Research Article

Quantitative risk assessment of haemolytic uremic syndrome associated with beef consumption in Argentina

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Abstract

We developed a quantitative microbiological risk assessment (QMRA) of haemolytic uremic syndrome (HUS) associated with Shiga toxin-producing *Escherichia coli* (STEC)-contaminated beef (intact beef cuts, ground beef and commercial hamburgers) in children under 15 years of age from Argentina. The QMRA was used to characterize STEC prevalence and concentration levels in each product through the Argentinean beef supply chain, including cattle primary production, cattle transport, processing and storage in the abattoir, retail and home preparation, and consumption. Median HUS probability from beef cut, ground beef and commercial hamburger consumption was $<10^{-15}$, $5.4 \times 10^{-8}$ and $3.5 \times 10^{-8}$, respectively. The expected average annual number of HUS cases was 0, 28 and 4, respectively. Risk of infection and HUS probability were sensitive to the type of abattoir, the application or not of Hazard Analysis and Critical Control Points (HACCP) for STEC (HACCP-STEC), stx prevalence in carcasses and trimmings, storage conditions from the abattoir to retailers and home, the joint consumption of salads and beef products, and cooking preference. The QMRA results showed that the probability of HUS was higher if beef cuts (1.7x) and ground beef (1.2x) were from carcasses provided by abattoirs not applying HACCP-STEC. Thus, the use of a single sanitary standard that included the application of HACCP-STEC in all Argentinean abattoirs would greatly reduce HUS incidence. The average number of annual HUS cases estimated by the QMRA (n = 32) would explain about 10.0% of cases in children under 15 years per year in Argentina. Since other routes of contamination can be involved, including those not related to food, further research on the beef production chain, other food chains, person-to-person transmission and outbreak studies should be conducted to reduce the impact of HUS on the child population of Argentina.
1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are foodborne pathogens associated with a wide spectrum of human diseases, from mild diarrhea to hemorrhagic colitis, thrombocytopenia and haemolytic uremic syndrome (HUS), which can lead to death [1]. Information about HUS cases around the world is scarce, particularly primary studies and notifiable disease data from different World Health Organization (WHO) regions, and population estimates on exposure, age distribution and clinical course of illness [2].

An estimated 2.5 million new STEC annual cases from different sources, including foodborne, have been reported globally, which have been responsible for 3,330 HUS cases, 269 deaths and 27,000 disability-adjusted life years [3]. In Argentina, STEC are the primary etiological agent of post-enteric HUS, and serotype O157:H7 is most frequently associated with HUS confirmed cases [4]. Between 2011 and 2015, 1,953 HUS cases were reported in Argentina, 70.7% of which corresponded to *E. coli* O157:H7 [5]. However, the food vehicle (ground beef and dry sausage) was identified in only four cases [6]. The last available report confirmed 290 HUS cases in 2019 [7].

Unlike Argentina, notification of HUS cases is not mandatory in most countries [8]. The annual HUS incidence rate in the general population of Argentina (0.6 cases per 100,000 inhabitants) [7] is similar to that reported in Canada (1.9) [9], Uruguay (0.4) (G. Varela, pers comm) and Australia (0.07) [10]). The Argentinean surveillance network has allowed the identification of most HUS cases, either in outbreaks or as sporadic cases [11], reporting one of the highest HUS incidence rates in populations younger than 5 and 1 year (6.3 and 12.9 per 100,000 children, respectively) [7]. In other countries, HUS incidence rates per 100,000 children under 5 years are 5.4 in Uruguay (G. Varela, pers comm), 4.2 in Canada [9] and 1.4 in USA [12]. Despite the high incidence rate, HUS-associated mortality rate in Argentina is higher (1.7%) [6] than that reported in Uruguay (1.2%) [G. Varela, pers comm] and lower than that reported in the USA (2.5%) [12], Chile (2.7%) [13] and Australia (12.0%) [10].

Cattle are the main animal reservoir of STEC currently known [14]. Recent reports have also pointed out the role of asymptomatic carriers in person-to-person STEC transmission (fecal-oral route) [6,15–17]. A study conducted in Argentina also showed that living in a farm or being in contact with farm animals and the presence of children <5 years of age in the family attending daycare or kindergarten were among the highest risk factors for STEC infection [18].

It has been recently shown that around 60.0% of all STEC reported cases worldwide cannot be attributed to a food source [19], despite 40.0% of cases were associated with food, mainly beef (18.2%), vegetables (15.6%) and dairy products (5.5%) [19]. In Argentina, beef per capita consumption is 51.0 kg/person [20]. Beef abattoirs can be classified into two main categories, namely, abattoirs with a Hazard Analysis and Critical Control Point (HACCP) system, that defines STEC as hazardous (hereinafter referred to as “applying HACCP-STEC”), and abattoirs with no HACCP plans or HACCP plans that do not define STEC as a hazard (hereinafter referred to as “not applying HACCP-STEC”) [21]. Abattoirs applying HACCP-STEC (38.0%) include cattle from arrival up to the production of vacuum-packaged beef cuts, commercial hamburgers and ground beef for supermarkets (with health authority permission), all within the abattoir plant. In abattoirs classified as “not applying HACCP-STEC” (62.0%), half carcasses are transported to retailers for cutting and deboning to produce beef cuts and for mincing to produce ground beef. In the case of butcher shops, they do not apply HACCP plans and they exceptionally apply good manufacturing practices (GMP) [22], considering that they should mince ground beef in the presence of the consumer according to the Argentine Food Code [23].
Beef can be cross-contaminated with STEC at different stages of the supply chain, from the abattoir to retail and the home environment [24–27]. In intact beef cuts, contamination is superficial, so that STEC can be easily destroyed by cooking [28]. Ground beef is not only considered a high-risk product due to the contamination spread during production, but it is normally associated with eating undercooked meat [29,30]. Additionally, home-made ground beef and commercial hamburgers have also been associated with STEC cases [31]. The prevalence of STEC in different beef products varies globally, ranging from 1.8–57.6% in Argentina to 0.7–60.6% in the rest of the world (S1 Table).

The use of risk analysis has been accepted internationally as a logical sequence of steps that contributes to the implementation of risk management measures based on scientific evidence. Risk assessment, the scientific process component, is the most relevant tool for assessing the association between existing foodborne hazards and public health risks [32]. Several quantitative microbial risk assessment (QMRA) models have been developed to link the presence of STEC in beef products with the risk of developing HUS in a certain population [25,27,30,33–36]. In 2009, a QMRA was developed in Argentina to model STEC contamination of beef hamburgers, using a farm-to-table risk approach [37]. More recent studies about STEC prevalence and contamination levels have been performed in other beef commodities, including hamburger, ground beef and beef cuts [38–43]. In this context, an updated QMRA including this new information would provide an accurate estimate of the incidence of HUS attributed to beef consumption in different age groups.

The aim of this study was to perform a quantitative risk assessment of HUS associated with the consumption of STEC-contaminated beef (intact beef cuts, ground beef and commercial hamburgers) from two abattoir systems in children under 15 years of age from Argentina.

2. Materials and methods

2.1. Study design

A probabilistic risk assessment model was developed to characterize STEC prevalence and contamination levels through the beef supply chain (Fig 1). The beef supply chain was divided into five production modules: cattle primary production, cattle transport, processing and storage in the abattoir, retail and home preparation, and consumption. Three beef products were modelled using the production modules described in Fig 1: 1) ground beef (any foodstuff containing ground meat, excepting commercial hamburgers), 2) commercial hamburgers, and 3) intact beef cuts.

The model was implemented in Microsoft Excel 2016 with the @Risk add-on package (version 7.5, Palisade Corporation, New York, USA) using inputs derived from data collected in Argentina and information gathered from experts, whenever possible. A Monte Carlo simulation with Latin Hypercube Sampling was used to assess all potential scenarios. Each simulation performed 5,000 iterations of the model, which allowed to achieve an adequate level of convergence (<1%). Model outputs were estimated as risk per serving of contaminated beef and population risk (median and 95.0% confidence intervals). To analyze the validity of the model, the predicted number of HUS cases was compared with data reported in the Argentinean Epidemiological Surveillance System [44].

2.2. Hazard identification

For the purpose of this study, all STEC were included in the model, assuming a similar pathogenic potential. Data of STEC prevalence at different production stages of the beef supply chain in Argentina were obtained by screening results of stx genes and/or STEC isolation reported in the literature (S2–S5 Tables).
2.3. Hazard characterization

A dose-response model was used to describe the relationship between the ingested dose of STEC from beef consumption and the probability of health endpoints of interest. The probability of illness (\(P_{ill}\)) was estimated using a Beta-Poisson model relating the ingested dose of the pathogen and the probability of illness [45,46]. The variability in \(\alpha\) and \(\beta\) parameters was modelled using PERT distributions based on the 5%, 50% and 95% percentiles estimated by Teunis et al. [45].

The probability of evolution to HUS (\(P_{HUSill}\)) of all STEC cases (3.0–9.0%) and HUS mortality rate (\(P_{mortHUS}\)) (2.6% in children and 12.0% in adults) were estimated from the data reported by Exeni in Argentina [46].

2.4. Exposure assessment

The five production modules of the beef supply chain were characterized by inputs (Fig 1). They were connected so that output distributions from each module served as inputs to the next module or as final outputs of the estimated ingested STEC dose (CFU) per serving portion (Table 1).

2.4.1. Cattle primary production. The prevalence of STEC in cattle was estimated according to three categories: a) season (spring-summer; fall-winter) [47,83], b) age of the animals (young, <18 months; adult, >18 months) [48], and c) production system (semi-intensive, feedlot) [48]. This classification resulted in eight different production scenarios (Table 1).

The proportion of animals in each age group (\(P_{Age}\)) and season (\(P_{Sc}\)) was modelled using cattle census data corresponding to 2018 [51] (S2 Table). The probability that a slaughtered animal belonged to a feedlot or semi-intensive production system (\(P_{PS}\)) was modelled using slaughter data from feedlot animals (S2 Table). Slaughter data from 2018 showed that the majority of animals were young (59.6%), slaughtered in spring-summer (50.1%) and from
Table 1. Input parameters used in the risk assessment model of STEC due to beef consumption.

| Variable | Symbol | Unit | Equation/Distribution | Reference |
|----------|--------|------|------------------------|-----------|
| Proportion of animals slaughtered in different seasons (autumn-winter vs. spring-summer) | $P_{(Se)}$ | Probability | $\sim$ Beta$[(6751434+1);(13468819−6751434+1)]$ | [47] |
| Proportion of animals slaughtered according to age ($<18$ months vs. $>18$ months) | $P_{(Age)}$ | Probability | $\sim$ Beta$[(8037782+1);(13468819−8037782+1)]$ | [48] |
| Proportion of animals slaughtered according to the production system (feedlot vs. semi-intensive system) | $P_{PS}$ | Probability | $\sim$ Beta$[(3651421+1);(13468819−3651421+1)]$ | [48] |
| $stx$ prevalence in animals slaughtered in autumn-winter, $<18$ months and from feedlot production systems | $P_{(1)}$ | Probability | $\sim$ Beta$[(38+1;95−38+1)]$ | [38,49–51] |
| $stx$ prevalence in animals slaughtered in autumn-winter, $<18$ months and from semi-intensive production systems | $P_{(2)}$ | Probability | $\sim$ Beta$[(36+1;166−36+1)]$ | [38,51,52] |
| $stx$ prevalence in animals slaughtered in autumn-winter, $>18$ months and from feedlot production systems | $P_{(3)}$ | Probability | $\sim$ Beta$[(0+1;6−0+1)]$ | [38,51] |
| $stx$ prevalence in animals slaughtered in spring-summer, $<18$ months and from feedlot production systems | $P_{(4)}$ | Probability | $\sim$ Beta$[(401+1;1865−401+1)]$ | [38–40,47,49,51] |
| $stx$ prevalence in animals slaughtered in spring-summer, $>18$ months and from feedlot production systems | $P_{(5)}$ | Probability | $\sim$ Beta$[(7+1;61−7+1)]$ | [38,49,51] |
| $stx$ prevalence in animals slaughtered in spring-summer, $<18$ months and from semi-intensive production systems | $P_{(6)}$ | Probability | $\sim$ Beta$[(145+1;238−145+1)]$ | [38,51,52] |
| $stx$ prevalence in animals slaughtered in spring-summer, $>18$ months and from semi-intensive production systems | $P_{(7)}$ | Probability | $\sim$ Beta$[(3+1;18−3+1)]$ | [4,38,51] |
| Change in $stx$ prevalence due to transport | $E_{(Tr)}$ | Odds Ratio | $\sim$ PERT$(0,561;1,028;1,882)$ | [54–61] |
| $stx$ prevalence in beef cattle after transport from farm to abattoir | $P_{(Tr)}$ | Prevalence | $\frac{\text{Prevalence} - E_{(Tr)} \cdot \text{Prevalence}}{\text{Prevalence} - E_{(Tr)}}$ | Where “Prevalence” is $P_{(1)}, P_{(2)}, \ldots$, or $P_{(8)}$ |

