, and Wang et al. [72] found O6:H34 in 10% of the isolated strains in a persistence study in cattle from Canada. Previously, Delannoy et al. [73] analyzed O1 and O2 STEC strains in human and cattle feces. Serogroups O113 and O179 have been also reported in bovine feces and carcasses in Argentina [50, 51], as well as in minced meat [68]. No information has been found about the O185:H19 serotype, yet O185:H9 and O185:NT have been previously reported in bovine feces in Germany [74].

As far as the serotype O187:H7 is concerned, there are not many findings yet. Bai et al. [75] found 4 human hybrid STEC/ETEC strains in Sweden belonging to rare serogroups, including O187. Hybrid strains represent a high risk for public health, and although they have been found in cattle before [76–78], we did not find any hybrid strain in our study.

Our aEPEC strain belonged to the O152:H25 serotype (Table 1). HeLa essays did not reveal a defined adhesion pattern. However, this did not affect the O152:H25 capability to produce A/E lesion. As far as we know, this serotype has not been associated with human diarrhea, yet clinical cases of EIEC O152 serogroup have been reported [79–81].

Regarding additional STEC virulence factors, the *ehxA* gene was found in 57% (16/28) of strains and 12/16 were able to produce the expected hemolysis (Table 1). Strain capability to produce this hemolysin has been associated with strains with clinical impact and has been used for screening detection [82]. The aEPEC strain (93fRG) was one of the carrier strains of gene *ehxA* without the capability to produce hemolysis, as has been also reported in previous studies [11, 83–85].

None of the analyzed strains carried either the *saa* gene or the four modules of the LAA island at the same time (Table 1). In their analysis of LEE negative strains from various sources (cattle, food, environment), Colello et al. [86] found the complete LAA sequence in 46% of the strains, without correlation between the detection of LAA and serogroup. Although the high variation of LEE negative strains makes it difficult to establish their virulence, Montero et al. [87] described the gene *hes* among the most prevalent virulence factors. In our study the *hes* gene was not prevalent (5/27) (Table 1). To establish the importance of LAA in our strains, it will be necessary to carry out further functional assays, as well as analyze the presence of other adhesins.

Finally, our susceptibility tests detected ampicillin resistance in all strains (Table S2). Other studies conducted in Latin America revealed similar results [88, 89]. In the analysis of feces from different animal sources, Pantozzi et al. [90] found ampicillin resistance in up to 4.4% of cattle strains. In 2017, SENASA (Servicio Nacional de Sanidad y Calidad Agroalimentaria) reported levels of ampicillin resistance related mainly to swine and poultry intensive production in Argentina. The resistance to this antibiotic in cattle was 6.5% [91]. Regarding other antibiotics, some strains in our work detected resistance or intermediate resistance to aminoglycosides, specifically streptomycin (71%), and amikacin (21%) (Table S2). Pantozzi et al. [90] also found strain resistance in cattle to streptomycin and amikacin, yet in lower proportions (2.2% and 9%, respectively). None of our strains revealed phenotypic resistance to colistin, none was resistant to more than two families of antibiotics, and there was no beta-lactamase extended spectrum resistance.

There are not many studies that allow the comparison between two *E. coli* pathovars in cattle feces samples [14, 23, 46, 92]. The analysis of 194 samples from local TDF cattle detected 30/194 positives to STEC at the screening stage

and 10/194 positives to EPEC. None of the strains was classified as AE-STEC. The correlation between STEC/EPEC was 3 to 1, so it is three times more likely (considering the same source of infection) for TDF infants to be exposed to STEC, with all the health risks it implies. Considering the gene homology between these two pathovars in LEE, Calderon Toledo et al. [93] suggested crossed immunity between EPEC and AE-STEC strains. They inoculated BALB/c mice with these strains and reported that the infection from EPEC could provide immunity to AE-STEC infection. However, all the STEC strains isolated in our work were LEE-negative. In this sense, the immunity provided by EPEC would not be effective for a consequent STEC LEE-negative infection. This could contribute to the high HUS rates in TDF.

This is the first study of STEC and EPEC prevalence in cattle in TDF. High HUS rates in TDF highlight the need for further research, in particular serial sampling.

We need to know if the proportion of serotypes, the relationship between *E. coli* detection and cattle production facility, and the prevalence obtained are stable over time. It is of importance as well the study of other sources of infection and reservoirs.

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Declarations

Conflict of interest The authors declare no competing interests.

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