DETECTION OF SHIGA TOXIN PRODUCING *Escherichia coli* BY PCR IN BEEF CATTLE AT SLAUGHTERING LEVEL IN ARGENTINA.

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Background

Shiga toxin-producing *Escherichia coli* (STEC) was recognized as a foodborne pathogen in 1982, when *E. coli* O157:H7 was identified as cause of hemorrhagic colitis in humans attributed to the under-cooked beef hamburgers (Riley et al., 1983). Several studies have pointed out that ruminants, particularly cattle, are reservoirs of STEC (Hancock et al., 1997). Cattle have been studied as source of outbreaks of human foodborne illness associated to the consumption of contaminated meat and milk and other foods contaminated by animal products or waste. STEC is present in the lower intestinal tract, in the rumen or on hides and fleece and it can be transferred to the carcass during slaughter. *E. coli* O157 is more commonly isolated from calves than from adult cattle. The relative high prevalence in young animals is consistent with the fact that calves shed the organism for a longer period of time than do adult cattle (Amstrong et al., 1996). Hancock et al. (1997) recovered 1.41% of *E. coli* O157 when analyzing fecal samples from heifers in 36 farms, while Wells et al. (1991) detected 28 different non-O157 serotypes of STEC from cattle, thirteen of them previously associated with human disease. There are several methods with different sensitivity for STEC detection and the isolation rate depends on the methodology applied. Recently, the polymerase chain reaction (PCR) method has gained acceptance for the rapid and specific detection of STEC. Prevalence studies of STEC on cattle from Argentine will help to assess the risk of foodborne disease and to propose control actions.

Objective

To evaluate different PCR protocols for isolation and characterization of STEC in feces of healthy steers at slaughtering level.

Material and Methods

Fecal sampling: Fecal samples from large intestine (cecum) were collected at the killing floor and put into sterile plastic bags. A total of 200 steers from main beef cattle producing areas of Argentina were sampled.

Laboratory methods: The PCR protocols applied are shown in scheme 1.Two gram of fecal sample were incubated in 10 ml of modified Tryptic Soy Broth (m-TSB) containing novobiocine at 37°C for 6 and 18 hours. Eighty from 200 samples were also simultaneously analyzed by direct culture (DC), on MacConkey agar (MAC) and MacConkey sorbitol with cefixime and tellurite (CT-SMAC). **Protocol I (PI):** PCR was performed on 200 µl of the enriched culture media at both incubation times. The remaining enriched culture media was stored at –70°C. Samples that were PCR+ were plated on MAC and CT-SMAC and ten colonies isolated from each culture media. **Protocol II (PII)**: PCR was performed on colony picked (mean of 20 colonies) from MAC and CT-SMAC. A total of 103 samples were analyzed by this protocol (80 samples of DC plus 23 enriched culture stored at –70°C). **Protocol III (PIII):** 64 samples were performed analyzing with PCR in confluent growth zone on MAC and CT-SMAC. Colonies (mean=20) were analyzed by the same technique when the PCR result was positive.

PCR assay: PCR for *stx*1 and *stx*2 genes was performed on enriched cultures, isolated colonies and confluent zone growth. PCR amplification was performed using primers to amplify 130-bp fragment of *stx*1 sub-unit B and 346-bp fragment of *stx*2 sub-unit A. Amplification products were analyzed in electrophoresis in agarose gels (2%) including positive and negative controls and weight molecular markers (Pollard et al., 1990).

Serotyping: The serotype was performed employing antiserum correspondent. This serotyping was completed by Dra. Beatriz Guth,UNIFESP, Sao Paulo, Brazil.

DNA hybridization assay: presence of genes for attaching and effacing (*eae* gene) and *stx*1 and *stx*2 was determined using probes derived from plasmids pCVD434, pJN37-19 and pNN110-18 respectively and probes were labeled with UTP-digoxigenin by random priming method.

Enterohemolisine (E-Hly) assay: haemolysis was studied in tryptose base agar media with 10 mM de Cl_2Ca and 5% of desfibrinated sheep blood (Schmidt, 1995). E-*hly* gene harboring was also established by PCR assay.