3. Processing and storage in the abattoir

Type of abattoir (applying HACCP-STECS vs. not applying HACCP-STECS) | Abatt | $\sim$ Bernoulli$(0,38)$ | [62] |
Table 1. (Continued)

| Variable | Symbol | Unit | Equation/Distribution | Reference |
|----------|--------|------|------------------------|-----------|
| Change in stx prevalence due to slaughter in abattoirs not applying HACCP-STE C | $TT_{(\text{noH})}$ | Odds ratio | $\frac{\text{BETA}(\text{A}+2;\text{H}+2;\text{i}+1)}{(\text{H}-\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1))}$ | [24,43,63,64] |
| stx prevalence in beef carcasses slaughtered in abattoirs not applying HACCP-STE C | $P_{(\text{noH})}$ | Prevalence | $\frac{\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1)}{(1-\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1))}$ | [38,41,42,65]; Brusa et al. (unpublished work) |
| Change in stx prevalence due to slaughter in abattoirs applying HACCP-STE C | $TT_{(\text{H})}$ | Odds ratio | $\frac{\text{BETA}(\text{A}+2;\text{H}+2;\text{i}+1)}{(\text{H}-\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1))}$ | [38,41,42,65]; Brusa et al. (unpublished work) |
| stx prevalence in carcasses slaughtered in abattoirs applying HACCP-STE C | $P_{(\text{H})}$ | Prevalence | $\frac{\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1)}{(1-\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1))}$ | [38,41,42,65]; Brusa et al. (unpublished work) |
| STEC concentration in carcasses slaughtered in abattoirs not applying HACCP-STE C | $C_{(\text{noH})}$ | Log cfu/100 cm$^2$ | $\text{Normal}(3.10, 71(\text{Truncated}(1.4,5.0)))$ | [43] |
| STEC concentration in carcasses slaughtered in abattoirs applying HACCP-STE C | $C_{(\text{H})}$ | Log cfu/100 cm$^2$ | $\text{Normal}(2.367, 0.89(\text{Truncated}(0.18,5.06)))$ | [42] |
| Storage temperature in abattoirs not applying HACCP-STE C | $\text{Temp}_{(\text{noH})}$ | ºC | $\text{PERT}(1;4;11)$ | [43] |
| Storage temperature in abattoirs applying HACCP-STE C | $\text{Temp}_{(\text{H})}$ | ºC | $\text{PERT}(0;1;3)$ | Industry communication |
| Storage time in abattoirs not applying HACCP-STE C | $T_{t_{(\text{noH})}}$ | h | $\text{Triangular}(24;52;192)$ | [43] |
| Storage time in abattoirs applying HACCP-STE C | $T_{t_{(\text{H})}}$ | h | $\text{Triangular}(24;27;30)$ | Industry communication |
| STEC growth during the storage period | $C_{(\text{stg})}$ | Log cfu/100 cm$^2$ | $c(stg) = C(A) + z(t) - \ln\left(1 - \frac{e^{-\alpha t}}{\alpha t}\right)$ with $z(t) = k \times \text{Temp}(A) + \frac{\alpha k}{2} \times \left(\text{Temp}(A) - 1\right)$, $k = 0.00658 + \frac{\text{BETA}(1.4;5.0))}{\text{Temp}(A) - 22.6}$, $\text{Temp}(A) = 24.367 \times \left[1 - \exp(-0.108 \times \text{Temp}(A))\right]$, $\text{BETA}(1,4;5,0))$, $\alpha = 8.53$, $Ym = 1.2683$, $\mu = \text{Normal}(0.001,0.004) \times (T(A) - 10\text{Normal}(6;1))$ | [66] |
| Change in stx prevalence due to deboning process | $\text{OR}_{(\text{deb})}$ | Odds ratio | $\text{BETA}(\text{A}+2;\text{H}+2;1.25;179+1) - \text{BETA}(\text{A}+2;\text{H}+2;1.25;0)$ | [41] |
| stx prevalence in beef cuts | $P_{(\text{BC})}$ | Prevalence | $\frac{\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1)}{(1-\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1))}$ | Industry communication |
| Storage temperature | $\text{Temp}_{(\text{bc})}$ | ºC | $\text{PERT}(0.2;4.0;5)$ | Industry communication |
| Storage time | $T_{t_{(\text{bc})}}$ | Hours | $\text{Uniform}(7,15) \times 24$ | Industry communication |
| STEC growth in beef cuts during storage | $C_{(\text{bc})}$ | Log cfu/cm$^2$ | Growth equation reported by Huang et al. | Industry communication |
| Surface area per gram of beef cuts | $Sa$ | cm$^2$/g | $\text{Uniform}(0,1;0,5)$ | Industry communication |
| Grams in 100 cm$^2$ of beef cuts | $Gcm^2$ | Grams | $\frac{100}{\text{Sa}}$ | Industry communication |
| STEC concentration in beef cuts in the abattoir | $C_{(\text{bcA})}$ | cfu/g | $\text{Normal}(0.001,0.004) \times (T(A) - 10\text{Normal}(6;1))$ | Industry communication |

3.b.- Commercial hamburger

| Variable | Symbol | Unit | Equation/Distribution | Reference |
|----------|--------|------|------------------------|-----------|
| Change in stx prevalence due to trimming process | $\text{OR}_{(\text{trm})}$ | Odds ratio | $\frac{\text{BETA}(\text{A}+2;\text{H}+2;1.25;179+1)}{(1-\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1))}$ | [41] |
| stx prevalence in trimmings | $P_{(\text{trm})}$ | Prevalence | $\frac{\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1)}{(1-\text{BETA}(\text{A}+\text{H}|\text{i}+1)+\text{BETA}(\text{A}+\text{H}|\text{i}+1))}$ | Industry communication |
| Storage temperature | $\text{Temp}_{(\text{trm})}$ | ºC | $\text{PERT}(-25;20;10)$ | Industry communication |
| Storage time | $T_{t_{(\text{trm})}}$ | Hours | $\text{Uniform}(2;5) \times 24$ | Industry communication |
| STEC growth during storage | $C_{(\text{trm})}$ | Log cfu/cm$^2$ | Growth equation reported by Huang et al. | Industry communication |

(Continued)
### Table 1. (Continued)

| Variable | Symbol | Unit | Equation/Distribution | Reference |
|----------|--------|------|-----------------------|-----------|
| STEC concentration at abattoir cuts to butcher’s hands | \(C_{(bA)}\) | cfu/g |  |  |
| Number of STEC in unwashed mincing machine to ground beef | \(P_{(mm-gb)}\) | cfu | |  |
| Transfer rate of STEC from beef cuts to butcher’s hands | \(T_{(bc)}\) | % | \(10^{\text{PERT}(0.48;0.55;1.72)}\) | [68] |
| Concentration change in beef grinding | \(p_{(gb)}\) | Odds ratio | \(\frac{\text{Beta}(1+1;86−1+1)}{\text{Beta}(1+1;86−1+1)}\) | [22,63,69–73]; Lopez et al. (unpublished work) |
| STEC concentration change in beef cuts after storage | \(C_{(stg)}\) | cfu/100cm² | Growth equation reported by Huang et al. [66] |  |
| Transfer rate of STEC from faucet | \(T_{(FH)}\) | % | \(10^{\text{PERT}(−2.99;−1.08;1.09)}\) |  |
| Number of STEC in unprocessed beef | \(P_{(bcAB)}\) | cfu | \((C(bA)\times T(bcA))/100\) |  |
| STEC concentration in beef cuts | \(C_{(bcB)}\) | cfu/g | \((C(bA)\times T(bcA))/100\) |  |

#### 4.a. - Beef cuts

| Probability of washing hands (butchers) | \(P_{(wh)}\) | Probability | \(-\text{Beta}(1+1;86−1+1)\) | [22] |
| Probability of washing the cutting board and table | \(P_{(cwb)}\) | Probability | \(-\text{Beta}(19+1;86−19+1)\) |  |
| Concentration change due to hand washing | \(R_{(wh)}\) | % | \(10^{-\text{Normal}(−0.2;1.42;\text{Truncated}(22))}\) |  |
| Transfer rate of STEC from beef cuts to butcher’s hands | \(T_{(bcH)}\) | % | \(10^{-\text{PERT}(−0.44;0.59;2)}\) | [68] |
| STEC concentration change in unwashed hands | \(P_{(nonWH)}\) | cfu | \((C(bc)\times T(bcH))/100\) |  |
| STEC concentration change in washed hands | \(P_{(WH)}\) | cfu | \((p(nonWH)\times R(wh))/100\) |  |
| Transfer rate of STEC from hands to faucet | \(T_{(HF)}\) | % | \(10^{-\text{PERT}(−2.99;−1.08;1.09)}\) |  |
| Number of STEC in faucet | \(p(F)\) | cfu | \((p(nonWH)\times T(HF))/100\) |  |
| Transfer rate of STEC from faucet to hands | \(T_{(FH)}\) | % | \(10^{-\text{PERT}(−1.7;0.169;2)}\) |  |
| Number of STEC in washed hands | \(P_{(WH)}\) | cfu | \((p(F)\times T(FH))/100\) |  |
| Number of STEC in beef cuts | \(p(bc)\) | cfu | In washed hands: \((p(WH)\times T(Hbc))/100\) |  |
| In unwashed hands: \((p(nonWH)\times T(Hbc))/100\) |  |
| Transfer rate of STEC from beef cuts to cutting board and table | \(T_{(bcCB)}\) | % | \(10^{-\text{PERT}(0.48;0.55;1.72)}\) | [68] |
| Number of STEC in unprocessed beef | \(P_{(bc)}\) | cfu | \((C(CB)\times T(bcCB))/100\) |  |
| Transfer rate of STEC from cutting board and table to beef cuts | \(T_{(CBbc)}\) | % | \(10^{-\text{PERT}(−0.79;−0.43;1.73)}\) |  |
| Number of STEC in unprocessed beef | \(P_{(bcnonW)}\) | cfu | \((p(CB)\times T(bcnonW))/100\) |  |
| Final number of STEC in beef cuts at butcher shops | \(C_{(bcB)}\) | cfu | \(C(bc)+p(bc)+p(nonW)\) |  |
| Storage temperature at butcher shops | \(\text{Temp}_{(B)}\) | °C | \(-\text{Triangular}(0.48;1.05;1.72)\) | C Adriani pers. comm |
| Storage time at butcher shops | \(T_{(B)}\) | Hours | \(-\text{Uniform}(2.5;24)\) | C Adriani pers. comm |

#### 4.b. - Ground beef

| Change in the stx prevalence due to beef grinding | \(OR_{(bc-gb)}\) | Odds ratio | \(\frac{-\text{Beta}(176+1436−173+1)}{-\text{Beta}(1+1;86−1+1)}\) |  |
| Number of STEC in ground beef | \(P_{(gb)}\) | Prevalence | \(\frac{(p(bc)\times T(bc-gb))}{(1−p(bc))+(p(bc)\times T(bc-gb))}\) |  |
| Probability of washing mincing machine | \(P_{(W-mm)}\) | Probability | \(-\text{Beta}(0+1;86−0+1)\) |  |
| Transfer rate of STEC from beef cuts to mincing machine | \(T_{(bc-mm)}\) | % | \(10^{-\text{PERT}(0.48;0.55;1.72)}\) |  |
| Number of STEC in unprocessed beef | \(P_{(bcnonW)}\) | cfu | \((C(CB)\times T(bcnonW))/100\) |  |
| Transfer rate of STEC from mincing machine to ground beef | \(T_{(mm-gb)}\) | % | \(10^{-\text{PERT}(−0.79;−0.49;1.73)}\) |  |
Table 1. (Continued)

| Variable                                      | Symbol | Unit | Equation/Distribution                                                                                     | Reference |
|-----------------------------------------------|--------|------|-----------------------------------------------------------------------------------------------------------|-----------|
| Number of STEC in ground beef                 | \( p_{(gb)} \) | cfu  | In washed mincing machine: 0 \( \frac{c(stg)}{c(mm)} \) In unwashed mincing machine: \( c(stg)+p(mm) \) | [22,68]   |
| Final number of STEC in ground beef (cm)      | \( C_{(cm)} \) | cfu  | \( C_{(cm)} \)                                                                                        |           |
| STEC concentration in ground beef             | \( C_{(gb)} \) | cfu/g| \( \frac{C_{(cm)}}{100} \) \( \sim \) Uniform(0.1:1:0.5)                                            |           |

4.c. Commercial hamburger

Type of retail where hamburgers are sold
\( Ret_{(Hamb)} \) ~ Discret((supermarket; minimarket, butcher); (1636:27;1069)) S6 Table. Survey of Argentinean beef consumption habits

Type of storage in each retail
\( Stg_{(Ret)} \) ~ Discret((refrigerated; freezing); (26:92)) Refrigerated: \( \sim \) Discret((0,1,2,4,6,14); (195:22:23:26)) \( \times 24 \)

Storage time
\( Ti_{(Ret)} \) Hours Freezing: \( \sim \) Discret((0,1,2,4,6,14); (228:37:602:543:385:737)) \( \times 24 \)

STEC concentration in commercial hamburgers at retail
\( C_{(HRet)} \) cfu Growth equation reported by Huang et al. [66]

Final STEC concentration in commercial hamburgers at retail
\( C_{(Hg)} \) cfu/g \( \sim \) Uniform(0.1:1:0.5) \[5.1.4\]

5. Home and consumption

5.a. Beef cuts

Storage at home
\( Stg_{(Hom)} \) ~ Beta(2832+1;5466−2832+1) S6 Table. Survey of Argentinean beef consumption habits

Temperature of household refrigerators
\( Temp_{(re)} \) °C \( \sim \) Trinagular(−1.5;6.1;16.1) [74,75]

Temperature of household freezers
\( Temp_{(fr)} \) °C \( \sim \) Trinagular(−41,1;−20,1;−2) [74,75]

STEC concentration in beef cuts at home
\( C_{(bchome)} \) cfu/g Growth equation reported by Huang et al. [66]

Probability of eating salad with beef cuts
\( Salad \) ~ Beta(5430+1;5494−5430+1) S6 Table. Survey of Argentinean beef consumption habits

Probability of preparing beef cuts before salad
\( P_{(bc-Sa)} \) ~ Beta(1079+1;3748−1079+1)

Probability of washing hands (consumers)
\( P_{(WH)} \) ~ Beta(3148+1;4468−3148+1)

Probability of washing cutting board
\( P_{(Wcb)} \) ~ Beta(3418+1;4468−3418+1)

Change in STEC concentration due to washing hands
\( R_{(WH)} \) % \( \sim \) Normal(−0.2;142;Truncated(2))

Transfer rate of STEC from beef cuts to hands
\( T_{(bc-H)} \) % \( \sim \) PERT(−0.44;0.59;2) [68,76]

STEC concentration in unwashed hands
\( p_{(nonWH)} \) cfu \( (C_{(bcHome)}\times T_{(nonWH)})/100 \)

Number of STEC in washed hands
\( p_{(WH)} \) cfu \( (p_{(nonWH)}\times R_{(WH)})/100 \)

Transfer rate of STEC from hands to faucet
\( T_{(HF)} \) % \( \sim \) PERT(−2.59;−1.08;1.09)

Number of STEC in the faucet
\( p_{(F)} \) cfu \( (p_{(nonWH)}\times T_{(HF)})/100 \)

Transfer rate of STEC from faucet to hands
\( T_{(FH)} \) % \( \sim \) PERT(−1.7;0.169;2) [68,76]

Number of STEC in washed hands
\( p_{(WH)} \) cfu \( (p_{(F)}\times T_{(FH)})/100 \)

Transfer rate of STEC from hands to salad
\( T_{(HSal)} \) % \( \sim \) PERT(−2.54;0.21;2) [68,76]

