Characterization of Diarrheagenic Escherichia Coli Strains Isolated from Slaughtered Sheep in México

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Research Article

Keywords: diarrheagenic Escherichia coli, pathotypes, sheep and slaughterhouse

DOI: https://doi.org/10.21203/rs.3.rs-573147/v1

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Abstract

Sheep represent one of the main reservoirs of diarrheagenic Escherichia coli, this microorganism is an etiological agent of food-borne diseases, therefore, this work aimed to identify and characterize the principal pathotypes of diarrheagenic E. coli obtained through rectal swabs and samples from sheep carcasses slaughtered in an abattoir at the central region of Mexico. The isolates were subjected to bacteriological identification, serotyping; phylogenetic classification; detection for virulence factors, and antimicrobial sensibility. A total of 90 E. coli isolates were obtained, diarrheagenic serotypes with health public relevance were found: O76:H19 (5), O146:H21 (3), O91:H10 (2), O6:NM (1), and O8:NM (1). According to pathotype, 47.7% of total isolates were Shiga toxin-producing E. coli, while 3.3% were enteropathogenic, 2.2% enterotoxigenic, and 1.1% enteroinvasive E. coli; the remaining isolates did not express the genes used to assign them to some pathotype. Regarding the Shiga toxin subtypes, 31/43 (72.09%) were cataloged as stx1c, 11/43 (25.5%), stx1a-stx1c and 1/43 (2.3%) stx1a-stx1d, while for stx2 it was possible identify stx2g 4/7(57.14%), stx2b 1/7 (14.7%) and stx2b-stx2g 2/7 (28.5%). Almost all pathotypes (91–100%) belonged to phylogroup B1. Furthermore, it was observed that the 90 isolates showed an antimicrobial resistance of 100% to nitrofurantoin, followed by ampicillin, tetracycline, and trimethoprim-sulfamethoxazole. These results highlight the importance of diarrheagenic E. coli as a potential risk for public health during the slaughtering process.

Introduction

Sheep and other ruminants are regular carriers of commensal Escherichia coli; however, they may harbor some pathogenic E. coli and cause either, diarrhea or extraintestinal illness (Bettelheim 2000). The relevance of these diarrheagenic E. coli (DEC) isolates as causative agents of food-borne diseases (FBD) has been recently studied in Latin America, although there is a lack of information in some countries regarding the main reservoirs and infection routes (Torres 2017). Animal products, like sheep and beef meat, are at risk of contamination by poor hygiene practices during the slaughtering process in the abattoirs, hence, the implementation of good production practices (GPP) and good manufacture practices (GMP) are essential to prevent bacterial contamination of carcasses and ensure food safety (FAO 2005). Sheep without diarrhea usually are asymptomatic carriers of zoonotic pathogens and reservoirs of DEC, which could enter the production line, especially in the critical control points (Reyes-Rodríguez et al. 2015). The animals that arrive at the slaughterhouse are the principal focus of contamination towards drinking water and animal products, allowing the direct transmission of zoonotic microorganisms to the human population (Blanco et al. 2003).

At least 5 E. coli pathotypes have been related to gastrointestinal illness in humans: Shiga-toxin-producing E. coli (STEC), enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), and enteroaggregative E. coli (EAEC) (Nataro and Kaper 1998). These pathotypes are classified according to their virulence factors. The principal virulence factor of STEC is the production of a toxin that inhibits protein synthesis coded by stx1 and stx2 genes and their variants, moreover, other virulence factors like the intimin (encoded by eae gene) or autoagglutinating adhesins can be found (Paton et al, 2001).

Shiga toxins are classified as stx1 and stx2. The stx1 toxins are a homogeneous group with 3 subtypes: stx1a, stx1c, and stx1d. On the other hand, stx2 toxins are more heterogeneous with a greater number of subtypes that include stx2a, stx2b, stx2c, stx2d, stx2e, stx2f, and stx2g, being stx2c and stx2d strongly associated with HUS. Other relevant virulence factors include the intimin (encoded by eae gene), a plasmid-carried enterohaemolysin (encoded by ehxA gene), putative adhesins genes like ToxB, saa, espC, and espP (Prager et al., 2011, Beutin 2007).

The presence or absence of eae gene in STEC strains allows classifying them into the typical virulent (t-STEC) or atypical strains of low virulence (a-STECE). STEC strains induce gastroenteritis and further complications as hemolytic uremic syndrome (HUS) or hemorrhagic colitis (HC) which can lead to chronic kidney dysfunction, especially in infants and the elderly (Nataro and Kaper 1998).

EPEC produces the attachment and effacing (A/E) lesions onto intestinal mucosa. This pathotype is divided into two categories based on the presence or absence of the bundle-forming pilus (bfp) gene; strains that contain this gene are
classified as typical (t-EPEC), while the ones that lack this gene are atypical (a-EPEC). Curiously, the a-EPEC strains are more common in developing countries, in contrast, t-EPEC causes diarrhea in children from developed countries (Trabulsi et al. 2002). The main feature of ETEC is the production of two enterotoxins: the heat labile-toxin (LT) and the heat-stable toxin (ST). The ETEC strains are the leading cause of traveler’s diarrhea and is also related to children’s diarrhea (Sjöling et al. 2007).

The EIEC group and *Shigella* spp. are biochemically and genetically related. The pathogenicity mechanism is through the invasion of the colon’s epithelium; several involved proteins like Ipa and others are encoded in the 140 MDa plasmid *plnv*, generally, watery diarrhea is observed, but in some cases, inflammatory colitis can occur (Halet et al. 1983). Finally, EAEC pathotype increases the production of mucus allowing a pattern autoaggregative of bacteria and forming a layer in the epithelium. Adherence and is due to aggregative adherence fimbriae, especially the variant I (AAF/I) encoded by *aggA* gene, which in turn is located in a plasmid of 60 MDa (Nataro et al. 1992).

Several studies have reported DEC in carcasses from slaughterhouses, for example in Burkina Faso the five pathotypes mentioned in this work were isolated from bovine, poultry, and swine carcasses (Kagambega et al. 2012). In France Um et al. (2018) reported the presence of STEC and EPEC in bovines. In Mexico there are few reports about these pathotypes in ruminant carcasses (Navarro et al. 2018), such information is necessary to assert the risk factors that could affect the safety of sheep carcasses in this country, therefore, we investigated the prevalence of DEC isolates obtained from sheep slaughtered in an abattoir in Mexico, and determined the presence of virulence factors, the phylogenetic classification of isolates as well as their antimicrobial resistance profile. Therefore, the main objective of this work is to know which diarrheal pathotypes of *E. coli* are naturally present in sheep slaughtered in a slaughterhouse in the state of Mexico and to know if they could represent a risk factor for the consuming population.

**Material And Methods**

**Sample collection and bacteriological isolation**

A convenience sampling was performed in a slaughterhouse with the largest number of slaughtered sheep in the central region of Mexico. The sample size was estimated with a prevalence of 12.3% (Etcheverría et al. 2010) and a 95% confidence level through sample size determination for finite populations (Waine 1991). A non-destructive method employing a swab in 0.1% peptone + NaCl (0.85%) according to the European Union was used (Official Journal of the European Community 2001). From a total of 321 samples, 159 rectal swabs were taken before evisceration and 162 swab samples were taken from carcasses after final washing and before refrigeration. Finally, swabs were stored in sterile tubes with 25 mL of peptone water (1%).

Samples were transported to Centro de Investigación y Estudios Avanzados en Salud Animal (CIESA, Universidad Autónoma del Estado de México). Samples were streaked onto MacConkey Agar (MAC, Beckton Dickinson, USA). After 24 h of incubation at 37 °C, suspected pink colonies were grown in Eosin Methylene Blue Agar (EMB, Dickinson, USA), and colonies with a green metallic sheen were identified by biochemical tests (triple sugar iron, sulfide indole motility, methyl-red Voges-Proskauer, urea, malonate, phenylalanine, gluconate, citrate, and sorbitol) (USDA 2015).

