

The paradigm of ground meat as source of STEC in high risk area of HUS from Argentina

Abstract

Objectives: The aim of this work is to evaluate ground meat as infection source of Shiga toxin-producing *Escherichia coli* (STEC) to population of Tierra del Fuego, Argentina.

Background: Argentina is the country with the highest rate of hemolytic uremic syndrome (HUS). The main agent of HUS is STEC, which produce a food borne disease at a low infective dose (<100 CFU/g). Cattle is a natural reservoir of STEC, and ground meat is a high-risk food since surface contamination is distributed throughout the meat. Tierra del Fuego (TDF) is part of the patagonian region of Argentina, which presents the highest rate of hemolytic uremic syndrome (HUS).

Methods: In our study, all of the butcher shops in the island were sampled. Ninety-three samples of ground meat were taken from butcher shops enabled in three TDF communities. Samples were processed in accordance of the algorithm for STEC O157 and non-O157 STEC.

Results: A total of 2.15% of the samples of ground meat from the retail outlets of TDF were suspected of O157: H7 non-STEC. STEC were absent in the sampling round.

Conclusion: According to no one cases of HUS from TDF was reported at the National Vigilance System during our sampling period, we could not discard ground meat as the main source of STEC infection which cause HUS. Ground meat as infection source of STEC could not be discarded in TDF according to no report of HUS case was reported at National Vigilance System during to our sampling dates. Meat contamination could have a timely presentation coinciding with the cases of HUS. Also, other sources of infection could be involved locally. Prevention is a fundamental tool to use with HUS, it is necessary to implement various measures, including good manufacturing and hygiene practices, consumption and use of safe water at industry and home steps.

Keywords: HUS, STEC, ground meat, Tierra del Fuego, infection source

Volume 7 Issue 5 - 2019

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Received: September 11, 2019 | **Published:** September 18, 2019

Abbreviations: TDF, tierra del fuego; HUS, hemolytic uremic syndrome; STEC, shiga toxin-producing *Escherichia coli*; FBD, foodborne disease; FSIS, food safety and inspection service; USDA, united states department of agriculture; GMP, good manufacturing practices

Introduction

Argentina is the country with the highest rate of hemolytic uremic syndrome (HUS) worldwide.¹ HUS represents the first cause of acute renal failure in pediatrics, determines the need for renal transplant in 20% of children and adolescents, and is the second cause of chronic renal failure in Argentina.² This disease mainly affects children under 5 years of age³ who ingest food or drinking water contaminated with Shiga toxin-producing *Escherichia coli* (STEC).⁴⁻⁶ Cattle are a natural reservoir of STEC O157 and STEC non-O157, and are asymptomatic carriers.⁷ Foods mostly associated with this foodborne disease (FBD) are ground beef, unwashed fruits and vegetables, milk products and unpasteurized derivatives.^{8,9} Ground meat represents a great risk since the surface contamination of STEC is distributed throughout the mass of the product.^{10,11} Contamination occurs at a low infective dose

(<100 CFU/g).^{12,13} Regarding the prevalence in Argentina, several serotypes have been found, STEC O157:H7 and a group of serotypes that do not belong to that serogroup, STEC non-O157.^{14,15} Cases distribution are not the same throughout the Argentinean territory,¹⁶ southern provinces, mainly Tierra del Fuego (TDF), have the highest incidence of cases.¹⁷ TDF has a sub polar oceanic climate and has cold temperatures throughout the year, with an average annual temperature of 5-7°C and low annual temperature variations, ranging from -0.3°C in July to 9.4°C in January. The main population of TDF province live in the big island, which has 3 well-differentiated cities, Tolhuin and Ushuaia cities, at the north and south of the Andes Mountains, and Rio Grande city at the north end of the island, with geographical features of patagonian plateau, wide plains and a local population less influenced by international tourism (Figure 1). The island of TDF has two slaughter houses, used in the slaughter of local bovine and sheep production. Meat imported from the continent only allows whole cuts vacuum packed, boneless, which are not used to grind. Exposure's degree of the population to STEC is particularly unknown in ground meat. The aim of this work is to evaluate ground meat contamination level with O157 and non-O157 STEC in butcher shops in TDF.