(Continued)
| Variable | Symbol | Unit | Equation/Distribution | Reference |
|----------|--------|------|------------------------|-----------|
| Number of STEC in salad | \( p_{(Sal)} \) | cfu | \( p\text{ (WH) } \times T_{(HSal)} /100 \) | \[68,76\] |
| Transfer rate of STEC from beef cuts to cutting board | \( T_{(bc-cb)} \) | % | \( \text{Normal}(-2.54;0.21;2) \) | \[68,76\] |
| Number of STEC in unwashed cutting board | \( p_{(nonWcb)} \) | cfu | \( (C_{(bcHome) } \times T_{(bcHome)}) /100 \) | \[68,76\] |
| Transfer rate of STEC from cutting board to salad | \( T_{(cbSal)} \) | % | \( \text{Normal}(-1.7;0.19;2) \) | \[68,76\] |
| Number of STEC in salad | \( p_{(SanonWl)} \) | cfu | \( p\text{ (nonWcb) } \times T_{(cbSal)} /100 \) | \[68,76\] |
| Final number of STEC in salad | \( FC_{(Sal)} \) | cfu | \( C_{(Sal)} + p_{(Sal)} + p_{(SalnonW)} \) | \[68,76\] |
| Decimal reduction | \( D_{(BC)} \) | 10 | \( 10^{(11.22 + 0.18 \times \text{Temp}_{(cook)})} \) | \[79\] |
| Number of decimal reductions | \( ND_{(BC)} \) | Times \( p \) | \( \text{Normal}(-0.2;1.42;2) \) | \[79\] |
| STEC concentration in ready-to-eat beef cuts | \( C_{(bccons)} \) | cfu/g | \( C_{(bcHome)} + p_{(bcHome)} - ND_{(bcCons)} \) | \[8-b\] Ground beef |
Table 1. (Continued)

| Variable                                         | Symbol    | Unit | Equation/Distribution | Reference |
|--------------------------------------------------|-----------|------|-----------------------|-----------|
| Number of STEC in salad                          | $p_{(En)}$ | cfu  | In washed hands: $(p(WH)\times T(HSal))/10$ |           |
|                                                   |           |      | In unwashed hands: $(p(nonWH)\times T(HSal))/100$ |           |
| Transfer rate of STEC from ground beef to cutting board | $T_{(gb-cb)}$ | %    | $1\%_{\text{PERT}(0.48;1.05;1.49)}$ | [68,76] |
| Number of STEC in unwashed cutting board         | $p_{(nonWcb)}$ | cfu  | $(C(\text{gb})\times T(gbcM CB))/100$ |           |
| Transfer rate of STEC from cutting board to salad | $T_{(cbSal)}$ | %    | $1\%_{\text{PERT}(-0.79;-0.42;1.72)}$ | [68,76] |
| Number of STEC in salad                          | $p_{(SalnonWcb)}$ | cfu  | $(p(cb)\times T(TcbSal))/100$ |           |
| Final number of STEC in salad                    | $p_{(Sal)}$ | cfu  | $(p(WH)+p(nonWH)+p(SalnonWcb))/100$ |           |
| Cooking preference                               | $p_{(cookgb)}$ | ~    | $\text{Discret([1,2,3,4,5];0.003;0.011;0.109;0.086;0.791)}$ | S6 Table. Survey of Argentinean beef consumption habits |
| Cooking temperature                              | $\text{Temp}_{(cookgb)}$ | ℃    | Red: 54.4℃ | [80] |
|                                                   |           |      | Medium-Red: 58.6℃ |           |
|                                                   |           |      | Medium: 62.7℃ |           |
|                                                   |           |      | Medium-Well done: 65.6℃ |           |
|                                                   |           |      | Well done: 68.3℃ |           |
| Number of decimal reductions                     | $ND_{(gb)}$ |      | $10.165+(0.211\times \text{Temp}_{(cookgm)})$ | [81] |
| STEC concentration in ready-to-eat ground beef   | $C_{(gbcons)}$ |     | $10\left(\text{Log}_{10}(gm)–\text{D}_{(gm)}\right)$ |           |
| Probability of eating salad with hamburger       | $Salad_{(H)}$ | Probability | $\text{Beta}(3539+1;3858–3539+1)$ | S6 Table. Survey of Argentinean beef consumption habits |
| Probability of preparing hamburger before salad   | $H-Sal$ | Probability | $\text{Beta}(1079+1;3748–1079+1)$ |           |
| Probability of washing hands (consumers)         | $p_{(WH)}$ | Probability | $\text{Beta}(4485+1;5493–4485+1)$ |           |
| Probability of washing cutting board             | $p_{(Wcb)}$ | Probability | $\text{Beta}(5286+1;5549–5286+1)$ |           |
| Change in STEC concentration due to washing hands | $R_{(WH)}$ | %    | $1\%_{\text{Normal}(0;2.1;42;\text{Truncated}(2))}$ |           |
| Transfer rate of STEC from hamburger to hands    | $T_{(HH)}$ | %    | $1\%_{\text{PERT}(0.440;0.59;2)}$ | [68,76] |
| STEC concentration in unwashed hands              | $p_{(nonWcb)}$ | cfu  | $(C(\text{Hg})\times T(HH))/100$ |           |
| Number of STEC in washed hands                   | $p_{(WH)}$ | cfu  | $(p(nonWH)\times R(WH))/100$ |           |
| Transfer rate of STEC from hands to faucet       | $T_{(HF)}$ | %    | $1\%_{\text{PERT}(-2.99;–1.08;1.09)}$ | [68,76] |
| Number of STEC in the faucet                     | $p_{(F)}$ | cfu  | $(p(nonWH)\times T(HF))/100$ |           |
| Transfer rate of STEC from faucet to hands       | $T_{(HH)}$ | %    | $1\%_{\text{PERT}(-2.94;–0.21;2)}$ | [68,76] |
| Number of STEC in washed hands                   | $p_{(WH)}$ | cfu  | $[T(\text{F})\times T(\text{F})]/100]+p(\text{WH})$ |           |
| Transfer rate of STEC from hands to faucet       | $T_{(HH)}$ | %    | $1\%_{\text{PERT}(-2.94;–0.21;2)}$ | [68,76] |
| Number of STEC in salad                          | $p_{(Sal)}$ | cfu  | In washed hands: $(p(WH)\times T(HSal))/100$ |           |
|                                                   |           |      | In unwashed hands: $(p(nonWH)\times T(HSal))/100$ |           |
| Transfer rate of STEC from hamburger to cutting board | $T_{(Hcb)}$ | %    | $1\%_{\text{PERT}(0.481;1.05;1.49)}$ | [68,76] |
| Number of STEC in unwashed cutting board         | $p_{(nonWcb)}$ | cfu  | $(C(\text{Hg})\times T(Hcb))/100$ |           |
Table 1. (Continued)

| Variable | Symbol | Unit | Equation/Distribution | Reference |
|----------|--------|------|-----------------------|-----------|
| Transfer rate of STEC from cutting board to salad | \( T_{(chSal)} \) | \( \% \) | \( 10^{-PERT(-0.79,-0.43;1.73)} \) | [68,76] |
| Number of STEC in salad | \( p_{(SalnonWcb)} \) | cfu | \((p(nonWcb) \times T(chSal)) / 100\) | | |
| Final number of STEC in salad | \( FC_{(Sal)} \) | cfu | \( C(Sal) + p(Sal) + p(SalnonWcb) \) | | |
| Cooking preference | \( P_{(cookH)} \) | | DISCRETE((1,2,3);(0.01;0.183;0.806) | S6 Table, Survey of Argentinean beef consumption habits |
| Cooking temperature | \( Temp_{(cookH)} \) | ‘C | Medium-Red: ~UNIFORM(54.4;58.6) | [80] |
| Number of decimal reductions | \( ND_{(gb)} \) | | 10.165 + (0.211 × Temp(CookH)) | [81] |
| STEC concentration in ready-to-eat hamburgers | \( C_{(Hcons)} \) | cfu | \( \logNormal(83.54;50.26) \) | |
| Probability of death | \( P_{(dth)} \) | | \[45,46\] |
| Probability of HUS | \( P_{(HUS)} \) | | ~UNIFORM(0.03;0.09) | |
| Probability of death | \( P_{(dth)} \) | | ~Beta(0.056; 39.71; 398.9) | |
| Probability of HUS | \( P_{(HUS|ill)} \) | | \( P(ill) \times P(HUS) \) | [46] |
| Probability of death | \( P_{(dth|HUS)} \) | | \( P(HUS|ill) \times P(dth) \) | |
| Number of portions | \( N_{(porbc)} \) | Number | Children < 23 months: \( (2.029,712 \times 0.5176) \times 365 \) | Table 2, Food consumption by the Argentine population (6 months to 15 years of age) [82] |
| Number of cases of HUS per year due to beef cut consumption | \( N_{(HUSbc)} \) | Number | \( N(\text{porbc}) \times P(HUS|\text{ill}) \) | |
| 6. Consumption | | | | |
| 6.a. Beef cuts | | | | |
| Portion size | \( PS_{(bc)} \) | Grams | \( \logNormal(65.9;45.8) \) | | |
| Ingested dose of STEC from beef cut consumption | \( Dose_{(bc)} \) | cfu | \( C(Hcons) \times PS_{(bc)} + C(Sal) \) | |
| 6.b. Ground beef | | | | |
| Portion size | \( PS_{(gb)} \) | Grams | \( \logNormal(43.8;30.9) \) | | |
| Ingested dose of STEC from ground beef consumption | \( Dose_{(gb)} \) | cfu | \( C(gbcons) \times PS_{(gb)} + C(Sal) \) | |
| 6.c. Commercial hamburger | | | | |
| Portion size | \( PS_{(H)} \) | Grams | \( \logNormal(58.4;32.1) \) | | |
| Ingested dose of STEC from hamburger consumption | \( Dose_{(H)} \) | cfu | \( C(Hcons) \times PS_{(H)} + C(Sal) \) | |
| 7. Dose-response module | | | | |
| Probability of illness | \( P_{(ill)} \) | | \( 1 - \left(\frac{P_{(past)}}{1 + \left(\frac{P_{(past)}}{10} \right)\alpha} \right) \) | [45,46] |
| Probability of HUS | \( P_{(HUS)} \) | | ~UNIFORM(0.03;0.09) | |
| Probability of death | \( P_{(dth)} \) | | ~Beta(35+1;1302–35+1) | |
| Probability of HUS | \( P_{(HUS|ill)} \) | | \( P(ill) \times P(HUS) \) | [46] |
| Probability of death | \( P_{(dth|HUS)} \) | | \( P(HUS|ill) \times P(dth) \) | |
| 7.a. Beef cuts | | | | |
| Number of portions | \( N_{(porbc)} \) | Number | Children < 23 months: \( (2.029,712 \times 0.5176) \times 365 \) | Table 2, Food consumption by the Argentine population (6 months to 15 years of age) [82] |
| Number of cases of HUS per year due to beef cut consumption | \( N_{(HUSbc)} \) | Number | \( N(\text{porbc}) \times P(HUS|\text{ill}) \) | | |
| 7.b. Ground beef | | | | |

(Continued)
feedlots (70.4%). The probability of occurrence of the three variables (P\textsubscript{Age}, P\textsubscript{Se} and P\textsubscript{PS}) was modelled using Beta distributions.

Data describing stx prevalence in cattle feces were available from several peer-reviewed studies performed in Argentina (S3 Table). The combination of the three variables (P\textsubscript{Age}, P\textsubscript{Se} and P\textsubscript{PS}) allowed to model stx prevalence considering potential risk factors. A syllogism was used to combine the probability of occurrence of the eight level combinations (P\textsubscript{1}, P\textsubscript{2}, P\textsubscript{3}, P\textsubscript{4}, P\textsubscript{5}, P\textsubscript{6}, P\textsubscript{7}, and P\textsubscript{8}). Applying the method of moments [84], these data were used to determine parameters α and β of Beta distributions and to estimate stx prevalence in each combination of factors.

2.4.2. Cattle transport. Cattle transport to abattoirs generates stress and increases cross-contamination, which could in turn modify stx prevalence. A systematic review and meta-analysis search of parameters related to the effect of transport on stx prevalence was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Fig 2) [85]. Scopus, PubMed and Science Direct databases were searched for scientific papers unrestricted by language and published from 1980 to 2019. The research question was: “Is there evidence from the scientific literature that transport of beef cattle from farm to abattoir modifies STEC prevalence?” Search terms included “transport” AND “STEC” OR “O157:H7” OR “non-O157 STEC” OR “stx” AND “cattle” OR “beef cattle”. Initially, 8639 articles were identified. Abstracts and titles were assessed, selecting articles that met the a priori inclusion criteria. Random effect meta-analysis was performed using the Comprehensive Meta-Analysis software 2.2.064 version. Differences in stx prevalence in beef cattle before and after transportation were incorporated in the meta-analysis and used in the model as odds ratio (OR) values. Mean OR and 95.0% confidence interval (95.0% CI) values were used as parameters and included in a PERT distribution to model the effect of transport on STEC prevalence.

The new stx prevalence after transport was estimated using the transfer rate equation as follows:

$$P = \frac{P_i \times OR}{1 - P_i + P_i \times OR}$$

where \(P\) is the new stx prevalence after a specific scenario (e.g., beef cattle in the abattoir after transport) and \(P_i\) is the stx prevalence before the specific scenario (e.g., beef cattle in the farm)
Fig 2. Flowchart of the cattle transport literature search according to PRISMA.

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and OR is the odds ratio value between the scenarios compared. An OR less than 1 means a reduction in stx prevalence and an OR greater than 1 indicates an increase in stx prevalence [21]. This methodology was used to model the change in stx prevalence along the beef supply chain.

2.4.3. Processing and storage in the abattoir. The prevalence of stx and STEC levels was modelled at various stages along the slaughtering process, from arrival of live cattle to carcass storage in the cold chamber (Fig 1, Table 1). As already mentioned, abattoirs were classified as “applying HACCP-STEC” (38.0%) and “not applying HACCP-STEC” (62.0%) [62]. The probability of slaughter in each type of abattoir was modelled using the Bernoulli distribution model (Abatt). Each abattoir type was modelled differently: HACCP-STEC included the production of vacuum-packaged beef cuts and commercial hamburgers all within the abattoir plant, whereas abattoirs not applying HACCP-STEC were modelled from the production of half carcasses within the plant to the transport to retail for the production of beef cuts and ground beef.