Antibiotics susceptibility: STEC strains were tested for antibiotic resistance by the Kirby Bauer method. Antibiotics tested were nadilixc acid, ampicillin, cephalotin cefixime, cefotaxime, cefoxitin, cefuroxime, colistin, chloramphenicol, streptomycin, gentamicin, norfloxacin, tetracycline, trimethroprim and sulfamethoxazole.

Cytotoxicity assay: was assayed on Vero cells using Stx1 and Stx2 specific monoclonal antibodies.

Results and Discussion

All STEC strains recovered considering the three protocols are showed in Table 1. From 200 enriched samples analyzed by PI, only one sample was PCR+ (0.5%). STEC O116:NM *eae-*/*stx*1/E-*hly*-, susceptible to all the antimicrobials assayed, was recovered from MAC plates after 6 h of enrichment in m-TSB.

When PCR was performed on isolated colonies (PII), 2 STEC strains (2/103, 1.94%) were detected from two different animals but belonging to the same herd. STEC O8:H19 *eae-*/*stx*2/E-*hly*+ was detected in colonies isolated both in MAC and CT-SMAC after 18 h incubation in m-TSB and showed resistance to ampicillin and cephalotin. On the other hand, STEC O26:H11 *eae*+/*stx*1/E-*hly*+ was isolated from CT-SMAC at 6 and 18 h enrichment incubation times.

Finally, analyzing confluent growth by PCR (PIII), 5 STEC strains (5/64, 7.8%) were detected. Three strains were STEC O8:H19 (*eae-*/*stx*2/E-*hly*+) and the others O2:H25(*eae-*/*stx*2/E-*hly*-) and O11:H14(*eae-*/*stx*2/E-*hly*+). These strains were susceptible to all of

the antibiotics assayed. The *stx* gene results were in agreement with the toxin-phenotyping results obtained by the Vero cells citotoxicity assay. The pheno-genotypic characteristics from all STEC isolated are showed in Table 2.
PCR performed on confluent growth zone applied in PIII recovered the major number of STEC in the current study. The PI showed a low recovery level might be due to the presence of fecal inhibitors in feces (Schultsz et al., 1994). Considering total STEC strains recovered 7/8 were detected on isolated colonies from enrichment culture media (-70°C). This result is consistent with Sanderson et al. (1995) that showed that all broth enrichment methods were superior to direct plating when they were combined with subsequent plating. There is a large number of serotypes of Shiga like-producing *E. coli* causing human disease, but *E. coli* O157H7 continues to be the dominant cause of hemorragic colitis and HUS (Zhao et al., 1995). However in this study it was no found this serotype. Prevalence on Argentine of STEC strains was 4% (8/200) under the conditions being assayed in the current study.

Conclusion PCR applied on confluent growth of selective media resulted in a good STEC recovery from bovine feces. PCR assay is a sensitive and rapid method for STEC detection in prevalence studies.

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TABLE 1. STEC SEROTYPES RECOVERED FROM BOVINE FECAL SAMPLES USING THREE DIFFERENT PROTOCOLS.

SEROTYPE	CULTURE MEDIA	INCUBATION TIME	PROCEDURE
O8:H19	MAC CT-SMAC	18	II
O26:H11	CT-SMAC	6-18	II
O116:NM	MAC	6	I
O2:H25	CT-SMAC	18	III
O8:H19	CT-SMAC	18	III
O11:H14	MAC CT-SMAC	18	III
O8:H19	MAC	DC	III
O8:H19	MAC	18	III

TABLE 2. PHENO-GENOTYPIC CHARACTERISTICS OF STEC STRAINS RECOVERED FROM CATTLE.

SEROTYPE	GENOTYPE				FENOTYPE		
	eae probe	*stx* probe	*stx* PCR	*hly* PcR	Stx Vero	ATB	Hly
O8:H19	-	2	2	+	+	CTN AMP	+
O26:H11	+	1	1	+	+	S	+
O116:NM	-	1	1	-	+	S	-
O2:H25	-	2	2	-	+	S	-
O8:H19	-	2	2	+	+	S	+
O11:H14	-	2	2	+	+	S	+
O8:H19	-	2	2	+	+	S	+
O8:H19	-	2	2	+	+	S	+