**Serotyping**

The procedure described by Orskov and Orskov (1984) was employed. Specific rabbit sera against 187 *E. coli* somatic (O) antigens and 53 flagellar (H) antigens were used (SERUNAM, registered trademark in Mexico, with number 323158/2015).

**Virulence factors**

The identification and characterization of diarrheagenic *E. coli* pathotypes (STEC, EPEC, ETEC, EIEC, and EAEC) were performed by PCR. Fragments of several virulence genes were amplified and assigned to each pathotype employing primers and thermal cycling conditions as described previously (Scheutz et al. 2012; Kong et al. 2002; Gunzburg et al. 1995; Sjöling et al. 2007; Li et al. 2009 y Czeczulin et al.1999) (Table 1). The reaction products were visualized on 2% agarose containing ethidium bromide.
Detection of Shiga toxin subtypes

Identification of stx\textsubscript{1} and stx\textsubscript{2} subtypes genes (stx\textsubscript{1a}, stx\textsubscript{1c}, stx\textsubscript{1d}, stx\textsubscript{2a}, stx\textsubscript{2b}, stx\textsubscript{2c}, stx\textsubscript{2d}, stx\textsubscript{2e}, stx\textsubscript{2f}, and stx\textsubscript{2g}) was carried out with primers and PCR conditions described by Scheutz et al., 2010. Amplicons were visualized on a 2% agarose gel with ethidium bromide (Table 2).

Phylogenetic group determination

A quadruplex PCR was carried out to identify the phylogenetic groups (A, B1, B2, C, D, E, and F), the chu\textit{A}, yja\textit{A}, arp\textit{A}, and Tsp\textit{E4.C2} genes were amplified with primers and PCR conditions according to Clermont et al. (2013) (Table 1).

Antimicrobial susceptibility testing

Susceptibility to antibiotics was tested using a disk diffusion method according to Clinical and Laboratory Standard Institute guidelines (CLSI 2017). E. coli ATCC 25922 and ATCC 35218 were used as quality control. Commercial discs of ampicillin 10 μg (AMP), cephalothin 30 μg (CEF), ceftriaxone 30 μg (CAZ), amikacin 30 μg (AMK), ciprofloxacin 5 μg (CIP), gentamicin 10 μg (GEN), fosfomycin 50 μg (FOF), netilmicin 30 μg (NET), trimethoprim-sulfamethoxazole 25 μg (SXT), norfloxacin 10 μg (NOR), nitrofurantoin 300 μg (NIT), and tetracycline 30 μg (TET) (BBL™ Sensi-Disc™ Becton Dickinson, USA) were used. The ATCC 25922 Escherichia coli strain was used as a quality control microorganism.

Antimicrobial resistance genes

To identify antimicrobial resistance genes against β-lactams, tetracyclines, and sulfonamides, the genes \textit{bla\textsubscript{TEM}}, \textit{tetA}, \textit{tetB}, \textit{sul1}, and \textit{sul2} were analyzed by PCR technique using the primers and conditions described by Kern et al., 2002 Martí et al., 2006 and Dallene et al., 2010 respectively (Table 3). The PCR products were visualized by electrophoresis on a 2% agarose gel stained with ethidium bromide.

Statistical analysis.

The virulence factors, serotypes, antibiotics resistance, and phylogroups results were analyzed by descriptive statistics and the generated data arranged in tables.

Results

Sample collection and bacteriological isolation

A convenience sampling was performed in a slaughterhouse with the largest number of slaughtered sheep in the central region of Mexico. The sample size was estimated with a prevalence of 12.3% (Etcheverría et al. 2010) and a 95% confidence level through sample size determination for finite populations (Waine 1991). A non-destructive method employing a swab in 0.1% peptone + NaCl (0.85%) according to the European Union was used (Official Journal of the European Community 2001). From a total of 321 samples, 159 rectal swabs were taken before evisceration and 162 swab samples were taken from carcasses after final washing and before refrigeration. Finally, swabs were stored in sterile tubes with 25 mL of peptone water (1%).

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Detection of Shiga toxin subtypes

Identification of *stx*₁ and *stx*₂ subtypes genes (*stx*₁a, *stx*₁c, *stx*₁d, *stx*₂a, *stx*₂b *stx*₂c *stx*₂d *stx*₂e *stx*₂f, and *stx*₂g) was carried out with primers and PCR conditions described by Scheutz et al., 2010. Amplicons were visualized on a 2% agarose gel with ethidium bromide (Table 2).

Phylogenetic group determination

A quadruplex PCR was carried out to identify the phylogenetic groups (A, B1, B2, C, D, E, and F), the *chuA*, *yjaA*, *arpA*, and *TspE4.C2* genes were amplified with primers and PCR conditions according to Clermont et al. (2013) (Table 1).

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Antimicrobial resistance genes

To identify antimicrobial resistance genes against β-lactams, tetracyclines, and sulfonamides, the genes *bla*₅ TEM, *tetA*, *tetB*, *sul1*, and *sul2* were analyzed by PCR technique using the primers and conditions described by Kerrn et al., 2002 Martí et al., 2006 and Dallene et al., 2010 respectively (Table 3). The PCR products were visualized by electrophoresis on a 2% agarose gel stained with ethidium bromide.

Statistical analysis.

The virulence factors, serotypes, antibiotics resistance, and phylogroups results were analyzed by descriptive statistics and the generated data arranged in tables.

Declarations

Funding.

This work was supported by a grant from Universidad Autónoma del Estado de México (Registration No. 3998/2016R ED) and is part of the Doctor studies in Agricultural Sciences and Natural Resources of Enriquez-Gómez who received CONACyT scholarship (No. 394480).

Code availability 'Not applicable' for that section

Authors' contributions.

All authors contributed to the conception and design of the study. Material preparation, data collection, and data analysis were performed by Jorge Acosta-Dibarrat, Edgar Enriquez-Gómez, Martín Talavera-Rojas, Edgardo Soriano-Vargas Armando Navarro,
Rosario Morales-Espinosa. The first draft of the manuscript was written by Jorge Acosta-Dibarrat, Edgar Enriquez-Gómez, Martín Talavera-Rojas, Edgardo Soriano-Vargas Armando Navarro, Rosario Morales-Espinoza and Jorge Acosta-Dibarrat, Edgar Enriquez-Gómez, Martín Talavera-Rojas, Edgardo Soriano-Vargas Armando Navarro, Rosario Morales-Espinoza commented on previous versions of the manuscript. Jorge Acosta-Dibarrat, Edgar Enriquez-Gómez, Martín Talavera-Rojas, Edgardo Soriano-Vargas Armando Navarro, Rosario Morales-Espinoza read and approved the final manuscript.

Ethics approval. 'Not applicable' for that section.

Consent to participate. 'Not applicable' for that section.

Consent for publication. 'Not applicable' for that section.

Acknowledgments

We appreciate the help and cooperation of the technical processes laboratory to Luis Antonio León Alamilla, Gabriel Pérez Soto and Biol. Delia Licona Moreno, Biol. José Luis Méndez Sánchez and Dr. Gabriela Delgado Sapien, from Faculty of Medicine (UNAM) and Carlos Martín de la Luz Moreno from Centro de Investigación y Estudios Avanzados en Salud Animal, UAEMEX.

Statement Animal Rights.

It does not apply since the investigation was carried out with animals previously slaughtered by the municipal authorities of the slaughterhouse.

Conflict of interests.

No conflict of interest on the part of all authors.

Data availability statement

The data that support the findings of this study are openly available in UAEMex Institutional repository at http://hdl.handle.net/20.500.11799/110239, on the website http://ri.uaemex.mx/handle/20.500.11799/110239.