The prevalence of stx in carcasses varied according to the type of abattoir and was modelled using scientific publications conducted in Argentina (S4 Table). The OR value from cross-contamination during slaughtering was calculated using stx prevalence in carcasses and live cattle jointly for abattoirs applying HACCP-STEC (TTA-H) and not applying HACCP-STEC (TTA-noH) (S4 Table), using the previously mentioned Eq 1.

Enumeration levels of STEC were estimated by using generic E. coli counts in carcasses from abattoirs applying HACCP-STEC (C(A-H)) [42] and not applying HACCP-STEC (C(A-noH)) [43]. This was considered as the most conservative scenario as is expected STEC enumeration levels to be much lower than generic E. coli counts. The levels of STEC during cold chamber storage (C(stg)) were estimated using the growth equation reported by Huang et al. [66]. The growth of STEC in beef cuts, commercial hamburgers and ground beef in the cold chamber and at retail was estimated using the same equation. Cold chamber temperature (TempA-H) and storage times (TiA-H) of HACCP-STEC abattoirs were provided by the participating plants (Industry communication). Temperature (TempA-noH) and storage times (TiA-noH) of abattoirs not applying HACCP-STEC were obtained from the work by Costa et al. [43] (Table 1).

Beef cuts. Operators, equipment, the environment and beef are sources of STEC contamination during cutting and deboning. Both operations were modelled in HACCP-STEC abattoirs only because abattoirs not applying HACCP-STEC provided half-carcasses to retailers, where they were thus modelled. The OR value due to cross-contamination during deboning to obtain beef cuts (ORdeb) was modelled with data obtained in Argentina by Brusa et al. [41] in HACCP-STEC abattoirs. The stx prevalence in beef cuts (P(bcA)) was calculated from the stx prevalence in carcasses stored in cold chambers (P(c-H)) and the OR value due to deboning (ORdeb) (Table 1). The STEC concentration was estimated per 100 cm$^2$ of beef cuts and considered as superficial contamination. To convert load per cm$^2$ (log CFU/cm$^2$) to load per gram of product (log CFU/g) (CbcA), the relationship between the two measures was estimated. According to previous estimates, a gram of beef corresponds to 0.1–0.5 cm$^2$ cut surface (Sa) [37].

Commercial hamburgers. The transfer rate (OR) from carcasses to trimmings (ORtrm) was estimated using data published by Brusa et al. [41] in HACCP-STEC abattoirs (Table 1). The prevalence of stx in trimmings (P(trm)) was estimated by combining the prevalence in carcasses stored in cold chambers (P(c-H)) and the OR value from cutting and deboning. The growth of STEC in commercial hamburgers (C(hA)) was modelled using the storage temperature (TempA) and storage time (TiA) values provided by abattoirs (Table 1) (Industry communication).
2.4.4. Retail. Beef cuts. The cross-contamination rate during deboning to obtain beef cuts at retail was estimated using the same equation as in abattoirs applying HACCP-STE. To incorporate cross-contamination due to retail handling, the probability of occurrence of certain practices was estimated from behavioral surveys conducted in Argentina, which included the probability of hand washing ($P_{wh}$) and cutting board washing ($P_{wcb}$) during beef handling [22]. Bacterial transfer rate from beef to hands ($T_{(bcH)}$) and to cutting boards and tables ($T_{(bcCB)}$) and reduction rate by hand washing ($R_{(wh)}$) were estimated according to Montville and Schaffner [68] (Table 1, Fig 3). The growth of STEC at retail was modelled using the temperature ($Temp_{(B)}$) and storage time ($Ti_{(B)}$) values at retail provided by the Sanitary Authority of the city of Berisso, Buenos Aires, Argentina (C Adriani pers. comm).

Ground beef. The $stx$ transfer rate (OR) from beef cuts to ground beef ($OR_{bc-gb}$) at retail was estimated based on the $stx$ prevalence in beef cuts and ground beef reported in Argentina (Table 1, S5 Table). The $stx$ prevalence in ground beef ($P_{gb}$) was estimated from the prevalence in beef cuts ($P_{bcA}$), modified according to the estimated transfer rates resulting from handling scenarios at retail (S5 Table). The STEC concentration in ground beef ($C_{gb}$) was estimated by the probability of washing the mincing machine ($P_{(Wmm)}$) [22] and the bacterial transfer rate ($T_{(bc-mm)}$) [68].

Commercial hamburgers. The proportion of commercial hamburgers ($Ret_{(Hamb)}$) and conditions ($Stg_{(Ret)}$) (frozen, chilled, other) in each retail outlet (mini-markets, supermarkets and butcher shops) was modelled according to consumer preferences (S6 Table). The STEC concentration in hamburgers at retail ($C_{(HRet)}$) was modelled considering the storage temperature ($Temp_{(h)}$) of each outlet with data from Evans and Redmond (2015) and James et al. (2017). The storage period at retail ($Ti_{(Ret)}$) was modelled considering the answers provided by Argentinean consumers (S6 Table) [74,75].

2.4.5. Home and consumption. Beef consumption habits in Argentina were surveyed (S6 Table) using a descriptive epidemiological design. The survey was anonymous and self-administered. It consisted of 16 closed questions with different options to evaluate frequency and preference of beef consumption, place of acquisition, habit of beef storage and preparation. Informed consent was attached regarding anonymity, non-mandatory participation and use of research results.

The growth of STEC on each beef product (beef cuts, ground beef and hamburger) during storage at home ($Stg_{(Hom)}$) was modelled using the temperature values of household refrigerators ($Temp_{(re)}$) and freezers ($Temp_{(fr)}$) from Evans and Redmond [74] and James et al. [75].
Cross-contamination at home was modeled using the bacterial transfer rates among the different surfaces (cutting boards, hands, faucet) reported by Montville and Schaffner [68] and Chen et al. [68,76]. The probability that consumers prepared salads together with beef (Salad), hand washing (\( P_{(WH)} \)) and cutting board washing (\( P_{(Wcb)} \)) was estimated from the survey of Argentine consumers (S6 Table).

The effect of cooking at home on STEC concentration in beef cuts was modeled considering five cooking preferences (red, medium-red, medium-well, medium-well done, well done) (S6 Table). For each cooking preference (\( P_{(cooking)} \)), cooking time (\( T_{(cook)} \)) was estimated taking into account the time to achieve the desired beef doneness and cut thickness [78]. Cooking temperature (\( Temp_{(cook)} \)) at the surface of beef cuts (where bacterial contamination is present) was estimated to vary between 75 to 90°C [77]. Log STEC reduction during cooking of beef cuts (\( ND_{(BC)} \)) (log CFU/g) was estimated by dividing cooking time by the D-value (\( D_{(BC)} \)) at each cooking temperature, using the D-values obtained from several \( E.\ coli \) O157:H7 strains isolated from beef [79]. The STEC concentration after cooking (\( C_{(bccons)} \) (CFU/g) was estimated by the difference between the concentration in raw beef cuts (\( C_{(bchome)} \)) and the log reduction due to cooking (\( ND_{(BC)} \)).

The effect of cooking during the preparation of commercial hamburgers and ground beef was modeled as a function of the final internal product temperature (\( Temp_{(cook)} \)) in ground beef (\( P_{(cookinggb)} \)) and hamburgers (\( P_{(cookingH)} \)) for each cooking preference of Argentinean consumers (S6 Table). In order to compare our results with previous studies reporting the preference of consumption of ground beef and hamburgers as "pink" in the center of the mass, the categories "red" and "medium-red" of our survey were considered jointly as "pink". Each cooking preference was related to an internal temperature using the approach reported by Jackson et al. [80]. Within-variability of internal temperatures for each cooking preference was modeled using a uniform distribution. Log STEC reduction during cooking of ground beef (\( ND_{(gb)} \)) and hamburgers (\( ND_{(H)} \)) was estimated using the linear model reported by Juneja el al. [81]. Final STEC concentration was estimated using the same approach as explained in beef cuts.

### 2.5. Risk characterization

The QMRA model used the specific conditions for the production of each type of beef product (beef cuts, ground beef and hamburgers) under two abattoir systems in Argentina, considering the intrinsic variability and uncertainties of each process. Risk characterization was expressed as probability of illness (diarrhea due to STEC infection) and number of HUS cases after consuming STEC-contaminated beef products.

Children aged 6 months to 15 years were considered the target population of this study as they represent the age group with the highest HUS incidence in Argentina [7]. Final exposure to STEC was estimated as the combination of the ingested dose (CFU) in a beef serving (beef cuts, ground beef, hamburger) and the dose ingested during salad consumption in case both were consumed together. Portion sizes, frequency of consumption of each beef product (\( N_{(porbc)} \), \( N_{(porgb)} \), \( N_{(porH)} \)) and population stratum were obtained from the National Nutrition and Health Survey of Argentina [82] (Table 2). Population estimates of each stratum were assessed in accordance with the 2010 National Census of Population, Households and Housing [86]. The number of annual HUS cases due to beef consumption (\( N_{(HUSbc)} \), \( N_{(HUSgb)} \), \( N_{(HUSH)} \)) was estimated considering the probability of acquiring the disease (\( P_{(HUSill)} \)) and the frequency of beef consumption (Table 2).

### 2.6. Sensitivity analysis

Sensitivity analysis was performed using @Risk (Palisade Inc.) to identify the processing steps with the greatest impact on the risk of acquiring STEC infection and thereby identify the risk management strategies that would generate the greatest impact on public health.
3. Results

3.1. Cattle primary production

The *stx* prevalence during primary production for all production scenarios (season, age of the animals and production system) was 25.1% (6.2–64.4, 95.0% CI). Results differed when *stx* prevalence was calculated for each specific scenario, as follows: 26.2% (7.3–43.1) in fall-winter and 36.2% (9.3–64.6) in spring-summer; 36.9% (10.8–64.1) in young and 22.9% (5.1–31.5) in adult cattle; and 35.0 (3.6–45.2) and 22.4% (18.8–64.1) in semi-intensive and feedlot production systems, respectively. As it can be observed, spring-summer, young cattle and semi-intensive production system showed the highest prevalence.

3.2. Cattle transport

The systematic literature search yielded 30 scientific studies using the terms “transport”, “beef cattle”, “STEC prevalence” and “stx prevalence”. Reviews and prevalence studies in other animals or animals not producing food and reports with limited data to estimate *stx* prevalence before and after transport were excluded (n = 18). Twelve articles were used to estimate the impact of transport on *stx* prevalence. The estimated pooled OR was 1.0 (0.6–1.9), showing a significant heterogeneity (Q-statistic: *P* < 0.0001; *I²*-statistic = 91.6%).

3.3. Processing and storage in the abattoir

The prevalence of *stx* on carcass surfaces in abattoirs applying and not applying HACCP-STEC was 23.3 (18.8–41.6) and 42.7% (36.2–63.8), respectively. The enumeration of STEC levels was 1.7 (0.3–3.4) and 2.7 (1.3–4.2) log CFU/100 cm², respectively.

3.4. Retail

3.4.1. Beef cuts. The prevalence of *stx* and STEC concentration in beef cuts was estimated considering whether the carcass supplier applied HACCP-STEC or not. Thus, *stx* prevalence was 28.4 (19.9–49.4) and 48.8% (37.3–70.1), respectively and STEC concentration was -2.9 (-5.0 and 0.4) and -0.2 (-3.4 and 3.6) log CFU/g, respectively.

3.4.2. Ground beef. Both *stx* prevalence and STEC concentration were estimated considering the available information from abattoirs applying or not HACCP-STEC and the effect of handling beef at retail. Accordingly, *stx* prevalence was 73.6% (55.8–89.3) and STEC concentration was -2.82 log CFU/g (-3.4–2.5).

3.4.3. Commercial hamburgers. The model incorporated information of Argentinean abattoirs applying HACCP-STEC. Thus, *stx* prevalence in trimmings was 30.1% (20.3–52.2) and STEC concentration in hamburgers was -2.9 log CFU/g (-5.0 and 0.4).

Table 2. Beef consumption by the Argentine population (6 months to 15 years of age) [82].

| Age (Population) | 6–23 months (2,029,712) | 2–5 years (1,984,070) | 6–15 years (9,692,170) |
|------------------|------------------------|----------------------|-----------------------|
| Foodstuff        | Beef cuts              | Ground beef          | Commercial hamburger  |
| Daily consumption| 0.52                   | 0.11                 | 0.01                  |
| frequency        |                        |                      | 0.64                  |
| Mean portion size| 65.9 (45.8)            | 43.8 (30.9)          | 58.4 (32.1)           |
| (g) (SD)         |                        |                      | 83.5 (50.3)           |
| Total portions   | 383,461,310            | 81,270,683           | 11,112,673            |
| consumed         |                        |                      | 467,172,098           |

Table 2. Beef consumption by the Argentine population (6 months to 15 years of age) [82].

| Age (Population) | 6–23 months (2,029,712) | 2–5 years (1,984,070) | 6–15 years (9,692,170) |
|------------------|------------------------|----------------------|-----------------------|
| Foodstuff        | Beef cuts              | Ground beef          | Commercial hamburger  |
| Daily consumption| 0.52                   | 0.11                 | 0.64                  |
| frequency        |                        |                      | 0.01                  |
| Mean portion size| 65.9 (45.8)            | 43.8 (30.9)          | 58.4 (32.1)           |
| (g) (SD)         |                        |                      | 83.5 (50.3)           |
| Total portions   | 383,461,310            | 81,270,683           | 11,112,673            |
| consumed         |                        |                      | 467,172,098           |

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3.5. Home and consumption

A total of 5,658 surveys from 23 jurisdictions in Argentina were collected in April 2019 (S6 Table). Regarding beef cuts, 89.7% of surveyed consumers acquired this product chilled at retail and 56.7% stored beef cuts frozen at home. Most consumers (99.7%) preferred levels of cooking that ensured STEC removal from the surface of beef cuts. The most preferred levels of cooking were "well-done" (57.6%), "medium-well done" (17.4%) and "medium-well" (17.9%). In the case of ground beef, 46.6% of people acquired the product chilled at retail and 49.8% stored ground beef frozen at home. The preferred level of cooking was "well-done" (79.1%) followed by "medium-well" (10.9%). Finally, commercial hamburgers were obtained frozen at retail (56.9%), stored at home once frozen (78.0%), and people preferred them "well-done" (80.6%) and "medium-well" (9.9%).

