VIRULENCE CHARACTERIZATION OF *ESCHERICHIA COLI* O157:H7 ISOLATED FROM FECAL SAMPLES AND BEEF CARCASSES IN MEXICO

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Abstract –*Escherichia coli* O157:H7 are one of the most important foodborne pathogens, posing a serious human health risk worldwide. Information regarding virulence factors of *E. coli* O157:H7 isolates obtained from Mexican food chain is needed in order to provide scientific data for meat industry and government. A total of 56 strains of *E. coli* O157:H7 were obtained from different locations along the animal production and processing chain at a vertically integrated feedlot and at a Federal Inspection type (TIF) certified slaughter plant in Mexico. Fifty two isolates (92.9%) carried stx1$^-$ *stx*^{2}, *eae*A$^+$ and *hly*A$^+$. These data showed that the majority of the *E. coli* O157:H7 strains from beef animals tested in this research, have the potential of causing serious human infections. This information will help to understand risk factors in the Mexican meat supply chain and allow the Mexican government and industry to develop mitigation strategies by improving their microbiological conditions at the processing plant.

Key Words – *stx*1, *stx*2, *eae*A and *hly*A , *E. coli* O157:H7

I. INTRODUCTION

Shiga toxin producing *Escherichia coli* O157:H7 is one of the most important foodborne pathogens, representing the major etiological agents of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), an infection that can become a life-threatening disease [1,2]. The morbidity and mortality associated with foodborne outbreaks linked to *E. coli* O157:H7 have highlighted the threat these organisms pose to public health. Human isolates of *E. coli* O157:H7 that have caused infection have been reported to carry a variety of virulence genes, including genes that code for Shiga toxin 1 (stx1), Shiga toxin 2 (stx2), intimin (eaeA) and enterohemolysin (hlyA) [3].

Cattle have been identified as a major reservoir of *Escherichia coli* O157:H7 [4]. However it has been suggested that STEC isolates from gastrointestinal tracts of domestic animals may have a low degree of virulence to humans, because they are less likely to produce virulence factors such as intimin and enterohemolysin [5]. The consumption of meat and meat products, of bovine origin, has been associated with a number of outbreaks due to *E. coli* O157:H7 [6,7,8].

Food safety is a big concern in international trade. The microbial contamination of food can result in serious human health threats as well as cause complications and disputes during international food trade.

Mexico has forged trade accords with about 50 other countries. The North American Free Trade Agreement (NAFTA) is a comprehensive trade liberalizing agreement among Canada, Mexico, and the U.S. The agreement facilitates cross-border investment, requires that sanitary and phytosanitary standards for trade be scientifically based, and expands cooperation regarding the environment and labor [9]. Research that provides data with regards to *E. coli* O157:H7 virulence profiles are currently not available in Mexico. This information is essential to understand risk factors in the food chain and to help the Mexican government and industry to take preventive measures, in order to improve microbiological standards and guidelines for the meat industry. Therefore, the objective of this research was to determine the virulence profiles of *E. coli* O157:H7 strains isolated from a Mexican cattle feedlot, holding pens, hides and carcasses.

II. MATERIALS AND METHODS

Sample Collection:

Fecal Samples: At the feedlot, fresh fecal pat samples were collected from animals that were shipped to the harvest facility. Approximately 40 g of sample were taken from each fresh fecal pat using plastic spoons and placed aseptically, into a labeled sterile plastic container. At the holding pen area, fecal samples were taken in the same manner as described above at the feedlot. Fecal grabs samples were collected after evisceration at the viscera room, the recto-colon portion of the intestines was cut and approximately 40 g of feces were taken and placed aseptically in a labeled sterile plastic container.

Hide and Carcass Samples: Hide samples were collected after animals were stunned and bleed. Carcasses were collected at each step of the slaughter process, after de-hiding, pre-evisceration, pre-cooler, just prior to entering the hot box, and after 24 h of dry chilling in the coolers.

Composite samples were taken for both hides and carcasses from inside round, hind shank and fore shank using Spongesicle® 3M hydrated with 10 mL of buffered peptone water (BPW), (Difco® Laboratories, Sparks, MD). Approximately a 1000 cm^2 area of the hide was swabbed in the perineal area, and *ca.* a 250 cm^2 area was swabbed from each of the fore shanks and hind shanks.