Discussion

Different E. coli pathotypes are related to diarrhea in both, human and animal populations, with some serotypes capable of causing outbreaks (Eslava et al. 1994; Nataro and Kaper 1998). In this work, several serotypes associated with diarrhea in humans in Mexico and other countries were found (O8:NM, O76:H19 and O146:H21) (Eslava et al. 1994; Sheutz and Strockbine 2005), moreover, serotypes O6:NM, O91:H10, and O104:H2 have been related to HUS. It is important to highlight that serotype O146:H21 has been found in sheep from farms and slaughterhouses in Brazil (Maluta et al. 2014; Vettorato et al. 2008), while, the same serotype can be found in Mexico backyard sheep or adult sheep from Norway (Amezquita et al. 2014; Urdhal et al. 2003). Similarly, the serogroup O104 is considered of clinical importance in the European Economic Community; interestingly, this serogroup is disseminated in lambs and sheep from India and lambs in Mexico (Wan et al. 2003; Bhat et al. 2008; Kumar et al. 2014; Enriquez-Gómez et al. 2016) (Table 4).

According to Monaghan et al. (2011), 40% of putative pathogenic E. coli belongs to pathotype STEC (non-O157) and is the major agent of microbial contaminations in meat products in Europe and USA (EFSA 2013; USDA-FSIS 2012). In this work, STEC was the most frequent pathotype (47.7%) in sheep slaughtered in the abattoir; this finding is similar to that reported in Brazil in sheep abattoirs (11.3%), or slaughter-age sheep from Australia (72%), and with other ruminants like goats in Kenia (50%), Iran (16.4%) or bovines in Burkina Faso (37%) and Mexico (40.7%) (Maluta et al. 2014; Djordjevic et al. 2001; Njoroge et al. 2013; Jajarmi et al. 2015; Kagambéga et al. 2012; Navarro et al. 2018).
The most frequent stx subtype gene described worldwide in sheep is stx1c (Brett et al., 2003; Kumar et al., 2012; Erol et al., 2016), the results of our investigation corroborate this statement, howbeit, a small number of isolates carried stx1a and stx1d. The stx7c subtype gene is related to diarrhea without complications in humans (Beutin et al., 2007).

Recent research has shown that stx subtypes have a predilection toward different receptors, the Stx B subunit recognizes Gb3 as its principal receptor and to a lesser extent Gb4. Lee and Tesh (2019) highlight the relevance of this interaction as a key mechanism in the pathogenicity of STEC. Stx1a interacts strongly with Gb3 on the human glomerular endothelium. On the other hand, the subtype stx2e shows predilection for Gb4 and Gb5 present in the glomerular endothelium of ruminants and pigs.

In this work stx2g gene was predominant, followed by stx2b, these subtypes are not associated with HUS and HC development in humans, which could represent a low hazard to establish disease. In contrast, the presence of stx2c and stx2d genes that were not reported in this investigation that boost the development of HUS and HC, have been reported in sheep carcasses from Turkey and Switzerland. Amezquita et al. (2014) found stx2c and stx2d genes in backyard sheep in Mexico. Prager et al. (2011) demonstrated that isolates harboring stx2g gene obtained from humans, animals and environmental sources had a close phylogenetic relationship, reinforcing the idea of human infections as a potential zoonotic disease.

Identification of stx subtypes is a priority as lets an early prediction of the virulence potential of each STEC isolate. This observation generated enough evidence to know that stx2a and stx2d genes are crucial determinants in the severity of HUS; furthermore, the mere presence of stx2a gene is considered an independent risk factor to develop HUS in multivariate analysis. Therefore, the identification of stx subtypes should be performed routinely in diagnostic laboratories. (Weber and Sheutz 2019)

Pathotype EPEC was the second most frequent (3.3%) and is responsible for neonatal diarrhea in human and animal populations (Karmali et al. 2013), however, it also affects adult sheep in Australia and Brazil (Djordjevic et al. 2001; Maluta et al. 2014). The isolates in this work not expressed the bfp gene, so they were categorized as a-EPEC (Nataro and Kaper 1998).

ETEC was the third most frequent pathotype (2.2%) and is considered one of the main diarrheagenic pathogens in lambs and calves (Troeger et al. 2017). In Kenia, it was also reported as the third most frequent DEC (10%) in slaughtered goats, while an investigation in Mexico rated as the second most frequent in bovines, the same as in Burkina Faso (4%) (Njoroge et al. 2013; Navarro et al. 2018; Kagambégå et al. 2012). Previous reports about ETEC have been described in bovines with/without diarrhea in Brazil, Vietnam (with the same number of isolates as this work), and Burkina Faso (De Moura et al. 2012; Nguyen et al. 2011; Bako et al. 2017). There is a lack of information regarding this pathotype in slaughtered sheep in Mexico.

The last reported pathotype was EIEC with only 1.1%, this low percentage it is also observed by other authors in comparison with other pathotypes in other species, for example, Navarro et al (2018) only discovered 11.5% of EIEC in bovine feces, while Kagambégå et al. (2012) found 1% of this pathotype in slaughtered poultry in Burkina Faso.

In this work EAEC was not detected, however, other investigations report this pathotype along with STEC, EPEC, and ETEC in Mexico, Iran, and Burkina Faso in bovines and goats (Navarro et al. 2018; Jajarmi et al. 2015, Kagambégå et al. 2012).

The presence of most of the DEC pathotypes of public health concern can be isolated from sheep, goats, and bovines which raise the relevance of livestock as a reservoir of these pathogens (Mora et al. 2011); precarious hygiene conditions make it possible for DEC to contaminate meat products with feces during different processes in slaughterhouses in Mexico.

The high percentage (~90-100%) of isolates belonged to phylogenetic group B1 (commensal E. coli), which is similar to that reported in other countries in isolates from sheep, goats, and bovines (Ghanbarpour et al. 2012; Wang et al. 2013; Alizade et al. 2014; Carlos et al. 2016).

Antimicrobial resistance (AMR) was observed in STEC isolates against AMP (72%), TET (30 %), and SXT (9%); these percentages were lower in comparison to a study carried out in Turkey where a higher frequency of AMR to AMP and TET (100% and 50% respectively) was reported (Seker and Kus 2019). In Egypt, lower levels of AMR to AMP (66.7%) but higher to
SXT (73.3%) were described in a goat slaughterhouse (Elsayed et al. 2018). In Mexico, a study discovered 92% and 75% of AMR to AMP and TET respectively in bovines, this contrast with our study where both antibiotics showed a lower level of AMR. Another study in this country found AMR to cephalosporins in STEC isolates from bovines, interestingly, we did not find AMR to these antibiotics, despite this, both studies showed AMR to TET and AMP (Martinez-Vazquez et al. 2018; Navarro et al. 2018).

In the case of α-EPEC, a lower resistance rate in comparison with this study was found in adult sheep in Spain with a 1.9%, 0, and 1% for GEN, TET, and SXT respectively (Medina et al. 2011), conversely, a study from Brazil detected higher rates of resistance against CIP (22%), AMK (4%), GEN (9%) and cephalosporins (72%) in a sheep abattoir (Rigobelo et al. 2008). In the particular case of ETEC, we find out high resistance levels for AMP (100%) and TET (50%) in our work compared to Njoroge et al. (2013) with goat isolates in Kenya.

Multi-drug resistance (MDR) was found against 3 and 4 antibiotics (TET, NIT, AMP, and SXT) in 10 STEC isolates. The presence of MDR E. coli in the gut microbiota of the analyzed sheep could further disseminate to other microorganisms due to horizontal gene transfer (Penders et al. 2013).

In the present study, it was possible to detect resistance genes such as tetA, tetB, sul1, sul2 detected in isolates resistant to tetracycline and trimethoprim-sulfamethoxazole. Research studies around the world also reported the finding of some of these genes, in Portugal (Ramos et al., 2011) informed the presence of tetA, tetB and in a smaller number sul2, in sheep samples processed in a slaughterhouse. Medina et al, (2011) working with live sheep in Spain reported the presence of these same genes, being tetA the most frequent, finally, in France, bovine isolates harbored tetA and sul2 genes (Um et al., 2018).

This investigation is one of the few to characterize DEC from slaughtered sheep in Mexico, and to the author knowledge, the first to be carried out in the region with the highest production, processing, and consumption of sheep meat of the country (SIAP 2016). We identified several serotypes related to gastrointestinal illness in Mexico, along with some stx subtypes genes that have been reported worldwide as low virulent (stx1a, stx1c, stx1d, stx2b, and stx2g), nevertheless, some serotypes are implicated in diarrhea and MDR isolates could pose a threat for treatment in case of intestinal and extra-intestinal illness in people who consume sheep meat. These findings reflect the potential concern of sheep as a primary reservoir of STEC non-O157 and the possible transmission through the food chain.