According to the type of side dish, 45.1–66.8% of surveyed individuals preferred the consumption of any beef product along with fresh vegetables; 51.8% reported having two separate tables to prepare beef and vegetables, whereas 15.8% used the same table for both, always washing the table with detergent in between handling these foods. After handling beef, 16.2% of consumers reported to wash their hands and 4.2% reported to wash the utensils.

The STEC concentration in raw beef cuts, ground beef and commercial hamburgers was 1.3 (-3.4–3.4), -2.7 (-3.4–3.9) and -2.8 (-3.4–3.0) log CFU/g, respectively. The STEC transfer rates from beef cuts, ground beef and commercial hamburgers to salad was -5.0 (-5.0–3.9), -5.0 (-5.0–0.5) and -5.0 (-5.0–0.9) log CFU/g, respectively.

3.6. Risk characterization

Median HUS probability from consumption of beef cuts, ground beef and commercial hamburgers was $<10^{-15}$ ($<10^{-15}$–6.0x10$^{-3}$, 90.0% CI), 5.4x10$^{-8}$ (3.5x10$^{-10}$–3.9x10$^{-4}$) and 3.5x10$^{-8}$ (3.0x10$^{-10}$–2.0x10$^{-4}$), respectively (Table 3). The expected average annual number of HUS cases from consumption of beef cuts, ground beef and commercial hamburgers was 0, 28 and 4, respectively. The expected annual number of deaths due to ground beef and commercial hamburger consumption was 0, 2 and 0, respectively.

3.7. Sensitivity analysis

3.7.1. Beef cuts. The risk of STEC infection from beef cut consumption and subsequent outcomes correlated with abattoirs applying HACCP-STEC, stx prevalence in carcasses at retail, storage temperature in cold chambers of abattoirs not applying HACCP-STEC or at retail, joint consumption of salad and beef cuts, hand washing after handling raw meat, transfer of STEC from hands to salad, refrigeration temperature at home, STEC concentration in carcasses from abattoirs not applying HACCP-STEC, and bacterial transfer from beef cuts to hands (Fig 4(A)).

Table 3. Probability of illness, HUS and death and annual number of HUS cases from consumption of beef cuts, ground beef and commercial hamburgers contaminated with STEC.

| Foodstuff           | Illness    | Probability* | Mortality | Expected median HUS cases per year |
|---------------------|------------|--------------|-----------|-----------------------------------|
| Beef cuts           | $<10^{-15}$ ($<10^{-15}$–8.0x10$^{-5}$) | $<10^{-15}$ ($<10^{-15}$–6.0x10$^{-3}$) | $<10^{-15}$ ($<10^{-15}$–7.9x10$^{-4}$) | 0                                 |
| Ground beef         | 9.0 x 10$^{-7}$ (6.3x10$^{-9}$–7.0x10$^{-3}$) | 5.4x10$^{-8}$ (3.5x10$^{-10}$–3.9x10$^{-4}$) | 6.4 x 10$^{-9}$ (4.2x10$^{-11}$–4.7x10$^{-5}$) | 28                                |
| Commercial hamburgers | 5.8x10$^{-7}$ (8.2x10$^{-9}$–4.1x10$^{-5}$) | 3.5 x 10$^{-8}$ (3.0x10$^{-10}$–2.0x10$^{-4}$) | 4.2x10$^{-9}$ (5.4x10$^{-11}$–2.9x10$^{-5}$) | 4                                 |

*Median (90% CI).

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The most significant input for the risk of STEC infection was the type of abattoir for beef production. This model input negatively correlated with the risk of infection (the higher the percentage of abattoirs applying HACCP-STEC, the lower the probability of illness). Such effect may be explained by the lower \(stx\) prevalence and STEC concentration in carcasses produced in abattoirs applying HACCP-STEC (28.4%; mean concentration, \(-2.9\) log CFU/g) as compared with abattoirs not applying HACCP-STEC (48.8%; mean concentration, \(-0.2\) log CFU/g). Thus, consumers eating beef cuts produced in an abattoir not applying HACCP-STEC had 1.7 times higher probability of being exposed to STEC as compared with abattoirs applying HACCP-STEC.

Likewise, hand washing negatively correlated with the probability of infection, proving the impact of this practice on disease occurrence. Storage temperature in the abattoir, at retail and home had a great influence on the probability of infection, with a 3.5 and 7.4 times increased risk of HUS if beef cuts were stored at 8 and 10˚C, respectively.

**3.7.2. Ground beef and commercial hamburgers.** The risk of STEC infection from ground beef and commercial hamburger consumption and subsequent outcomes correlated positively with \(stx\) prevalence in carcasses at retail and trimmings in the abattoir, storage temperature at home, storage type at home, carcass STEC concentration in abattoirs not applying HACCP-STEC (ground beef) and applying HACCP-STEC (commercial hamburgers), transfer of STEC from the mincing machine to ground beef, and joint consumption of salad and ground beef (Fig 4(B) and 4(C)). The probability of HUS was 1.2 times higher if ground beef was elaborated with beef provided by abattoirs not applying HACCP-STEC. Ground beef cooking preference was the only input with a negative correlation, i.e., the higher
percentage of consumers who preferred a higher degree of beef doneness (well-done was selected by 79.1% of consumers), the higher the STEC reduction after cooking and the lower the probability of infection. The positive correlation between STEC transfer from the mincing machine to ground beef and the probability of acquiring HUS due to ground beef consumption evidenced the impact of good hygiene practices (GHP) at retail.

4. Discussion

This risk assessment study allowed to shed light into the potential role of beef consumption in the development of HUS cases in the Argentinean child population, considering the very limited epidemiological information on food sources in the country [6]. The QMRA included all the available information throughout the Argentinean beef production chain, from primary production to home consumer habits [38–43,50]. Although a risk assessment of HUS from hamburger consumption had already been carried out in Argentina [37], the relevance of the current QMRA is concerned with the inclusion of new information that responds to the uncertainties identified in the previous risk assessment [37], such as a) risk factors associated with the presence of STEC in primary production, b) effect of the transport of live animals, c) identification of abattoirs with different risk levels, d) evaluation of the effect of cross-contamination in butcher shops, e) application of a survey to assess beef consumption habits at home at national level, not just regional, and f) consideration of other meat matrices. Despite the quantity and quality of the information used in the current QMRA were better, the risk of HUS from hamburger consumption was very similar in both models (3.5x10^{-8} vs. 4.6x10^{-8}).

4.1. Cattle primary production

Cattle are the major STEC reservoir, and beef has been essentially identified as the main vehicle associated with the transmission of this group of microorganisms [52]. The mean stx prevalence in cattle estimated by the QMRA (25.1%, 6.2–64.4%, 95.0% CI) was in the range of that reported in Brazil, USA, Italy and Spain (21.3–36.2%) [87–91]. The STEC prevalence reported in studies conducted in Argentina is also within the same range (11.8–38.9%) [38,39,52,53]. However, other authors have reported higher mean STEC prevalence in cattle feces in Paraguay, Canada, Germany, Ireland, UK, France and Australia (44.8–84.8%) [92–99]. International studies have identified differences in cattle stx prevalence according to season, cattle age and feeding practices [48,83,87,97,100,101]. However, the QMRA model did not show any statistical association between these primary production variables and the risk of developing HUS from any beef product. More detailed prevalence studies including different production scenarios in Argentina could validate the model conclusions.

4.2. Cattle transport

The impact of transport on STEC prevalence in cattle is controversial. In this study, the pooled OR impact of transport on STEC prevalence in cattle was 1.0 (0.6–1.9). Other studies have observed an increase [57,102,103], a reduction [58], no change [60,61,101] and even contradictory results [54,104] in the prevalence and spread of STEC in bovine faeces caused by transport.

4.3. Processing and storage in the abattoir

Carcass contamination with STEC can occur during the slaughtering process in the abattoir, and STEC-contaminated carcasses can carry over the contamination to beef cuts and trimmings [41,42,63]. The prevalence of stx in carcasses was 23.3% (18.8–41.6) in HACCP-STECPLOS ONE | https://doi.org/10.1371/journal.pone.0242317 November 13, 2020 20 / 32
abattoirs and 42.7% (36.2–63.8) in abattoirs not applying HACCP-STEC. A similar or higher prevalence has been reported in the USA (23.0 and 60.6%) and UK (27.0%) [95,105,106]. In Canada, the proportion of STEC confirmed by isolation from carcasses was 5.4% [107]. In Argentina, the isolation rate of STEC strains was 5.8 to 9.0% in abattoirs applying HACCP-STEC [24,38,41].

The concentration of STEC in carcasses was also associated with the type of abattoir. The probability of developing HUS from beef cut consumption was lower (1.7x) if carcasses were provided by abattoirs applying HACCP-STEC, evidencing the impact of targeting the food safety mitigation strategies against STEC. In these abattoirs, beef cuts are vacuum-packaged, avoiding later product contamination until consumption. On the other hand, abattoirs not applying HACCP-STEC do not cut and bone carcasses; these processes are performed in butcher shops that do not even apply GMP [22]. As the type of abattoir was one of the most influential model inputs on the risk of HUS, the use of a single sanitary standard (application of HACCP-STEC) in all Argentinean abattoirs and during transportation of packaged beef cuts would have the greatest impact on HUS reduction.

4.4. Retail

The prevalence of stx in beef cuts at retail was also higher if carcasses were produced in abattoirs not applying HACCP-STEC (48.8 vs. 28.4%). Studies conducted in Uruguay and the USA have reported 28.0% and 36.0% stx detection in beef cuts, respectively [105,108]. In Argentina, stx detection in retail beef cuts was 12.1% [63], and even lower in Chile, Brazil, Canada and Italy (0.7–8.4%) [109–111].

Food products elaborated with ground beef are considered an epidemiologically important source of STEC infections due to contamination spread during mincing [22,112]. Although the stx prevalence estimated by the QMRA in ground beef at retail (73.6%) was similar to that reported in Chile, Brazil, USA, Italy, Spain and Australia (2.1–49.3%) [113–120], studies conducted in Argentina have reported a lower prevalence (6.1–45.3%) (S5 Table). Differences may be due to true differences in STEC shedding rates in cattle, GMP and HACCP practices in the abattoir and storage conditions at retail. It is important to note that the laboratory methodologies or criteria (screening or isolation) to consider positivity for STEC differed, which may also account for differences in prevalence levels between studies.

Commercial hamburgers are elaborated with beef trimmings obtained from deboning in abattoirs applying HACCP-STEC. The stx prevalence in trimmings was 30.1%, including activities that could lead to cross-contamination (slaughtering, quartering, deboning). In this regard, the only study conducted in Argentina reported 1.4% stx prevalence in trimmings [41], whereas studies in New Zealand, Australia, USA and Uruguay informed a higher stx prevalence (9.7–30.0%) [108,121].

4.5. Risk characterization

4.5.1. Beef cuts. In the present study, the mean probability of illness, HUS and death from beef cut consumption in children under 15 years was 10^{-15}, with an expected number of zero HUS cases per year (95.0% CI 0–0). In a risk assessment carried out in Canada [30], the mean probability of illness (2.9x10^{-9}) from beef cut consumption was six orders of magnitude greater than in our study. In our QMRA, storage temperature at retail (>5˚C) was a risk variable for HUS development due to beef cut consumption, as identified in the sensitivity analysis. Application of GMP along the beef chain and storage of beef at temperatures below 5˚C were identified as protective factors against HUS. Since microbial contamination in beef cuts is superficial and STEC are not heat-resistant, exposure to recommended cooking
temperatures eliminates STEC [122]. In Argentina, children prefer beef cuts to ground beef and commercial hamburgers (Table 2) [82]. Even though most Argentinean consumers (99.7%) prefer eating beef cuts "medium-red" to "well-done", the sensitivity analysis did not identify the level of cooking as a factor that impacted on HUS risk.

4.5.2. Ground beef and commercial hamburgers. The mean probability of illness, HUS and death from ground beef consumption in Argentine children under 15 years was 9.0x10^{-7}, 5.4x10^{-8} and 6.4x10^{-9}, respectively, and 5.8x10^{-7}, 3.5x10^{-8} and 4.2x10^{-9}, respectively, from commercial hamburger consumption. The expected annual number of HUS cases from ground beef and commercial hamburger consumption was 28 and 4, respectively. The present HUS QMRA is similar to other risk assessments developed in Canada [30,33], Australia [34], the Netherlands [123], USA [35,124], Ireland [27] and Argentina [37], all of which considered primary production conditions, distribution, storage and consumption. The probability estimates reported in those studies (P_{illness} 6.0x10^{-7}–1.8x10^{-6}, (P_{HUS} 4.2x10^{-9}–6.4x10^{-7}) and (P_{death} 5.9x10^{-10}–2.3x10^{-8}) were within the values informed here. In a previous risk assessment carried out in Argentina [37], the probability of HUS from home-made and commercial hamburger consumption was 4.6x10^{-8} (95.0% CI, 7.4x10^{-11}–1.6x10^{-4}), similar to the one obtained with the present QMRA. In agreement with a study conducted in Canada, home storage conditions were a protective factor against HUS from ground beef consumption [30]. On the other hand, cross-contamination at retail, specifically the transfer of STEC from the mincing machine to ground beef due to lack of standardized sanitation operating procedures (SSOP) and GHP, significantly increased bacterial loads and the public health risk associated with ground beef consumption [22,125]. In Argentina, most consumers (70.0%, S6 Table) purchase ground beef in butcher shops, the majority of which do not apply SSOP, GHP or GMP [22]. The probability of HUS was 1.2 times higher if ground beef was elaborated with carcasses provided by abattoirs not applying HACCP-STEC. Thus, applying HACCP-STEC in all abattoirs could help reduce HUS incidence. In this context, it would be interesting to evaluate the impact of HACCP-STEC from ground beef production to immediate packaging after processing.

The stx prevalence in trimmings was also associated with higher risk of HUS from commercial hamburger consumption. Storage at refrigeration temperatures (<5˚C) at retail and home were protective factors against HUS. In agreement with other risk assessments, cooking was the most influential model input for ground beef and hamburgers [27,30,33–36]. Opposite to other survey studies conducted in Ireland and Norway reporting 65.0% and 45.7% of consumers eating hamburgers well-done [126,127], most consumers in Argentina preferred eating ground beef (79.1%) and commercial hamburgers (80.6%) well-done. Such preference for a higher degree of meat doneness was seen as a protective factor against the risk of acquiring HUS.