Figure 1 Geographical location of Tierra del Fuego in Argentina and southern cone.

Material and methods

All butcher shops enabled in the three towns of the island of TDF were geo referenced. A sample of 500grams of ground meat were bought from all the markets, during one week sampling in spring 2016. Samples were collected in sterile bags avoiding contamination among them, and were immediately frozen to send by air transport to the laboratory in cooled boxes. At the laboratory of Microbiology from Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, each sample was defrozen at 4°C overnight and two portions of each sample (65g) was stomached in 585 mL of different culture media for 2 min. Tryptic Soy Broth (TSB) (Oxoid) was used for screening for STEC non-O157 group and TSB with novobiocin plus casaminoacids (mTSB+n) for screening for STEC O157 sero group. The samples were pre-enriched at 37°C±0.5 for STEC non-O157 and 41.5°C±0.5 for STEC O157 respectively, for 18h. Cultures from TSB were streaked onto MacConkey agar (MAC). After pre-enriched mTSB+n culture, immuno absorption for O157 serogroup (Reveal) was performed according to the manufacturer's instructions. In O157 positive cultures, immunomagnetic separation (IMS) with paramagnetic beads (beads anti-*E. coli* O157, Neogen) was executed according to the manufacturer's instructions. After IMS, the beads were spread out on sorbitol MacConkey agar (Oxoid) supplemented with 0.05mg/L cefixime and 2.5mg/L of potassium tellurite (bioMérieux) (CT-SMAC). After incubation at 37°C for

24h, screening for *stx1*, *stx2*, and *rfbO157* genes was performed by PCR as previously described from the confluence zone of MAC and non-sorbitol fermenting colonies from CT-SMAC as templates for suspected non-O157 STEC and STEC O157 respectively¹⁰. *E. coli* strains ATCC 25922 (*stx1*-, *stx2*-, and *rfbO157*-), EDL 933 (O157:H7, *stx1*+, *stx2*+, and *rfbO157*+), and UNCPBA O91:H21 (*stx1*+, *stx2*+, and *rfbO157*-) were used as controls. From each positive plate, isolation of a representative strain was investigated in up to 50 CFU through pools with up to ten colonies. Isolated strains of each positive pool were retested by PCR. The confirmed strains were kept in TSB + glycerol (20%) at -196°C. Samples were processed in accordance of the algorithm for STEC O157 and non-O157 STEC in meat products.^{18,19}

Results

A total of 93 butcher shops were identified in the island. The distribution of butchers from each city in TDF was 21 in Ushuaia, 5 in Tolhuin and 49 in Río Grande city (Figures 2–4). Two O157:H7 serogroup out of 93 samples were detected by immunoabsorption. After IMS and subsequent PCR screening, the Shiga toxin genes were not detected. In both routes of the 93 samples analyzed, STEC O157 and STEC non-O157 were not detected. According to serogroup detection by immunoabsorption test, 2.15% of the ground meat samples from TDF were suspected of O157 non-STEC.

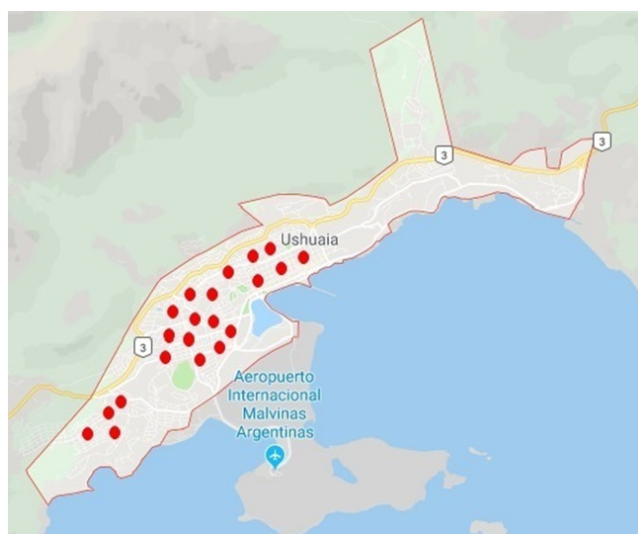


Figure 2 Butcher shops location from Ushuaia city.