References

1. Alizade, H., Ghanbarpour, R., Nekoubin, M., 2014. Phylogenetic of Shiga Toxin-Producing Escherichia coli and a typical Enteropathogenic Escherichia coli Strains Isolated from Human and Cattle in Kerman, International Journal of Enteric Pathogens. 2(1): e15195. https://doi: 10.17795/ijep15195.

2. Amézquita-López, B.A., Quiñones, B., Lee, B.G., Chaidez, C., 2014. Virulence profiling of Shiga toxin producing Escherichia coli recovered from domestic farm animals in Northwestern Mexico. Frontiers in Cellular and Infection Microbiology. 31; 4:7. https://doi: 10.3389/fcimb.2014.00007.

3. Bako, E., Kagambéga, A., Traore, K.A., Serge, Bagre, T., Ibrahim, H.B., Bouda, S.C., Bonkoungou, I.J.O., Kaboré, S., Zongo, C., Traore, S.A., Barro, N., 2017. Characterization of Diarrheagenic Escherichia coli Isolated in Organic Waste Products (Cattle Fecal Matter, Manure and, Slurry) from Cattle's Markets in Ouagadougou, Burkina Faso. International Journal of Environmental Research in Public Health. 14, 1100; https://doi: 10.3390/ijerph14101100.

4. Bettelheim, K.A., 2000. Role of non-0157 VTec. Journal of Applied Microbiology Symposium Supplement, 88, 388–508. https://onlinelibrary.wiley.com › doi › pdf › j.1365–2672. Accessed 18 august 2018.

5. Beutin, L., Miko, A., Krause, G., Priès., Haby, K.S., Steege, K., Albrecht, N., 2007. Identification of Human-Pathogenic Strains of Shiga Toxin-Producing Escherichia coli from Food by a Combination of Serotyping and Molecular Typing of Shiga Toxin Genes. Applied environmental microbiology. Aug, p. 4769–4775 https://doi:10.1128/AEM.00873-07

6. Bhat, M.A., Nishikawa, Y., Wani, S.A., 2008. Prevalence and virulence gene profiles of Shiga toxin-producing Escherichia coli and enteropathogenic Escherichia coli from diarrheic and healthy lambs in India. Small ruminant research. 75 (1): 65–70. https://doi.org/10.1016/j.smallrumres.2007.08.006
1. Blanco, M., Blanco, J.E., Mora, A., Rey, J., Alonso, J.M., Hermoso, M., Alonso, M.P., Dahbi, G., González, E.A., Bernárdez, M.I., Blanco, J., 2003. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from healthy sheep in Spain. Journal of Clinical and Microbiology. 41, 1351–5. https://doi.org/10.1128/jcm.41.4.1351-1356.2003

2. Brett, K., Ramachandran, V., Hornitzky, M., Bettelheim, K.A., Walker, M.J., Djordjevic, S.P., 2003. *stx*1c Is the Most Common Shiga Toxin 1 Subtype among Shiga Toxin-Producing *Escherichia coli* Isolates from Sheep but Not among Isolates from Cattle. Journal of clinical microbiology. 41(3), 926–936. https://doi.org/10.1128/jcm.41.3.926-936.2003

3. Carlos, C., Pires, M.M., Stoppe, N.C., Hachich, E.M., Sato, M.I.Z., Gomes, T., Amaral, L.A., Ottobon, L. M.M., 2010. *Escherichia coli* phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. BMC Microbiology. 10, 161. https://doi.org/10.1186/1471-2180-10-16

4. Clermont, O., Christenson, J.K., Denamu, E., Gordon, D.M., 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environmental Microbiology Reports. 5(1):58–65. https://doi.org/10.1111/1758-2229.12019

5. Clinical and Laboratory Standards Institute (CLSI) (2017) performance standards for antimicrobial susceptibility testing. 27th ed CLSI supplement M100 (ISBN 1-56238-1-56238-805-3). https://clsi.org/media/1469/m100s27_sample.pdf. Accessed 10 September 2017

6. Czeczulin, J.R., Whittam, T.S., Henderson, I.R., Navarro-Garcia, F., Nataro, J. P., 1999. Phylogenetic analysis of enteroaggregative and diffusely adherent *Escherichia coli*. Infection and Immunity. 67 (6) 2692–2699; DOI: 10.1128/IAI.67.6.2692-2699.199

7. Dallenne, C., Da Costa, A., Decré, D., Favier, C., Arlet, G., 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important b-lactamases in *Enterobacteriaceae*. Journal of Antimicrobial Chemotherapy. 65: 490–495. doi: 10.1093/jac/dkp498

8. De Moura, C., Ludovic, M., Valadares, G.F., Gatti, M.S.V., d Leite, D.S., 2012. Detection of virulence genes in *Escherichia coli* strains isolated from diarrheic and healthy feces of dairy calves in Brazil. Arquivos do Instuto Biologico. vol.79 no.2 São Paulo Apr. / June. https://doi.org/10.1590/S1808-16572012000200016

9. Djordjevic, S. P., Hornitzky, M. A., Bailey, G., Gill, P., Vanselow, B., Walker, K., Bettelheim, K.A., 2001. Virulence properties and serotypes of Shiga toxin-producing *Escherichia coli* from healthy Australian slaughter-age sheep. Journal of Clinical Microbiology, 39(5), 2017–2021. https://doi.org/10.1128/JCM.39.5.2017-2021.2001EFSA 2013. Scientific opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. EFSA Journal, 11(4), 3138. https://doi.org/10.2903/j.efsa.2013.3138

10. Elsayed, M.S.A.E., Awad, A., Trabees, R., Marzouk, A., 2018. Virulence repertoire and antimicrobial resistance profile of shiga toxin-producing *E.coli* isolated from sheep and goat farms from Al-Buhayra Egypt. Pakistan Veterinary Journal 38(04):18–186. DOI:10.29261/pakvetj/2018.082

11. Enriquez-Gómez, E., Talavera-Rojas, M., Soriano-Vargas, E., Navarro-Ocaña, A., Vega-Sanchez, V., Aguilar-Montes de Oca, S., Acosta, D.J.P., 2017. Serotypes and antimicrobial resistance patterns in Shiga-toxin-producing *Escherichia coli* isolates from healthy lambs in México. Small Ruminant Research. 153, 41–47. http://doi: 10.1016/j.smallrums.2017.05.003.

12. Erol, I., Goncuoglu, M., Ayaz, N.D., Orm, F.S.B., 2016. Comparison of prevalence and genetic diversity of *Escherichia coli* o157:h7 in cattle and sheep. Journal of Microbiology, Biotechnology and Food Science. 6 (2) 808–812. https://doi: 10.15414/jmbfs.2016.6.2.808-812.

13. Eslava, C., Mateo, J., Cravioto, A., 1994. Cepas de *Escherichia coli* relacionadas con la diarrea. En: diagnóstico de laboratorio de infecciones gastrointestinales. Giono S, Escobar A, Valdespino JL. Secretaria de Salud. México: 251

14. Etcheverria, A.I, Padola, N.L, Sanz, M.E., Polifroni, R., Krüger, A., Passucci, J., Rodríguez, E.M., Taraborelli, A.L., Ballerio, M., Parma, A.E., 2010. Occurrence of Shiga toxin-producing *E. coli* (STEC) on carcasses and retail beef cuts in the marketing chain of beef in Argentina. Meat Science. 86; 418–421. https://doi.org/10.1016/j.meatsci.2010.05.027FAO, Food and Agriculture Organization. (2005) Code of hygienic practice for meat. CAC/RCP 58–200. Codex Alimentarius, FAO, Rome. http://www.fao.org/tempref/codex/Circular_Letters/CxCL2013/cl13_11e.pdf. Acceded 10 september 2018
21. Ghanбарpour, R., Kiani, M., 2013. Characterization of non-O157 shiga toxin-producing Escherichia coli isolates from healthy fat-tailed sheep in southeastern of Iran. Tropical Animal Health and Production. 45(2):641-8. https://doi:10.1007/s11250-012-0271-5.