Differences in the probabilities estimated by the different models worldwide reflected the diverse conditions of food production, distribution, storage and preparation [36]. However, all models were markedly similar in terms of the factors having the highest risk impact. The prevalence and concentration of the pathogen in faeces and carcasses and the cooking temperature of beef were the most influential variables in all the published models.

The cross-contamination module "at home" regarding Argentinean habits was incorporated to capture the effect of food preparation practices on disease transmission. Storage temperature was identified in the sensitivity analysis of all beef products of our model. This coincided with other authors [123] and reinforced the idea of the impact of storage and processing practices at home on the risk of HUS. Cross-contamination has been previously proposed as a factor associated with illness and increased HUS risk [27,36,67]. Vegetables have been associated with STEC cases and outbreaks worldwide [19,128–133], and STEC cross-
contamination from beef to vegetables as well as the effect of hygiene measures have also been studied [134–136]. In our QMRA, the joint consumption of salads with beef was identified as a risk factor for HUS due to improper hygiene practices at home and vegetable contamination from meat, although the effect of the possible level of STEC contamination of vegetables was not included. Other QMRA did not consider or identify the joint consumption of salads with beef as a risk factor for HUS. The sensitivity analysis of all foodstuffs in our model estimated that the impact of consumers’ habits during food preparation at home was lower than that of variables such as type of abattoir, stx prevalence in carcasses or storage of beef at retail. However, their influence on the probability of HUS should not be underestimated.

4.6. Is beef consumption the only responsible for endemic HUS in Argentina?

Haemolytic-uremic syndrome is considered a multifactorial disease [18] and, for this reason, HUS endemicity in Argentina cannot be explained only by beef consumption. Although the consumption of raw beef, raw milk, lettuce, sprouts, fruit juices and vegetables is recognized as a potential source of STEC infection in human beings [137], environmental exposure, direct contact with animals and person-to-person transmission have also been identified as important risk factors [18,138–141]. In Argentina, information on potential food sources and transmission routes other than beef is scarce. However, an epidemiological study showed that eating undercooked beef outside home, living or visiting a place with pets and being in contact with children <5 years old with diarrhoea were risk factors for HUS [142]. The routes of transmission have expanded from direct or indirect contact with cattle or animal food products to include direct contact with infected people that may be actively shedding STEC [18].

The rate of HUS cases reported in Argentina ranges from 300 to 500 new cases per year, with a median of 349 cases in the period 2010–2016 [6]. The average number of annual HUS cases in this study was 32, all related to the consumption of beef products. On average, 10.0% of HUS cases reported in children under 15 years in Argentina would be due to beef consumption, especially ground beef. Official reports of the period 2002–2015 only attributed 0.1–0.06% of cases to beef consumption [6]. The last epidemiological report in Argentina has shown a slight decrease in HUS cases, totalling 290 cases [7]. Such tendency could be explained by consumers’ habits, the improvements implemented along the beef production chain and specific legislation on beef products. However, HUS primarily affects 1-year-old children. The annual rate slightly increased from 12.3 cases per 100,000 in 2018 [44] to 12.9 cases in 2019 [7]. According to the Argentinian National Nutrition and Health Survey [82], beef cuts are the beef food most consumed by this sub-population. In the present evaluation, no HUS cases from beef cut consumption would be expected. In this context, other potential sources of infection should be included to implement actions tending to reduce HUS in the affected sub-population. For example, in 2011–2015, 39 HUS outbreaks were reported in Argentina; 30 were associated with home origin, 5 with kindergarten and 4 with the community [6]. Fernandez Brando et al. [17,143] reported that 75.0% of children in urban and suburban areas and 68.7% of healthy adults working in kindergartens from Buenos Aires had antibodies against Shiga-toxins. Also, it was recognized that human beings can be carriers and eliminate STEC in faeces, without presenting disease symptoms [6]. These findings allowed us to hypothesize about the role of person-to-person transmission, particularly if we consider that more than 54.0% of disease outbreaks caused by STEC worldwide were not associated with any specific food source [32,140,144].

Cooking preference impacted on the probability of HUS among Argentine consumers, but the responsibility cannot rest exclusively on consumers and their consumption habits. The
origin of beef (abattoirs applying or not HACCP-STE)
was also associated with HUS risk. It would be very
important to continue working in the beef production chain and to deepen the
knowledge of other food production chains and sources of water supply. Additionally, person-
to-person transmission should be evaluated and epidemiological studies strengthened to iden-
tify the origin of HUS cases in order to reduce the impact of HUS on the child population of
Argentina.

5. Conclusion
In summary, the QMRA developed in the present study did not find any statistical association
between primary production variables (cattle age, season and production system) and the
probability of developing HUS. The model predicted almost double stx prevalence and higher
STE enumeration levels in carcasses and beef cuts produced in abattoirs not applying
HACCP-STE. The abattoir type (applying or not applying HACCP-STE), storage tempera-
tures (higher temperatures from abattoir to home) and lack of hygienic practices at retail were
the most influential factors increasing significantly HUS probability. Beef consumption in the
Argentinian children population (mainly ground beef) was able to explain only about 10.0% of
the HUS median cases per year in children under 15 years. This study highlights the multi-
factorial nature of HUS disease and the plausibility of other STEC infection routes (other food
sources, animal contact, person-to-person) and the need to investigate the contribution of
these additional risk factors on the overall HUS disease burden in the children population of
Argentina.

Supporting information
S1 Table. Prevalence of stx and STEC in beef products from all over the world.
(DOCX)
S2 Table. Bovine slaughtered during 2018. Total amount and amount provided by feedlots.
(DOCX)
S3 Table. Peer-reviewed sources of stx prevalence in Argentinean cattle feces used for
input into the model.
(DOCX)
S4 Table. Scientific publications of samplings conducted in Argentinean abattoirs used to
model the prevalence of stx-positive carcasses after slaughter.
(DOCX)
S5 Table. Scientific publications of samplings conducted in Argentinean retails used to
model stx prevalence at retail.
(DOCX)
S6 Table. Survey of Argentinean beef consumption habits.
(DOCX)

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References

1. Karmali MA, Gannon V, Sargeant JM. Verocytotoxin-producing *Escherichia coli* (VTEC). Vet Microbiol. 2010; 140(3–4):11. https://doi.org/10.1016/j.vetmic.2009.04.011 PMID: 19410388.

2. Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ, et al. Global incidence of human Shiga toxin-producing *Escherichia coli* infections and deaths: a systematic review and knowledge synthesis. Foodborne Pathog Dis. 2014; 11(6):17. https://doi.org/10.1089/fpd.2013.1704 PMID: 24750096; PubMed Central PMCID: PMC4607253.

3. Kirk MD, Pires SM, Black RE, Caipo M, Crump JA, Devleeschauwer B, et al. World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: A data synthesis. PLoS Med. 2015; 12(12):21. https://doi.org/10.1371/journal.pmed.1001921 PMID: 26633831; PubMed Central PMCID: PMC4668831.

4. Leotta GA, Milliwebskey ES, Chinen I, Espinosa EM, Azzopardi K, Tennant SM, et al. Characterisation of Shiga toxin-producing *Escherichia coli* O157 strains isolated from humans in Argentina, Australia and New Zealand. BMC Microbiol. 2008; 8:8. https://doi.org/10.1186/1471-2180-8-8 PMID: 18197985; PubMed Central PMCID: PMC2277424.

5. JEMRA. Joint FAO/WHO Core Expert Group Meeting on VTEC/STEC. 2016.

6. Área de Vigilancia de la Salud de la Dirección de Epidemiología MdSdNA. Boletín Integrado de Vigilancia | N° 329 –SE 39–2016. Ciudad Autónoma de Buenos Aires2016.

7. Área de Vigilancia de la Salud de la Dirección de Epidemiología MdSdNA. Boletín Integrado de Vigilancia | N° 481—SE 02–2020. Ciudad Autónoma de Buenos Aires2020.

8. Rivas M, Chinen I, Guth BEC. Enterohemorrhagic (Shiga Toxin-Producing) *Escherichia coli*. In: Torres AG, editor. *Escherichia coli* in the Americas 2016. p. 97.

9. Alberta Health AG. Haemolytic Uremic Syndrome. 2018.

10. Vally H, Hall G, Dyda A, Raupach J, Knope K, Combs B, et al. Epidemiology of Shiga toxin producing *Escherichia coli* in Australia, 2000–2010. BMC Public Health. 2012; 12:12. https://doi.org/10.1186/1471-2458-12-12 PMID: 22221851

11. Torres AG. *Escherichia coli* in the Americas. 1 ed. Switzerland Springer International Publishing 2016. 384 p.

12. CDC. FoodNet Fast’s Hemolytic Uremic Syndrome (HUS) USA2020 [cited 2020 1rst January]. Available from: https://www.cdc.gov/foodnethu/HUS.
13. Di Pillo S, Sotomayor G, Alimentaria. ACpiCel. *Escherichia coli* productoras de toxinas Shiga O157 y no O157 en carne bovina, Chile. In: Alimentaria ACpiCel, editor. Chile2018. p. 77.

14. Kim JS, Lee MS, Kim JH. Recent Updates on Outbreaks of Shiga Toxin-Producing *Escherichia coli* and Its Potential Reservoirs. Front Cell Infect Microbiol. 2020; 10:10. https://doi.org/10.3389/fcimb.2020.00010 PMID: 32117794

15. Morita-Ishihara T, Iyoda S, Iguchi A, Ohnishi M. Secondary Shiga Toxin-Producing *Escherichia coli* from patients and asymptomatic food handlers in Japan. Plos One. 2019: 13. https://doi.org/10.1371/journal.pone.0225340 PMID: 31743366

16. Baba H, Kanamori H, Kudo H, Kuroki Y, Higashi S, Oka K, et al. Genomic analysis of Shiga toxin-producing *Escherichia coli* from broiler chickens: Evaluating processing interventions. Food Control. 2019: 100:14.

17. Fernández-Brando RJ, Amaral MM, Ciocihini AE, Bentancor LE, Trelles JA, Da Rocha M, et al. Microbiological and serological control of *Escherichia coli* O157: H7 in kindergarten staff in Buenos Aires city and suburban areas. Medicina (B Aires). 2017; 77:6. PMID: 28643674

18. Rivas M, Sosa-Estani S, Rangel J, Caletti MG, Vallés P, Roldán CD, et al. Risk factors for sporadic Shiga toxin–producing *Escherichia coli* infections in children, Argentina. Emerg Infect Dis. 2008; 14 (5):9.

19. FAO WHO. Shiga toxin-producing *Escherichia coli* (STEC) and food: attribution, characterization, and monitoring. Microbiological risk assessment series. 2018; 31.

20. IPCVA. Estadísticas Consumos Promedio 2020 [cited 2020 08/05]. Available from: http://www.ipcva.com.ar/estadisticas/vista_consumos_promedio.php.

21. Dogan OB, Clarke J, Mattosc F, Wang B. A quantitative microbial risk assessment model of *Campylobacter* in broiler chickens: Evaluating processing interventions. Food Control. 2019: 100:14.

22. Leotta GA, Brusa V, Galli L, Adriani C, Linares L, Etcheverria A, et al. Comprehensive evaluation and implementation of improvement actions in butcher shops. PLOS One. 2016; 11(9):16. https://doi.org/10.1371/journal.pone.0162635 PMID: 27618439; PubMed Central PMCID: PMC5019392.

23. Código Alimentarioa Argentino. Capítulo VI. Alimentos carnosos y afines. Carnes de consumo frescas y envasadas, (2007).

24. Cap M, Carboni CC, D’Asteck BA, Zolezzi G, Deza N, Palladino MP, et al. Frequency, characterization and genotypic analysis of Shiga toxin-producing *Escherichia coli* in beef slaughterhouses of Argentina. Rev Argent Microbiol. 2019; 51(1):7. https://doi.org/10.1016/j.ram.2018.03.005 PMID: 29937134.

25. Duffy G, O’Brien SB, Carney E, Sheridan JJ, McDowell DA, Blair IS. Characterisation of *E. coli* O157 isolates from bovine hide and beef trimming in Irish abattoirs by pulsed field gel electrophoresis. J Microbiol Methods. 2005; 60(3):8. https://doi.org/10.1016/j.mimet.2004.10.014 PMID: 15649539.

26. Asakura H, Masuda K, Yamamoto S, Igimi S. Molecular approach for tracing dissemination routes of Shiga toxin-producing *Escherichia coli* O157 in bovine offal at slaughter. BioMed Res Internat. 2014: 2014:5. https://doi.org/10.1155/2014/739139 PMID: 24592396; PubMed Central PMCID: PMC3925628.

27. Duffy G, O’Brien S, Carney E, Butler F, Cummins E, Nally P, et al. A quantitative risk assessment of *E. coli* O157:H7 in Irish minced beef. In: Teagasc, editor. Ireland2005.

28. FSAl. Microbiological safety of raw minced beef and beef burgers on retail sale in Ireland (11NS1). Microbiology ed. Dublin, Ireland2013. p. 25.

29. Luchansky JB, Porto-Fett ACS, Shoyer BA, Phillips J, Chen V, Eblen DR, et al. Fate of Shiga toxin-producing O157:H7 and non-O157:H7 *Escherichia coli* cells within blade-tenderized beef steaks after cooking on a commercial open-flame gas grill. J Food Prot. 2012; 75(1):10. https://doi.org/10.4315/0362-028X.JFP-11-267 PMID: 22221356

30. Smith BA, Fazil A, Lammerding AM. A risk assessment model for *Escherichia coli* O157:H7 in ground beef and beef cuts in Canada: Evaluating the effects of interventions. Food Control. 2013; 29(2):18. https://doi.org/10.1016/j.foodcont.2012.03.003

31. Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis. 2005; 11(4):7. https://doi.org/10.3201/eid1104.040739 PMID: 15829201

32. FAO WHO. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Microbiological risk assessment series. 2004; 5:311.

33. Cassin MH, Lammerding AM, Todd ECD, Ross W, Stephen McColl R. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. Int J Food Microbiol. 1998; 41:23. https://doi.org/10.1016/s0168-1605(98)00028-2 PMID: 9631335
34. Lammerding AM, Fazil A, Paoli G, Desmarchelier P, Vanderlinde P. Shiga-toxin-producing *E. coli* in ground beef manufactured from Australian beef: Process improvement. *Australia: Meat and Livestock Australia.*, 1999.