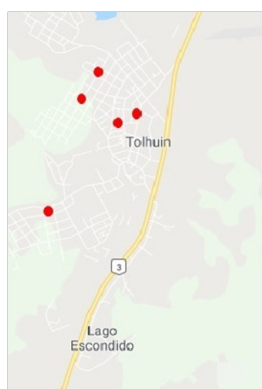


Figure 3 Butcher shops location from Tolhuin city.

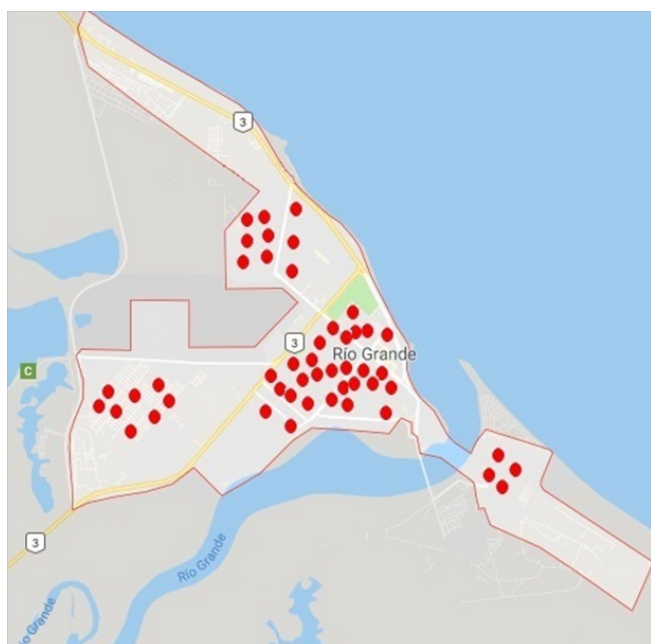


Figure 4 Butcher shops location from Rio Grande city.

Discussion and conclusion

In this work we hypothesized a causality between contamination level of ground meat in TDF and the high rate of HUS in the region, which also contributing to the major rate of HUS from patagonian area. During sampling period, no cases of HUS from TDF were reported to the National Health Surveillance System. According to the results over our round sampling, we could not accept nor reject the hypothesis to TDF. The chain of events that induce a disease state can be identified as effective causal complexes, and is known that in HUS the main microorganisms associated is STEC. Moreover, STEC O157 causes around 83% of HUS cases in Argentina.²⁰ Our work is the first record that serogroup O157 circulating in the meat chain of the region, but serotype could not be confirmed because isolated was not obtained and the presence of *stx* + strains was not observed in this sampling round. Our results allow us to consider that other sources of infection could be involved locally. On another hand, meat contamination could have a seasonal presentation coinciding with HUS. It is necessary to point out that food handler could be an asymptomatic carrier of STEC and performing bad practices it can spread the microorganism contaminating food. Along food chain (industrial, small merchant or household) food handlers plays a fundamental role in preparing food to be consumed.²¹ Bad manufacturing practices are due to various cultural factors and an inadequate perception risk.²² Food safety, good manufacturing practices (GMP) and training by the food handler-butcher becomes essential in preventing this and other FBD. Detecting and evaluating risk parameters becomes necessary to identify possible action points in order to reduce the contamination of sources of infection.

Funding details

None.

Acknowledgments

Méd. Vet. Juan Facundo Petrino, Méd. Vet. Nora Loekemeyer, Ing. Agr. Victor Canalis, Ushuaia, Tolhuin and Rio Grande bromatology departments.

Conflicts of interest

Authors declare that there is no conflict of interest.

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