22. Gunzburg, S.T., Tornieporth, N.G., Riley, L.W., 1995. Identification of Enteropathogenic Escherichia coli by PCR-Based Detection of the Bundle-Forming Pilus Gene. Journal of Clinical and Microbiology. 33(5): 1375–1377. https://doi.org/10.1128/JCM.33.5.1375-1377.1995

23. Halet, T., Sansonetti, P., Schad, P., Austin, S., Formal, S.B., 1983. Characterization of virulence plasmids and plasmid-associated outer membrane proteins in Shigella exneri, Shigella sonnei and Escherichia coli. Infection and Immunity. 40(1): 340–350. doi:10.1128/IAI.40.1.340-350.1983

24. Jajarmi, M., Ghanbarpour, R., Shari, R.H., Golchin. M., 2015. Distribution Pattern of EcoR Phylogenetic Groups Among Shiga Toxin-Producing and Enteropathogenic Escherichia coli Isolated from Healthy Goats. International Journal of Enteric Pathogens. 3(3): e27971. https://doi:10.17795/ijep27971.

25. Kagambéga, A., Martikainen, O., Siitonen, A., Traore, A.S., Barro, N., Haukka, K., 2012. Prevalence of diarrheagenic Escherichia coli virulence genes in the feces of slaughtered cattle, chickens, and pigs in Burkina Faso. Microbiology Open. 1(3): 276–284. https://doi:10.1002/mbo3.30.

26. Karmali, M.A., Mascarenhas, M., Shen, S., Ziebell, K, Johnson, S., Reid-Smith, R., Isaac-Renton, J., Clark, C., Rahn, K., Kaper, J.B., 2013. Association of genomic O island 122 of Escherichia coli EDL933 with verocy o toxin-producing Escherichia coli seropathotypes that are linked to epidemic and / or serious disease. Journal of Clinical Microbiology. 41: 4930-40. https://doi:10.1128/jcm.41.11.4930-4940.2003

27. Kerrn, M.B., Klemmensen, T., Frimodt-Møller, N., Espersen, F., 2002. Susceptibility of Danish Escherichia coli strains isolated from urinary tract infections and bacteremia, and distribution of sul genes conferring sulphonamide resistance. Journal of Antimicrobial Chemotherapy. 50;513–516. doi:10.1093/jac/dkf164.

28. Kumar A, Taneja N, Kumar Y, Sharma, M., 2012. Detection of Shiga toxin variants among Shiga toxin–forming Escherichia coli isolates from animal stool, meat and human stool samples in India. Journal of Applied Microbiology. 113, 1208—1216. https://doi:10.1111/j.1365-2672.2012.05415.x

29. Kumar, A., Taneja, N., Sharma, M., 2014. An epidemiological and environmental study of Shiga toxin-producing Escherichia coli in India. Foodborne Pathogens and Disease. 11(6):439–46. https://doi:10.1089/fpd.2013.1613.

30. Lee., M.S., Tesh, V.L., 2019. Review Roles of Shiga Toxins in Immunopathology. Toxins. 11(4), 212; https://doi.org/10.3390/toxins11040212.

31. Li, Y., Cao, B., Liu, B., Liu, D., Gao, Q., Peng, X., Wu, J., Bastin, D.A., Feng, L., Wang, L., 2009. Molecular detection of all 34 distinct-antigen forms of Shigella. Journal of Medical Microbiology. 58(Pt 1):69–81. doi:10.1099/jmm.0.000794-0. PMID: 19074655.

32. Maluta, R.P., Fairbrother, J.M., Stella, A.E., Rigobelo, E.C., Martinez, R.F., de Ávila, A., 2014. Potentially pathogenic Escherichia coli in healthy, pasture-raised sheep on farms and at the abattoir in Brazil. Veterinary Microbiology. 169; 89–95. http://dx.doi.org/10.1016/j.vetmic.2013.12.013.

33. Martínez-Vázquez, A.V., Rivera-Sánchez, G., Lira-Méndez, K., Reyes-López, M.Á, Bocanegra-García, V., 2018. Prevalence, antimicrobial resistance and virulence genes of Escherichia coli isolated from retail meat in Tamaulipas, Mexico. Journal of Global Antimicrobial Resistance. 14:266–272. doi: 10.1016/j.jgar.2018.02.016. Epub 2018 Mar 6. PMID: 29501529.

34. Martí, S., Fernández-Cuenca, F., Pascual, Á, Ribera, A., Rodríguez-Baño, J., Bou, G, Cisneros, J.M., Pachón, J., Martínez-Martínez, L., Vila, J., 2006. Prevalencia de los genes tetA y tetB como mecanismo de resistencia a tetraciclina y minociclina en aislamientos clínicos de Acinetobacterbaumannii. Enfermedades Infecciosas y Microbiología Clínica. 24(2):77–80. DOI: 10.1157/13085012

35. Medina, A., Horcajo, P., Jurado, S., De La Fuente, R., Ruiz-Santa-Quiteria, J.A., Domínguez-Bernal, Orden, J., 2011. Phenotypic and genotypic characterization of antimicrobial resistance in enterohemorrhagic Escherichia coli and atypical enteropathogenic E. coli strains from ruminants. Journal of Veterinary Diagnostic Investigation. 23(1):91–5. https://doi:10.1177/104063871102300114
36. Monaghan, Á., Byrne, B., Fanning, S., Sweeney, T., McDowell, D., Bolton, D.J., 2011. Serotypes and virulence profiles of non-O157 Shiga toxin-producing *Escherichia coli* isolates from bovine farms. Applied Environmental Microbiology 77, 8662–8668. https://doi:10.1128/AEM.06190-11.

37. Mora, A., Herrera, A., López, C., Dahb, G., Mamani, R., Pita, J.M., Alonso, M.P., Llovo, J., Benárdez, M.I., Blanco, J.E., Blanco, M., Blanco, J., 2011. Characteristics of the Shiga-toxin-producing enteroaggregative *Escherichia coli* O104:H4 German outbreak strain and of STEC strains isolated in Spain. International microbiology. 14:121–141. https://doi:10.2436/20.1501.01.142

38. Nataro, J.P, Kaper, J.B., 1998. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews. 11, 142–201. DOI: 10.1128/CMR.11.1.142

39. Njoroge, S., Muigai, A.W.T., Njiruh, P.N, Kariuki, S., 2013. Molecular Characterization and antimicrobial resistance patterns of *Escherichia coli* isolates from goats slaughtered in parts of Kenya. East African Medical Journal. 90(3):72–83. https://www.ajol.info/index.php/eamj/article/viewFile/103233/93447. Official Journal of the European Community L165/48. laying down rules for the regular checks on the general hygiene carried out by the operators in establishments according to Directive 64/433/EEC on health conditions for the production and marketing of fresh meat and Directive 71/118/EEC on health problems affecting the production and placing on the market of fresh poultry meat. European Directive 2001/471/EC.https://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:165:0048:0053:EN:PDF. Accessed 11 october 2017

40. Orskov, F., Orskov, I., 1984. Serotyping of *Escherichia coli*. In: T. Bergan, editor. Methods in Microbiology. Academic Press Ltd, London. p 43–112.

41. Paton, A.W., Srimanote, P., Woodrow, M.C., Paton, J.C., 2001. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli* strains that are virulent for humans. Infection and Immunity. 69(11):6999–7009. DOI: 10.1128/IAI.69.11.6999-7009.2001

42. Penders, J., Stobberingh, E.E., Savelkoul, P.H., Wolffs, P., 2013. The human microbiome as a reservoir of antimicrobial resistance. Frontiers in Microbiology. 4:87. https://doi:10.3389/fmicb.2013.00087.