35. Kiermeier A, Jenson I, Sumner J. Risk Assessment of *Escherichia coli* O157 illness from consumption of hamburgers in the United States made from Australian manufacturing beef. *Risk Anal.* 2015; 35 (1):13. [https://doi.org/10.1111/risa.12248](https://doi.org/10.1111/risa.12248) PMID: 24984959.

36. Duffy G, Cummins E, Nally P, O’ Briena S, Butler F. A review of quantitative microbial risk assessment in the management of *Escherichia coli* O157:H7 on beef. *Meat Sci.* 2006; 74(1):13. [https://doi.org/10.1016/j.meatsci.2006.04.011](https://doi.org/10.1016/j.meatsci.2006.04.011) PMID: 22062718

37. Signorini M, Tarabila H. Quantitative risk assessment for verocytotoxigenic *Escherichia coli* in ground beef hamburgers in Argentina. *Int J Food Microbiol.* 2009; 132(2–3):9. [https://doi.org/10.1016/j.ijfoodmicro.2009.04.022](https://doi.org/10.1016/j.ijfoodmicro.2009.04.022) PMID: 19446904.

38. Masana MO, D’Astel BA, Palladino PM, Galli L, Del Castillo LL, Carbonari C, et al. Genotypic characterization of non-O157 Shiga toxin-producing *Escherichia coli* in beef abattoirs of Argentina. *J Food Prot.* 2011; 74(12):10. [https://doi.org/10.4315/0362-028X.JFP-11-189](https://doi.org/10.4315/0362-028X.JFP-11-189) PMID: 22186039.

39. Fernández D, Irino K, Sanz M, Padola NL, Parma AE. Characterization of Shiga Toxin-producing *Escherichia coli* isolated from dairy cows in Argentina. *Lett Appl Microbiol.* 2010; 51:6. [https://doi.org/10.1111/j.1472-765X.2010.02849.x](https://doi.org/10.1111/j.1472-765X.2010.02849.x) PMID: 20438618.

40. Tanaro JD, Galli L, Lound LH, Leotta GA, Piaggio MC, Carbonari CC, et al. Non-O157:H7 Shiga toxin-producing *Escherichia coli* in bovine rectums and surface water streams on a beef cattle farm in Argentina. *Foodborne Pathog Dis.* 2012; 9(10):7. [https://doi.org/10.1089/fpd.2012.1182](https://doi.org/10.1089/fpd.2012.1182) PMID: 22994915.

41. Brusa V, Restovich V, Galli L, Teitelbaum D, Signorini M, Brasesco H, et al. Isolation and characterization of non-O157 Shiga toxin-producing *Escherichia coli* from beef carcasses, cuts and trimmings of abattoirs in Argentina. *PLoS One.* 2017; 12(8):16. [https://doi.org/10.1371/journal.pone.0183248](https://doi.org/10.1371/journal.pone.0183248) PMID: 28829794; PubMed Central PMCID: PMC5568767.

42. Signorini M, Costa M, Teitelbaum D, Restovich V, Brasesco H, Garcia D, et al. Evaluation of decontamination efficacy of commonly used antimicrobial interventions for beef carcasses against Shiga toxin-producing *Escherichia coli*. *Meat Sci.* 2018; 142:8. [https://doi.org/10.1016/j.meatsci.2018.04.009](https://doi.org/10.1016/j.meatsci.2018.04.009) PMID: 29656275.

43. Costa M, Pracca G, Sucari A, Galli L, Ibargoyen J, Gentiliuomo J, et al. Comprehensive evaluation and implementation of improvement actions in bovine abattoirs to reduce pathogens exposure. *Prev Vet Med.* 2020; 176:8. [https://doi.org/10.1016/j.prevetmed.2020.104933](https://doi.org/10.1016/j.prevetmed.2020.104933) PMID: 32105562.

44. Área de Vigilancia de la Salud de la Dirección de Epidemiología MdSdlNA. Boletín Integrado de Vigilancia | N° 439—SE 06—2018. Ciudad Autónoma de Buenos Aires 2018.

45. Teunis PF, Ogden ID, Strachan NJ. Hierarchical dose response of *E. coli* O157:H7 from human outbreaks incorporating heterogeneity in exposure. *Epidemiol Infect.* 2008; 136:10. [https://doi.org/10.1017/S0950268807008242](https://doi.org/10.1017/S0950268807008242) PMID: 17352837; PubMed Central PMCID: PMC2870861.

46. Exeni RA. Síndrome urérmico hemolítico. Manifestaciones clínicas. *Tratamiento. Medicina (B Aires).* 2006; 66(3):5.

47. Fernandez D, Rodriguez EM, Arroyo GH, Padola NL, Parma AE. Seasonal variation of Shiga toxin-encoding genes (stx) and detection of *E. coli* O157 in dairy cattle from Argentina. *J Appl Microbiol.* 2009; 106(4):8. [https://doi.org/10.1111/j.1365-2672.2008.04088.x](https://doi.org/10.1111/j.1365-2672.2008.04088.x) PMID: 19187162.

48. Venegas-Vargas C, Henderson S, Khare A, Mosci RE, Lehnert JD, Singh P, et al. Factors associated with Shiga toxin-producing *Escherichia coli* shedding by dairy and beef cattle. *Appl Environ Microbiol.* 2016; 82(16):8. [https://doi.org/10.1128/AEM.00829-16](https://doi.org/10.1128/AEM.00829-16) PMID: 27342555; PubMed Central PMCID: PMC4968536.

49. Favier GI, Estrada CL, Cortinas TI, Escudero ME. Detection and Characterization of Shiga Toxin Producing *Escherichia coli*, *Salmonella spp.*, and *Yersinia* Strains from Human, Animal, and Food Samples in San Luis, Argentina. *Int J Microbiol.* 2014; 2014:12. [https://doi.org/10.1155/2014/284649](https://doi.org/10.1155/2014/284649) PMID: 25177351; PubMed Central PMCID: PMC4142171.

50. Padola NL, Sanz ME, Blanco JE, Blanco M, Blanco J, Etcheverria AI, et al. Serotypes and virulence genes of bovine Shigatoxigenic *Escherichia coli* (STEC) isolated from a feedlot in Argentina. *Vet Microbiol.* 2004; 100:7. [https://doi.org/10.1016/S0378-1135(03)00127-5](https://doi.org/10.1016/S0378-1135(03)00127-5) PMID: 15135507.

51. IPCVA. [2019]. Available from: [http://www.ipcva.com.ar/](http://www.ipcva.com.ar/).

52. Meichtli L, Miliwetbsky E, Gioftrre A, Chinen I, Baschiker A, Chillemi G, et al. Shiga toxin-producing *Escherichia coli* in healthy young beef steers from Argentina: prevalence and virulence properties. *Int J Food Microbiol.* 2004; 96(2):10. [https://doi.org/10.1016/j.ijfoodmicro.2004.03.018](https://doi.org/10.1016/j.ijfoodmicro.2004.03.018) PMID: 15364473.

53. Sanz ME, Viñas MR, Parma AE. Prevalence of bovine verotoxin-producing *Escherichia coli* in Argentina. *Eur J Epidemiol.* 1998; 14:5. [https://doi.org/10.1023/a:1007427925583](https://doi.org/10.1023/a:1007427925583) PMID: 9690760
54. Barham RA, Barham BL, JohNSon AK, Allen DM, Blanton JR, Miller MF. Effects of the transportation of beef cattle from the feedyard to the packing plant on prevalence levels of *Escherichia coli* O157 and *Salmonella* spp. J Food Prot. 2002; 65(2):4. https://doi.org/10.3389/fmicb.2015.00384 PMID: 25983727; PubMed Central PMCID: PMC4415432.

55. AT M., BJ M., Brichta-Harhay D, Guerini MN, Kalchayanand SD, Shackelford SD, et al. Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:H7 on hides and carcasses of beef cattle at processing. J Food Prot. 2007; 70(2):7.

56. Stanford K, Bryan M, Peters J, González LA, Stephens TP, Schwartzkopf-Genswein KS. Effects of long- or short-haul transportation of slaughter heifers and cattle liner microclimate on hide contamination with *Escherichia coli* O157. J Food Prot. 2011; 74(10):6. https://doi.org/10.3389/fmicb.2015.00384 PMID: 25983727; PubMed Central PMCID: PMC4415432.

57. Minihan D, O‘Mahony M, Whyte P, Collins JD. An Investigation on the effect of transport and lairage on the faecal shedding prevalence of *Escherichia coli* O157 in cattle. J Vet Med B. 2003; 50:5.

58. Minihan D, O‘Mahony M, Whyte P, Collins JD. An Investigation on the effect of transport and lairage on the faecal shedding prevalence of *Escherichia coli* O157 in cattle. J Vet Med B. 2003; 50:5.

59. Escherichia coli

60. Minihan D, O‘Mahony M, Whyte P, Collins JD. An Investigation on the effect of transport and lairage on the faecal shedding prevalence of *Escherichia coli* O157 in cattle. J Vet Med B. 2003; 50:5.

61. Minihan D, O‘Mahony M, Whyte P, Collins JD. An Investigation on the effect of transport and lairage on the faecal shedding prevalence of *Escherichia coli* O157 in cattle. J Vet Med B. 2003; 50:5.

62. Consortium ABC. www.abc-consorci.com.ar 2019 [cited 2019 15–01]. Available from: http://www.abc-consorci.com.ar.

63. Jaros P, Cookson AL, Reynolds A, Withers H, Clemens R, Brightwell G, et al. The effect of transportation and lairage on faecal shedding and carcass contamination with *Escherichia coli* O157 and O26 in very young calves in New Zealand. Epidemiol Infect. 2018; 146(9):12. https://doi.org/10.1017/S0950268818000973 PMID: 29789035.

64. Martínez de M. Calidad microbiológica de la carne bovina y cuantificación del riesgo en plantas de procesamiento. Buenos Aires, Argentina. BioMed Res Internat. 2019; 25(6):6. https://doi.org/10.1177/1082013219836326 PMID: 30862194.

65. Jaros P, Cookson AL, Reynolds A, Withers H, Clemens R, Brightwell G, et al. The effect of transportation and lairage on faecal shedding and carcass contamination with *Escherichia coli* O157 and O26 in very young calves in New Zealand. Epidemiol Infect. 2018; 146(9):12. https://doi.org/10.1017/S0950268818000973 PMID: 29789035.

66. Jaros P, Cookson AL, Reynolds A, Withers H, Clemens R, Brightwell G, et al. The effect of transportation and lairage on faecal shedding and carcass contamination with *Escherichia coli* O157 and O26 in very young calves in New Zealand. Epidemiol Infect. 2018; 146(9):12. https://doi.org/10.1017/S0950268818000973 PMID: 29789035.

67. Jaros P, Cookson AL, Reynolds A, Withers H, Clemens R, Brightwell G, et al. The effect of transportation and lairage on faecal shedding and carcass contamination with *Escherichia coli* O157 and O26 in very young calves in New Zealand. Epidemiol Infect. 2018; 146(9):12. https://doi.org/10.1017/S0950268818000973 PMID: 29789035.

68. Montville R, Schaffner DW. Inoculum size influences bacterial cross contamination between surfaces. Appl Environ Microbiol. 2003; 69(12):6. https://doi.org/10.1128/aem.69.12.7188-7193.2003 PMID: 11948658.

69. Barham RA, Barham BL, JohnsOn AK, Allen DM, Blanton JR, Miller MF. Effects of the transportation of beef cattle from the feedyard to the packing plant on prevalence levels of *Escherichia coli* O157 and *Salmonella* spp. J Food Prot. 2002; 65(2):4. https://doi.org/10.3389/fmicb.2015.00384 PMID: 25983727; PubMed Central PMCID: PMC4415432.

70. Montville R, Schaffner DW. Inoculum size influences bacterial cross contamination between surfaces. Appl Environ Microbiol. 2003; 69(12):6. https://doi.org/10.1128/aem.69.12.7188-7193.2003 PMID: 14660365; PubMed Central PMCID: PMC309958.

71. Barriol PA, Soto SA, Jaureguiberry MV, Gottardi G, Bascur I, Leotta GA, et al. Microbiological risk characterization in butcher shops from the province of Neuquen, Patagonia Argentina. J Food Saf. 2018; 38(5):1–10. https://doi.org/10.1111/jfs.12488
73. J. RM, Sanz M, Elchiribey L, Villalobo C, Kruger A, Coello R, et al. Carnicerías saludables: detección de *Escherichia coli* O157:H7 y no-O157:H7 en carne picada fresca y en instalaciones de comercios minoristas. XIII Congreso Argentino de Microbiología II Congreso de Microbiología Agrícola y Ambiental; Ciudad Autónoma de Buenos Aires, Argentina2013.

74. Evans EW, Redmond EC. Analysis of older adults’ domestic kitchen storage practices in the United Kingdom: identification of risk factors associated with listeriosis. J Food Prot. 2015; 78(4):9. https://doi.org/10.4315/0362-028X-JFP-14-527 PMID: 25836399.

75. James C, Onarinde BA, James SJ. The Use and Performance of Household Refrigerators: A Review. Compr Rev Food Sci F. 2017; 16(1):20. https://doi.org/10.1111/1541-4337.12242

76. Chen Y, Jackson KM, Chea FP, Schaffner DW. Quantification and variability analysis of bacterial cross-contamination rates in common food service tasks. J Food Prot. 2001; 64(1):9. https://doi.org/10.4315/0362-028x-64.1.72 PMID: 11198444

77. Anderson ME, Marshall RT, Dickson JS. Estimating depths of bacterial penetration into post-rigor carcass tissue during washing. J Food Saf. 1991; 12:191–8.

78. Sporing SB. *Escherichia coli* O157:H7 risk assessment for production and cooking of blade tenderized beef steaks. Manhattan, KS, USA: Kansas State University; 1996.

79. Stringer SC, George SM, Peck MW. Thermal inactivation of *Escherichia coli* O157:H7. Journal of Applied Microbiology Symposium Supplement 2000; 88:11.

80. Jackson TC, Hardin MD, Acuff GR. Heat resistance of *Escherichia coli* O157:H7 in a nutrient medium and in ground beef patties as influenced by storage and holding temperatures J Food Prot. 1996; 59:8.

81. Juneja VK, Snyder OP, Williams AC, Marmer BS. Thermal Destruction of *Escherichia coli* O157:H7 in Hamburger. J Food Prot. 1999; 60:4.