E. coli *O157:H7 Detection and Isolation:*

Immunomagnetic separation (IMS) and PCR (Bax® system, Dupont Qualicon, Wilmington, DE). techniques) were used for detection and isolation of *E. coli* O157:H7 from fecal, hides and carcass samples.

Fecal and Hide Samples: *ca*. 1 g of the fecal sample was diluted in 9 mL Gram-negative broth supplemented with novobiocin (20 mg/L) (Sigma®, St. Louis, MO); (mGNB) (10:1 dilution). After dilution, each tube was vortexed thoroughly and incubated for 6±1 h at 37°C. For hides, each sponge bag was homogenized for 30 s using a stomacher (Stomacher®400) then a 1 mL sample from each bag was aseptically transferred into 9 mL of mGNB. After dilution, each tube was vortexed thoroughly and incubated for 6±1 h at 37°C.

After the incubation, IMS was performed from both kind of samples with Dynabeads using the BeadRetriever™ instrument following the manufacturer's recommendations. *E. coli* O157 cells were subjected to IMS by mixing 1 mL of the culture above, with 20 µL of anti-O157 beads (Dynal, Lake Success, NY). Beads were washed three times in PBS-Tween 20, and 50 µl of the bead-bacteria mixture of *E. coli* O157 and the beads were spread onto CHROMagar O157 (BBL™) plates. Plates were incubated at 37°C overnight. From each CHROMagar O157 plate typical colonies (mauve color) were tested against O157 serogroup-specific antisera by a slide agglutination test (DrySpot, Oxoid®). Final confirmation of isolates was performed by PCR analysis for the O157:H7 serotype using the Dupont Bax® system (Dupont Qualicon, Wilmington, DE).

Carcass Samples: Bax® system (Dupont Qualicon, Wilmington, DE) was used to detect positive samples to *E. coli* O157:H7 on carcass samples. Each spongesicle bag was homogenized for 30 s using a stomacher (Stomacher®400), then 1 mL of sample from each bag was aseptically transferred into 9 mL of modified Tryptic Soy Broth (mTSB) (novobiocin 20 mg/Liter), (Sigma®, St. Louis, MO). Each tube was vortexed thoroughly and incubated for 14 h at 37°C. *E. coli* O157:H7 were detected using the AOAC approved BAX® (Polymerase Chain Reaction) detection unit (DuPont Qualicon, Wilmington, DE), according to the manufacturer's published procedures. The IMS was used to recover *E. coli* O157:H7 strains from carcass samples Bax® positive, as described above. Recovered isolates were tested against O157 serogroup-specific antisera by a slide agglutination test (DrySpot, Oxoid®). Final confirmation of isolates was performed by PCR analysis for the O157:H7 serotype using the Dupont Bax® system (Dupont Qualicon, Wilmington, DE).

A total of 56 strains of *E. coli* O157:H7 were obtained from different locations along the animal production and processing lines at a vertically integrated feedlot and at a TIF (*Tipo Inspección Federal*; *i.e.* Federal Inspected) certified slaughter plant in Mexico. Eleven strains were isolated from fecal samples obtained from beef steers (fecal grabs), five from fresh fecal pats collected at the feedlot, five from fresh fecal pats collected at holding pen area, 33 from hides (at killing floor) and two from carcasses at pre-evisceration.

A multiplex PCR was performed to detect *stx*1, *stx*2, *eae*A and *hly*A following previous published procedures [10] with some modifications. Primers sets are shown in Table 1. A 25 µL reaction mixture consisted of 3µL template DNA, hotStart DNA polymerase (1U/25 µL rxn), 0.2 mM of each dNTP and 3.0 mM MgCl2 at 1X final concentration (KapaBiosystems®). PCR was performed in a thermocycler (Eppendorf®) with 35 cycles: 30 sec of denaturation at 94°C; 30 sec of annealing at 58°C and 1 min elongation at 72°C. The QIAxel Biocalculator (Qiagen®) was used to analyze PCR products.

Table 1. PCR Primers

Primer Sequence (5'-3')	Amplicon size
*stx*1: FATAAATCGCCATTCGTTGACTAC *stx*1R: AGAACGCCCACTGAGATCATC	180bp
*stx*2F: GGCACTGTCTGAAACTGCTCC *stx*2R:TCGCCAGTTATCTGACATTCTG	255bp
eaeAF: GACCCGGCACAAGCATAAGC eaeAR: CCACCTGCAGCAACAAGAGG	384bp
*hly*AF: GCATCATCAAGCGTACGTTCC, *hly*AR: AATGAGCCAAGCTGGTTAAGCT.	534bp

Source: Paton and Paton, 1998.