43. Prager, R., Frut, A., Busch, U., Tietze, E., 2011. Comparative analysis of virulence genes, genetic diversity, and phylogeny of Shiga toxin 2g and heat-stable enterotoxin STLa encoding *Escherichia coli* isolates from humans, animals, and environmental sources. International Journal of Medical Microbiology. 301(3):181 – 91. https://doi:10.1016/j.ijmm.2010.06.003

44. Ramos, S., Silva, N., Canic, M., Capelo-Martinez, J., Brito, F., Igrejas, G., Poeta, P., 2012. High prevalence of antimicrobial-resistant *Escherichia coli* from animals at slaughter: a food safety risk. Journal of Science of Food Agriculture. 93(3):517 – 26. doi: 10.1002/jsfa.5814. Epub 2012 Jul 27. PMID: 22836880.

45. Reyes-Rodríguez, N.E., Soriano-Vargas, E., Barba-León, J., Navarro, A., Talavera-Rojas, M., Sanso, A.M., Bustamante, A.V., 2015. Genetic characterization of *Escherichia coli* 15 isolated from cattle carcasses and feces in Mexico State. Journal of Food Protection. 78, E796-16 801. https://doi:10.4315/0362-028X.JFP-14-425.

46. Rigobelo, E.C., Takahashi, L.S., Nicodemo, D., de Ávila, F.A, Maluta, R.P., dos Santos Ruiz, U., Stella, A.E., 2008. Virulence of *Escherichia coli* strains isolated from ovine carcasses. Revista Acadêmica Ciências Agrárias e Ambientais. 6, 475–482. http://dx.doi.org/10.7213/cienciaanimal.v6i4.11618
50. Scheutz, F., Teel, L.D., Beutin, L., Pierard, D., Buvens, G., Karch, H., Mellmann, A., Caprioli, A., Tozzoli, R., Morabito, S., Stockbne, N.A., Melton-Celsa, A.R., Sanchez, M., Persson, S., O’Brien, A.D., 2012 Multicenter evaluation of a sequence-based protocol for subtyping shiga toxins and standardizing stx nomenclature. Journal of Clinical Microbiology. 50(9):2951-63. https://doi: 10.1128/JCM.00860-12.

51. Sheutz, F., Stockbne, N.A., 2015. Bergey's Manual of Systematics of Archaea and Bacteria, Online © 2015 Bergey's Manual Trust. Published by John Wiley & Sons, Inc., in association with Bergey's Manual Trust. https://doi: 10.1002/9781118960608.gbm01147

52. Sheutz, F., Strockbine, N.A., 2015. Bergey's Manual of Systematics of Archaea and Bacteria, Online © 2015 Bergey's Manual Trust. Published by John Wiley & Sons, Inc., in association with Bergey's Manual Trust. https://doi: 10.1002/9781118960608.gbm01147

53. Sjöling, A., Wiklund, G., Savarino, S.J., Cohen, D.I., Svennerholm, A.M., 2007. Comparative analyses of phenotypic and genotypic methods for detection of entero-toxigenic Escherichia coli toxins and colonization factors. Journal of Clinical Microbiology. 45:3295–301. https://doi: 10.1128/JCM.00471-07

54. Tasara, T., Bielaszewska, M., Nitzsche, S., Karch, H., Zweifel, C., Stephan, R., 2008. (Short communication) Activatable Shiga toxin 2d (Stx2d) in STEC strains. Isolated from cattle and sheep at slaughter. Veterinary Microbiology. 131(1–2):199–204. doi: 10.1016/j.vetmic.2008.03.001

55. Torres, A.G., 2017. MINIREVIEW Escherichia coli diseases in Latin America. ‘OneHealth’ multidisciplinary approach. Pathogens and Disease.75, Issue 2, March 2017, ftx012, https://doi.org/10.1093/femsdp/ftx012

56. Trabulsi, L. R., Keller, R., Tardelli, Gomes, T.A., 2002. Typical and atypical enteropathogenic Escherichia coli. Emerging infectious diseases. 8(5), 508–513. https://doi.org/10.3201/eid0805.010385

57. Troeger, C., Forouzanfar, M., Rao, P.C., Khalil, I., Brown, A., Reiner, R.C. Jr., et al 2017. Estimates of global, regional, and national morbidity, mortality, and etiologies of diarrheal diseases: a systematic analysis for the global burden of disease study 2015. The Lancet Infectious Disease. 17(9):909–948. doi: 10.1016/S1473-3099(17)30276-1

58. Um, M.M., Brugère, H., Kérioût, dan M., Oswald, E., Bibbal, D., 2018. Antimicrobial Resistance Profiles of Enterohemorrhagic and Enteropathogenic Escherichia coli of Serotypes O157:H7, O26:H11, O103:H2, O111:H8, O145:H28 Compared to Escherichia coli Isolated from the Same Adult Cattle. Microbial Drug resistance. 24(6):852–859. doi: 10.1089/mdr.2017.0106.

59. Urdahl, AM., Beutin, L., Skjerve, E., Zimmermann, S., Wasteson, Y., 2003. Animal host associated differences in Shiga toxin-producing Escherichia coli isolated from sheep and cattle on the same farm. Journal of Applied Microbiology. 95(1):92–101. doi: 10.1046/j.1365-2672.2003.01964.x USDA. 2015. United States Department of Agriculture Food Safety and Inspection Service MLG 5C.01 Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products and Carcass and Environmental Sponges. https://www.fsis.usda.gov/sites/default/files/media_file/2021-04/MLG-5C.01.pdf Accessed 7 May 2019 USDA-FSIS 2012. Risk profile for pathogenic non-O157 Shiga toxin-producing Escherichia coli (non-O157 STEC. https://www.fsis.usda.gov/shared/PDF/Non_O157_STEC_Risk_Profile_May2012.pdf. Accessed 6 august 2018.

60. Vettorato, M.P., De Castro, A.F.P., Cergole-Novella, M.C., Camargo, F.L.L., Irino, K., Guth, B.E.C., 2009. Shiga toxin-producing Escherichia coli and atypical enteropathogenic Escherichia coli strains isolated from healthy sheep of different populations in Sao Paulo, Brazil. Letters in Applied Microbiology. 49(1):53 − 9. doi: 10.1111/j.1472-765X.2009.02617. x.

61. Waine, W. Daniel., 1991. Bioestadística. Base para el análisis de las ciencias de la salud 4° Ed. Editorial Limusa S.A de C.V.

62. Wang, L., Wakushima, M., Aota, T., Yoshida., Y., Kita, T., Maehara, T., Ogasawara, J., Choi, C., Kamata, Y., Hara-Kudo, Y., Nishikawa, Y., 2013. Specific Properties of Enteropathogenic Escherichia coli Isolates from Diarrheal Patients and Comparison to Strains from Foods and Fecal Specimens from Cattle, Swine, and Healthy Carriers in Osaka City, Japan. Applied Environmental Microbiology. 79(4):1232-40. https://doi: 10.1128/AEM.03380-12

63. Wani, S.A., Bhat, M.A., Samanta, I., Nishikawa, Y., Buchh, A.S., 2003. Isolation and characterization of Shiga toxin-producing Escherichia coli (STEC) and enteropathogenic Escherichia coli (EPEC) from calves and lambs with diarrhea in...
Tables

Table 1 Primers used in the identification of virulence factors.
| Gene or probe | Description of target | Oligonucleotide sequence (5’ – 3’) | PCR Product (pb) | Reference |
|---------------|------------------------|------------------------------------|------------------|-----------|
| vtx1          | Verocytotoxintype 1    | GTACGGGGATGCAGATAAATCGC<br>AGCAGTCATTACATAAAGACGYCCACT | 209              | Scheutz et al., 2012 |
|               |                        |                                    |                  |           |
| vtx2          | Verocytotoxintype 2    | GGCACGTCTGCTGAAACTGCTCCTGT<br>ATTAAACTGCACTTCAGCAAATCC<br>CGCTGTCTGAGGCATCTCCGCT<br>TAATTTACCTGGGCAAGCC | 627              |           |
|               |                        |                                    |                  |           |
| eae           | Intimin                | TCAATGCAGTTCCGTTATCAGTT<br>GTAAGTCCGTTACCCCAACCTG | 482              | Kong et al., 2002 |
|               |                        |                                    |                  |           |
| Bfp           | bundle-forming pilus   | AATGGTGCTTGGCGCTTGCTGC<br>GGCCTTTTATCCAACTTGTA | 300              | Gunzburg et al., 1995 |
|               |                        |                                    |                  |           |
| LT            | Heat-labile toxins     | ACGCGGTCTACTATCTTC<br>TGGTCTCAGATTGATGTC | 273              | Sjöling et al., 2007 |
|               |                        |                                    |                  |           |
| STp           | Heat-stable toxins     | TCTTTCCCCCTTTTTTCTAGC<br>ACAGGCAGGATTACAAACAG | 166              |           |
|               |                        |                                    |                  |           |
| ipaH          | Invasion plasmid antigen | TGGAAAAACTCAGTGCTCT<br>CCAGTCCGTAATTCCTCTTCT | 423              | Li et al., 2009 |
|               |                        |                                    |                  |           |
| aggR          | Transcriptional activator of AAFs | CTAATTGTACAATCGAGTGA<br>ATGAAGTAAATTCTTAAT | 308              | Czeczulin et al., 1999 |
|               |                        |                                    |                  |           |
| chuA          | Outer membrane hemin receptor ChuA | ATGGTACCGGACGAACCAAC<br>TGCCGCCAGTACCAAGACA | 288              | Clermont et al., 2013 |
|               |                        |                                    |                  |           |
| yjaA          | Uncharacterized protein YjaA | CAAAAGTGAAGTGTCAAGAG<br>AATGCATTCCCTACACCTTG | 211              |           |
|               |                        |                                    |                  |           |
| TspE4.C2      | putative gene for a lipase | CACTATTCGTAAGGTCTAGCC<br>AGTTATCGGTGGGTCG | 152              |           |
|               |                        |                                    |                  |           |
| arpA          | Ankyrin repeat protein A | AACGCTATTCCAGCTTG<br>TCTCCCCCATACCTACGCTA | 400              |           |

Table 2 Primers used in the identification of variants of shiga toxin.
| Gene or probe | Oligonucleotide sequence (5’ – 3’) | PCR Product (pb) | Reference |
|---------------|----------------------------------|-----------------|-----------|
| vtx1a         |                                  |                 |           |
| vtx1a-F1      | CTTTCCAGGTACAACAGCGGTT           |                 |           |
| vtx1a-R2      | GGAAGCTCATCAGATGCCATTCTGG        | 478             | Scheutz et al., 2012 |
| vtx1c         |                                  |                 |           |
| vtx1c-F1      | CTTTCCCTGTTACAACCTCGGTT         |                 |           |
| vtx1c-R1      | CAAGGTGTGTACGAAATCCCCCTCTGA     | 252             |           |
| vtx1d         |                                  |                 |           |
| vtx1d-F1      | CAGTTAATGCAGTGCTAAGGAGTTTACC    |                 |           |
| vtx1d-R1      | CTCTTCTCTGTTCTAACCCATGATA       | 203             |           |
| vtx2a         |                                  |                 |           |
| vtx2a-F2      | GCGATACTGRGBACTGTGACC           |                 |           |
| vtx2a-R3      | CCGKCAACCTTCACTGTAATGTG         | 349             |           |
| vtx2a-R2      | GGCCACCTCAGTGTAATGTG            |                 |           |
| vtx2b         |                                  |                 |           |
| vtx2b-F1      | AAATAGAAGAGAATTTGTAGCGGC        |                 |           |
| vtx2b-R1      | CAGCAAACTCTGAACCTGACG           | 347             |           |
| vtx2c         |                                  |                 |           |
| vtx2c-F1      | GAAAGTCACAGTTTTATATACACGGGTA    |                 |           |
| vtx2c-R2      | CCGGCCAGYTTTACTGTAATGTA         | 251             |           |
| vtx2d         |                                  |                 |           |
| vtx2d-F1      | AAARTCACAGTCTTTTATACACGGGTT     |                 |           |
| vtx2d-R1      | TTYCCGGCCACTTTTACTGTA           | 179             |           |
| vtx2d-R2      | GCCTGATGACACAGGTACTGGAC         |                 |           |
| vtx2e         |                                  |                 |           |
| vtx2e-F1      | CGGAGTATCGGGGAGAGGC            |                 |           |
| vtx2e-R2      | CTCTCAGACACCTTCACAGTTAAAGT      | 179             |           |
| vtx2f         |                                  |                 |           |
| vtx2f-F1      | TGGGCTCATCTGTTCTGTTG            |                 |           |
| vtx2f-R1      | TAATGGCCGCTGCTTCC              | 411             |           |
| vtx2g         |                                  |                 |           |
| vtx2g-F1      | CACCGGTTATTTATTTTCTGTGGATATC    |                 |           |
| vtx2g-R1      | GATGGGAATTTCAGAATAACCGCT        | 573             |           |
Table 3 Primers used in the identification of resistance genes

| Gene or probe | Oligonucleotide sequence (5’ – 3’) | PCR Product (pb) | Reference          |
|---------------|-----------------------------------|------------------|--------------------|
| sul1          |                                   | 433              | Kern *et al.* (2002) |
| sul1 F        | CGG CGT GGG CTA CCT GAA CG        |                  |                    |
| sul1 R        | GCC GAT CGC GTG AAG TTC CG        |                  |                    |
| sul2          |                                   | 293              |                    |
| sul2 F        | GCG CTC AAG GCA GAT GGC ATT       |                  |                    |
| sul2 R        | GCG TTT GAT ACC GGC ACC CGT       |                  |                    |
| tetA          |                                   | 950              | Marti *et al.* (2006) |
| tet A F       | GTA ATT CTG AGC ACT GTC GC         |                  |                    |
| tet A R       | CTG CCT GGA CAACAT TGC TT         |                  |                    |
| tet B         |                                   | 650              |                    |
| tet B F       | GTT AGG GGC AAG TTT TG            |                  |                    |
| tet B R       | GTA ATG GGC CAA TAC CAC CG        |                  |                    |
| BlaTEM        |                                   | 800              | Dallenne *et al.* (2010) |
| MultiTSO-T BlaTEM F | CAT TTC CGT GTC GCC CTT ATT C  |                  |                    |
| MultiTSO-T BlaTEM R | CGT TCA TCC ATA GTT GCC TGA C  |                  |                    |

Table 4 DEC* pathotypes isolated from slaughtered sheep in México.

| Pathotype | No. isolates (%) |
|-----------|------------------|
| w/o VF†   | 41 (46%)         |
| STEC      | 43 (47.7%)       |
| EPEC      | 3 (3.3%)         |
| ETEC      | 2 (2.2%)         |
| EIEC      | 1 (1.1%)         |
| TOTAL     | 90               |

*DEC, diarrheagenic *E. coli*; †w/o VF, without virulence factor.

Table 5 Serotypes found in this work and reported as DEC* in Mexico.
| Serotype     | Pathotype | No.of isolates |
|--------------|-----------|---------------|
| O6: NM‡      | STEC      | 1             |
| O8: NM       | STEC      | 1             |
| O76:H19      | STEC      | 5             |
| O91:H10      | STEC      | 2             |
| O146.H21     | STEC      | 3             |
| O104:H2      | STEC      | 2             |

*DEC, diarrheagenic E. coli

‡NM, not mobile

Table 6 Association between serotype, phylogenetic group and virulence genes of DEC* isolated from sheep slaughtered in an abattoir.

*DEC, diarrheagenic E. coli

‡NM, not mobile

†Phylogenetic group

Table 7 Antimicrobial resistance profile of DEC* isolated from sheep slaughtered in an abattoir.