III. RESULTS AND DISCUSSION

Fifty two (92.9%) of 56 isolates contained *stx*2, *eae*A and *hly*A genes. None contained *stx*1. Figure 1 shows a representative gel image for two reference *E. coli* O157:H7 controls strains and 6 samples.

Table 2 shows results of PCR analysis of *E. coli* O157:H7 isolates.

Table 2. *E. coli* O157:H7 virulence characterization

Origin	*stx*1	*stx*2	*eae*A	*hly*A
BS	-	72.7% (8/11)	72.7% (8/11)	72.7% (8/11)
FL	-	100% (5/5)	100% (5/5)	100% (5/5)
Hide	-	96.9 (32/33)	96.9 (32/33)	100% (33/33)
HP	-	100% (5/5)	100% (5/5)	100% (5/5)
PE	-	100% (2/2)	100% (2/2)	100% (2/2)

BS: Beef steers
FL: Feedlot
Holding Pen: HP
PE: Pre-evisceration

Seventy two percent of the isolates obtained from steers carried *stx*2, *eae*A and *hly*A. However, three strains (27.2%) were negative for all four genes. The *stx*1 gene was not found in any isolate. The majority of the strains (96.9%) from cattle hides carried *stx*2, *eae*A and *hly*A genes, with an exception of one strain (3%) that only carried the *hly*A gene. All isolates from feedlots, holding pen and carcasses at pre-evisceration were positive for *stx*2, *eae*A and *hly*A, but negative for stx1.

The STEC strains producing stx2 toxin can cause more severe disease than STEC strains producing stx1 or both stx1 and stx2 toxins. [11,12,13]. Our data showed that the majority (92.9%) of the *E. coli* O157:H7 strains contain virulence determinants associated with the disease in humans, which may be of clinical significance.

In Mexico there is no mandatory regulation regarding *E. coli* O157:H7, for whenever *E. coli* O157:H7 is present in beef to be commercialized in the domestic market.

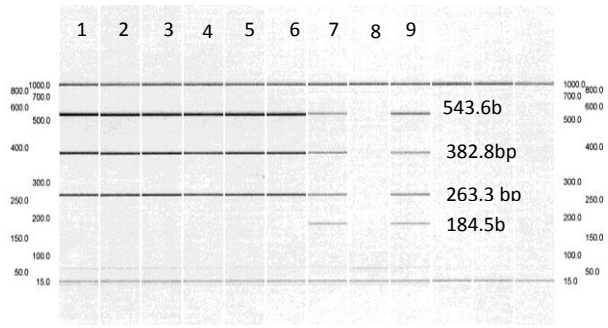

Figure 1. PCR products analyzed by QIAexcel BioCalculator
Lines 1 to 6: samples Steers (1), steers (2), feedlot (3), hide (4), hide (5), holding pen (6). Lines 7 and 8 positive controls (*E. coli* O157:H7 ATCC 43895), lane 9 negative control (*E. coli* ATCC 25922). The expected motilities for the various specific PCR products are also indicated.

The presence of *E. coli* O157:H7 in beef steers and carcasses could represent a health threat for the consumers if appropriate food safety hurdles and interventions are not implemented during the slaughter process.

Interestingly, there are no reports from Mexico about the human infection with *E. coli* O157:H7 despite the presence of *E. coli* O157:H7 in cattle. One possible reason may be due to the non-reporting of such infections by the hospitals. Furthermore lack of surveillance programs and effective regulations also contributes toward the absence of data. Therefore public health

surveillance of VTEC infections may play an important role in detecting human cases and furthermore for devising and implementing control measures. Additionally establish baselines surveillance for *E. coli* O157:H7 in feedlots and along the food chain across the country is also needed in order to establish a representative scientific data, therefore, more research in this area is needed.

IV. CONCLUSION

E. coli O157:H7 strains isolated from steers feces and hides, carcasses and their environment (feedlot and holding pens) were *stx*1$^-$, *stx*2$^+$, *eae*A$^+$ and *hly*A$^+$. This study demonstrated the potential of beef cattle and their environment in harboring *E. coli* O157:H7 with the potential to cause disease in humans.

This information is important for the industry and the government, and can be used to develop additional criteria and standards in the future (national regulations regarding *E. coli* O157:H7), to implement pre and post-harvest interventions, and to evaluate trends in bacteria prevalence.

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