*DEC, diarrheagenic E. coli

‡NM, not mobile

NIT: nitrofurantoin; AMP: ampicillin; TET: tetracycline, SXT: trimethoprim-sulfamethoxazole
| Isolate identification | Serotype | Source | Virulence factor | PG† |
|------------------------|----------|--------|------------------|-----|
|                        |          |        | stx₁  stx₂  eae  stp  ipah |     |
| E44                    | O53:H51  | rectal swab | 1c     |     | A   |
| Z15                    | 重整::H10 | rectal swab | 1c     |     | B1  |
| C28B                   | 重整::H14 | rectal swab | 1c     |     | B1  |
| D3                     | 重整::H34 | carcass | 1c     |     | B1  |
| Z2                     | 重整::H34 | rectal swab | 1c     |     | B1  |
| Z29                    | 重整::H16 | rectal swab | 1c     |     | B1  |
| V25                    | O100:H21 | rectal swab | 1c     |     | B1  |
| V22                    | O100:H28 | rectal swab | 1c     | 2g  | B1  |
| D28                    | O104:H2  | carcass | 1c     |     | B1  |
| D15                    | O105 AB: H16 | carcass | +    |     | B1  |
| D15B                   | O120: H16  | carcass | +    |     | B1  |
| V13                    | O146: H10  | rectal swab | 1c     |     | B1  |
| C23                    | O146: H21  | rectal swab | 1c     | 2b-2g | B1  |
| Z3                     | O146: H21  | rectal swab | 1c     |     | B1  |
| Z19                    | O146: H21  | rectal swab | 1c     |     | B1  |
| B7                     | O146: H8   | rectal swab | 1a-1c | 2g  | B1  |
| B13C                   | O150: NM   | carcass | 1c     |     | B1  |
| Z5                     | O174: H16  | rectal swab | 1c     |     | B1  |
| Z25                    | O174: H16  | rectal swab | 1a-1c |     | B1  |
| V15                    | O176: NM   | rectal swab | 1c     |     | B1  |
| C24                    | O185: NM   | rectal swab | +     |     | B1  |
| D53                    | O28 AC: H21  | carcass | +    |     | B1  |
| E3                     | O28 AC: H21  | rectal swab | +    |     | B1  |
| E15B                   | O32: H27   | rectal swab | 1c     |     | B1  |
| E20                    | O32: H7    | rectal swab | 1c     |     | B1  |
| E30                    | O32: H7    | rectal swab | 1c     |     | B1  |
| D26                    | O34: H14   | carcass | 1c     |     | B1  |
| Z9                     | O34:0145: H45  | rectal swab | 1c     |     | B1  |
| Z16                    | O37: H10   | rectal swab | 1a-1c |     | B1  |
| B1                     | O6: H16    | rectal swab | 1c     |     | B1  |
| Z20                    | O70: H10   | rectal swab | 1a-1c |     | B1  |
| Z17                    | O76: H19   | rectal swab | 1a-1c |     | B1  |
| Z21                    | O76: H19   | rectal swab | 1c     |     | B1  |
| Sample No | Serotype | Sample Type   | Id | Location |
|-----------|----------|---------------|----|----------|
| Z26       | O76:H19  | rectal swab   | 1c | B1       |
| Z32       | O76:H19  | rectal swab   | 1a-1c | B1 |
| Z34       | O76:H19  | rectal swab   | 1a-1c 2b-2g | B1 |
| E16       | O8:NM    | rectal swab   | 1c | B1       |
| E18       | O8:H2    | rectal swab   | 1c-1d | B1 |
| C28       | O84:H14  | rectal swab   | 1c | B1       |
| C27       | O91:H10  | rectal swab   | 1c | B1       |
| Z12       | O91:H28  | rectal swab   | 1a-1c | B1 |
| Z13       | O91:H42  | rectal swab   | 1a-1c | B1 |
| Z14       | O91:H47  | rectal swab   | 1a-1c | B1 |
| Z18       | O91:H47  | rectal swab   | 1c | B1       |
| C3        | O96:H20  | carcass       | 1c | B1       |
| V5        | O176:H21 | rectal swab   | 1c 2g | B1 |
| B3        | O176:NM  | rectal swab   | 1c | B2       |
| B15       | O6:NM    | rectal swab   | 1c | C        |
| V11       | O153:NM  | rectal swab   | 1c 2g | F |


| Isolate identification | Serotype   | Source    | STEC | EPEC | ETEC | EIEC | Antimicrobial resistance profile | Resistance gene |
|------------------------|------------|-----------|------|------|------|------|----------------------------------|-----------------|
| Z3                     | O146:H21   | rectal swab | +    |      |      |      | NIT, AMP, TET, SXT               | +               |
| V15                    | O176:NM    | rectal swab | +    |      |      |      | NIT, AMP, TET, SXT               |                |
| C28                    | O84:H14    | rectal swab | +    |      |      |      | NIT, AMP, TET, SXT               |                |
| V22                    | O100:H28   | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| B7                     | O146:H8    | rectal swab | +    |      |      |      | NIT, AMP, TET                   | +               |
| V11                    | O153:NM    | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| D26                    | O34:H14    | carcass    | +    |      |      |      | NIT, AMP, TET                   | +               |
| E44                    | O53:H51    | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| Z26                    | O76:H19    | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| E16                    | O8:NM      | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| V25                    | O100:H21   | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| D28                    | O104:H2    | carcass    | +    |      |      |      | NIT, AMP, TET                   | +               |
| C23                    | O146:H21   | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| Z5                     | O174:H16   | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| Z25                    | O174:H16   | rectal swab | +    |      |      |      | NIT, AMP, TET                   | +               |
| B3                     | O176:NM    | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| C24                    | O185:NM    | rectal swab | +    |      |      |      | NIT, AMP, TET                   | +               |
| E15B                   | O32:H27    | rectal swab | +    |      |      |      | NIT, AMP, TET                   | +               |
| E20                    | O32:H7     | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| E30                    | O32:H7     | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| Z9                     | O34:O145:H45 | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| Z16                    | O37:H10    | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| B1                     | O6:H16     | rectal swab | +    |      |      |      | NIT, AMP, TET                   |                |
| Sample ID | Specificity | Type          | Result | Antibiotics |
|-----------|-------------|---------------|--------|-------------|
| B15       | O6:NM       | rectal swab   | +      | NIT, AMP    |
| Z17       | O76:H19     | rectal swab   | +      | NIT, AMP    |
| Z32       | O76:H19     | rectal swab   | +      | NIT, AMP    |
| Z34       | O76:H19     | rectal swab   | +      | NIT, AMP    |
| E18       | O8:H2       | rectal swab   | +      | NIT, AMP    |
| C27       | O91:H10     | rectal swab   | +      | NIT, AMP    |
| Z12       | O91:H28     | rectal swab   | +      | NIT, AMP    |
| Z13       | O91:H42     | rectal swab   | +      | NIT, AMP    |
| Z14       | O91:H47     | rectal swab   | +      | NIT, AMP    |
| Z18       | O91:H47     | rectal swab   | +      | NIT, AMP    |
| C3        | O96:H20     | carcass       | +      | NIT, AMP    |
| V13       | O146:H10    | rectal swab   | +      | NIT, TET    |
| V5        | O176:H21    | rectal swab   | +      | NIT, TET    |
| C28B      |             | rectal swab   | +      | NIT         |
| Z29       |             | rectal swab   | +      | NIT         |
| Z19       | O146:H21    | rectal swab   | +      | NIT         |
| B13C      | O150:NM     | carcass       | +      | NIT         |
| Z20       | O70:H10     | rectal swab   | +      | NIT         |
| Z21       | O76:H19     | rectal swab   | +      | NIT         |
| Z2        |             | rectal swab   | +      | NIT, AMP, TET, SXT |
| D3        |             | carcass       | +      | NIT, AMP, TET, SXT |
| Z15       |             | rectal swab   | +      | NIT, AMP    |
| D53       | O28 AC:H21  | carcass       | +      | NIT, AMP    |
| D15       | O105 AB:H16 | carcass       | +      | NIT, AMP, TET |
| D15B      | O120:H16    | carcass       | +      | NIT, AMP    |
|     |     |     |     |     |
|-----|-----|-----|-----|-----|
| E3  | O28 AC:H21 | rectal swab | + | NIT, AMP, TET |