82. Ministerio de Salud PdlN. Encuesta Nacional de Nutrición y Salud 2004/5. Argentina2012. p. 54.

83. Ferens WA, Hovde CJ. *Escherichia coli* O157:H7: Animal Reservoir and Sources of Human Infection. Foodborne Pathog Dis. 2011; 8(4):24. https://doi.org/10.1089/fpd.2010.0673 PMID: 21171940

84. Vose D. Quantitative risk analysis: A guide to Monte Carlo simulation modelling. England: John Wiley & Sons, Ltd.; 1996.

85. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015; 4:9. https://doi.org/10.1186/2046-4053-4-9 PMID: 25588564

86. INDEC. Censo Nacional de Población, Hogares y Viviendas 2010 Censo del Bicentenario Resultados definitivos, Serie B N° 2. Ciudad Autónoma de Buenos Aires, Argentina 2012.

87. Mir RA, Weppelmann TA, Elzo M, Ahn S, Driver JD, Jeong KC. Colonization of Beef Cattle by Shiga Toxin-producing *Escherichia coli* during the First Year of Life: A Cohort Study. PLoS One. 2016; 11 (2):16. https://doi.org/10.1371/journal.pone.0148518 PMID: 26849041; PubMed Central PMCID: PMC4743843.

88. Bonardi S, Alpigiani I, Tozzoli R, Vismarra A, Zecca V, Greppi C, et al. Shiga toxin-producing *Escherichia coli* O157, O26 and O111 in cattle faeces and hides in Italy. Vet Rec Open. 2015; 2(1):9. https://doi.org/10.1136/vetreco-2014-000061 PMID: 26392887

89. Ferreira MRA, Stella AE, Freitas-Filho EG, Silva TS, Nascimento KA, Pinto JFN, et al. Distribution of the stx1 and stx2 genes in *Escherichia coli* isolated from milk cattle according to season, age, and production scale in southwestern region of Goiás, Brazil. Arq Bras Med Vet Zoot. 2018; 70(9):7.

90. Thiry D, De Rauw K, Takaki S, Duprez JN, Iguchi A, Pierard D, et al. Low prevalence of the ‘gang of seven’ and absence of the O80:H2 serotypes among Shigatoxigenic and enteropathogenic *Escherichia coli* (STEC and EPEC) in intestinal contents of healthy cattle at two slaughterhouses in Belgium in 2014. J Appl Microbiol. 2018; 124(3):7. https://doi.org/10.1111/jam.13677 PMID: 29280544.

91. Oporto B, Ocejo M, Alkorta M, Marimon JM, Montes M, Hurtado A. Zoonotic approach to Shiga toxin-producing *Escherichia coli*: integrated analysis of virulence and antimicrobial resistance in ruminants and humans. Epidemiol Infect. 2019; 147:e164:9. https://doi.org/10.1017/S0950268819000556 PMID: 31063106; PubMed Central PMCID: PMC6518511.

92. Geue L, Segura-Alvarez M, Connath FJ, Kuczius T, Bockemuhl J, Karoh H, et al. A long-term study on the prevalence of shiga toxin-producing *Escherichia coli* (STEC) on four German cattle farms. Epidemiol Infect. 2002; 129(1):13. https://doi.org/10.1017/S0950268802007288 PMID: 12211585; PubMed Central PMCID: PMC2869863.

93. Mellor GE, Fegan N, Duffy LL, Mc MK, Jordan D, Barlow RS. National survey of Shiga toxin-producing *Escherichia coli* serotypes O26, O45, O103, O111, O121, O145, and O157 in Australian beef cattle faeces. J Food Prot. 2016; 79(11):7. https://doi.org/10.4315/0362-028X-JFP-15-507 PMID: 28221921.
94. Lynch MJ, Fox EM, O’Connor L, Jordan K, Murphy M. Surveillance of verocytotoxigenic Escherichia coli in Irish bovine dairy herds. Zoonoses Public Health. 2012; 59(4):8. https://doi.org/10.1111/j.1863-2378.2011.01443.x PMID: 22128857.

95. Monaghan Á, Byrne B, Fanning S, Sweeney T, McDowell D, Bolton DJ. Serotypes and virulotypes of non-O157 shiga-toxin producing Escherichia coli (STEC) on bovine hides and carcasses. Food Microbiol. 2012; 32:7. https://doi.org/10.1016/j.fm.2012.06.002 PMID: 22986184.

96. Ahmed W, Gyawali P, Toze S. Quantitative PCR measurements of Escherichia coli including Shiga Toxin producing E. coli (STEC) in animal feces and environmental waters. Environ Sci Technol. 2015; 49(5):3084–90. https://doi.org/10.1021/es505477n PMID: 25648758.

97. Bibbal D, Loukiadis E, Kerouedan M, Ferre F, Dilasser F, Peytavin de Garam C, et al. Prevalence of carriage of Shiga toxin-producing Escherichia coli (STEC) serotypes O157:H7, O26:H11, O103:H2, O111:H8, and O145:H28 among slaughtered adult cattle in France. Appl Environ Microbiol. 2015; 81(4):9. https://doi.org/10.1128/AEM.03315-14 PMID: 25527532; PubMed Central PMCID: PMC4309698.

98. Wang LYR, Jokinen CC, Laing CR, Johnson RP, Ziebell K, Gannon VPJ. Multi-Year persistence of Verotoxigenic Escherichia coli (VTEC) in a closed Canadian beef herd: A cohort study. Front Microbiol. 2018; 9:24. https://doi.org/10.3389/fmicb.2018.00024 PMID: 29410658; PubMed Central PMCID: PMC6127291.

99. Rivelli Zea SM, Padola NL, Etcheverria AI, Florentin M, Acuna P, Rodriguez F, et al. Molecular characterization of Shiga toxin producing Escherichia coli isolated from 2 livestock establishments of Paraguay. Rev Argent Microbiol. 2018; 5. https://doi.org/10.1016/j.jram.2018.07.001 PMID: 31635897.

100. Dewsbury DM, Renter DG, Shridhar PB, Noll LW, Shi X, Nagaraja TG, et al. Summer and winter prevalence of Shiga toxin-producing Escherichia coli (STEC) O26, O45, O103, O111, O121, O145, and O157 in feces of feedlot cattle. Foodborne Pathog Dis. 2015; 12(8):7. https://doi.org/10.1089/fpd.2015.1987 PMID: 26075548.

101. Stanford K, Johnson RP, Alexander TW, McAllister TA, Reuter T. Influence of season and feedlot location on prevalence and virulence factors of seven serogroups of Escherichia coli in Feces of western-Canadian slaughter cattle. PLoS One. 2016; 11(8):18. https://doi.org/10.1371/journal.pone.0159866 PMID: 27482711; PubMed Central PMCID: PMC4970572.

102. Arthur TM, Bosilevac JM, Brichta-Harhay DM, Kalchayanad N, Shackelford SD, Wheeler TL, et al. Effects of a minimal hide wash cabinet on the levels and prevalence of Escherichia coli O157:H7 and Salmonella on the hides of beef cattle at slaughter. J Food Prot. 2007; 70:4. https://doi.org/10.4315/JF-0362-026x-70.5.1076 PMID: 17536663.

103. Bach SJ, McAllister TA, Meard GJ, Schwartzkopf-Genswein KS. Long-Haul transport and lack of preconditioning increases fecal shedding of Escherichia coli and O157:H7 by calves. J Food Prot. 2004; 67(4):6.

104. Mather AE, Reid SW, McEwen SA, Ternent HE, Reid-Smith RJ, Boerlin P, et al. Factors associated with cross-contamination of hides of Scottish cattle by Escherichia coli O157. Appl Environ Microbiol. 2008; 74(20):8. https://doi.org/10.1128/AEM.00770-08 PMID: 18723662; PubMed Central PMCID: PMC2570309.

105. Cobbold RN, Davis MA, Rice DH, Szymanski M, Tarr PI, Hancock DD. Associations between bovine, human, and raw milk, and beef isolates of non-O157 Shiga toxigenic Escherichia coli within a restricted geographic area of the United States. J Food Prot. 2008; 71(5):5.

106. Stromberg ZR, Baumann NW, Lewis GL, Sevart NJ, Cernichiaro N, Renter DG, et al. Prevalence of Enterohemorrhagic Escherichia coli O26, O45, O103, O111, O121, O145, and O157 on Hides and Preintervention Carcass Surfaces of Feedlot Cattle at Harvest. Foodborne Pathog Dis. 2015; 12(7):8. https://doi.org/10.1089/fpd.2015.1945 PMID: 26125496.

107. Bohaychu VM, Gensler GE, Romero Barrios P. Microbiological baseline study of beef and pork carcasses from provincially inspected abattoirs in Alberta, Canada. Can Vet J. 2011; 52(6). PMID: 22467964.

108. Bosilevac JM, Guerini MN, Brichta-Harhay DM, Arthur TM, Koohmaraie M. Microbiological characterization of imported and domestic boneless beef trim used for ground beef. J Food Prot. 2007; 70(2):10. https://doi.org/10.4315/JF-0362-026x-70.2.440 PMID: 17340881.

109. Baeza Quiroz CB. Aislamiento y caracterización de cepas de Escherichia coli productor de toxina Shiga desde carne de vacuno nacional e importada, distribuida en los principales supermercados de la provincia de Santiago. Chile: Escuela de Salud Pública. Universidad Mayor; 2013.

110. Jones TH, Nattress FM, Dilts B, Olsen D, Muelhauser V. Numbers of coliforms, Escherichia coli, F-RNA phage, rotavirus, bovine enteric calcivirus and presence of non-O157 STEC on commercial vacuum packaged beef. Food Microbiol. 2014; 42:7. https://doi.org/10.1016/j.fm.2014.04.001 PMID: 24929741.
Varcasia BM, Tomassetti F, De Santis L, Di Giamberardino F, Lovari S, Bilei S, et al. Presence of Shiga toxin-producing Escherichia coli (STEC) in fresh beef marketed in 13 regions of ITALY (2017). Microorganisms. 2018; 6(12):12. https://doi.org/10.3390/microorganisms6040126 PMID: 30563244; PubMed Central PMCID: PMC631577.

EFSA ECDC. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J. 2018; 16(12):262. https://doi.org/10.2903/j.efsa.2018.5500 PMID: 32625785

Barlow RS, Gobius KS, Desmarchelier PM. Shiga toxin-producing Escherichia coli in ground beef and lamb cuts: results of a one-year study. Int J Food Microbiol. 2006; 111(1):5. https://doi.org/10.1016/j.ijfoodmicro.2006.04.039 PMID: 16793157.

Bosilevac JM, Koohmaraie M. Prevalence and characterization of non-O157 shiga toxin-producing Escherichia coli isolates from commercial ground beef in the United States. Appl Environ Microbiol. 2011; 77(6):10. https://doi.org/10.1128/AEM.02833-10 PMID: 21257806; PubMed Central PMCID: PMC3067332.

Ju W, Shen J, Li Y, Toro MA, Zhao S, Ayers S, et al. Non-O157 Shiga toxin-producing Escherichia coli in retail ground beef and pork in the Washington D.C. area. Food Microbiol. 2012; 32(2):7. https://doi.org/10.1016/j.fm.2012.07.017 PMID: 22986203.

Nobili G, Franconieri I, La Bella G, Basanisi MG, La Salandra G. Prevalence of Verocytotoxigenic Escherichia coli strains isolated from raw beef in southern Italy. Int J Food Microbiol. 2017; 257:5. https://doi.org/10.1016/j.ijfoodmicro.2017.06.022 PMID: 28672173.

Toro M, Rivera D, Jimenez MF, Diaz L, Navarrete P, Reyes-Jara A. Isolation and characterization of non-O157 Shiga toxin-producing Escherichia coli (STEC) isolated from retail ground beef in Santiago, Chile. Food Microbiol. 2017; 75:8. https://doi.org/10.1016/j.fm.2017.07.015 PMID: 30596963.

Wasilenko JL, Fratamico PM, Sommers C, DeMarco DR, Varkey S, Rhoden K, et al. Detection of Shiga toxin-producing Escherichia coli (STEC) O157:H7, O26, O45, O103, O111, O121, and O145, and Salmonella in retail raw ground beef using the DuPont BAX(R) system. Front Cell Infect Microbiol. 2014; 4(81):7. https://doi.org/10.3389/fcimb.2014.00081 PMID: 24995164; PubMed Central PMCID: PMC4061970.

Mora A, Blanco M, Blanco JE, Dahbi G, Lopez C, Justel P, et al. Serotypes, virulence genes and intimin types of Shiga toxin (verocytotoxin)-producing Escherichia coli isolates from minced beef in Lugo (Spain) from 1995 through 2003. BMC Microbiol. 2007; 7:9. https://doi.org/10.1186/1471-2180-7-9 PMID: 17263893; PubMed Central PMCID: PMC1810539.

Rodolpho D, Marín JM. Isolation of Shiga toxinogenic Escherichia coli from butcheries in Taquaritinga city, State of São Paulo, Brazil. Braz J Microbiol. 2007; 38:4. https://doi.org/10.1590/s1517-83822007000400004

Hill WE, Suhalim R, Richter HC, Smith CR, Buschow AW, Samadpour M. Polymerase chain reaction screening for Salmonella and enterohemorrhagic Escherichia coli on beef products in processing establishments. Foodborne Pathog Dis. 2011; 8(9):10. https://doi.org/10.1089/fpd.2010.0825 PMID: 21561381.

FSAI. Microbiological safety of raw minced beef and beef burgers on retail sale in Ireland (11NS1). MicroBiology ed2013.

Nauta MJ, Evers EG, Takumi K, Havelaar AH. Risk assessment of Shiga-toxin producing Escherichia coli O157 in steak tartare in the Netherlands. In: Environment NIfPHat, editor. The netherlands: RIVM; 2001. p. 169.

USDA, Assessment FPR, Academies IoMoTN. Escherichia coli O157:H7 in ground beef: Review of a draft risk assessment. In: Board BoHPaDPFa N, editor. Washington, D.C.: The National Academies Press; 2002.

EFSA-BIOHAZ. Scientific Opinion on the public health risks related to the maintenance of the cold chain during storage and transport of meat. Part 2 (minced meat from all species ). EFSA J. 2014; 12 (7):30. https://doi.org/10.2903/j.efsa.2014.3783

FSAI. An investigation of the most appropriate z-value to be used in calculating ‘equivalent cooks’ for beef burgers in food business establishments. Dublin, Ireland2018. p. 36.

Rassvoll E, Sorheim O, Heir E, Moreto T, Olsen NV, Langsrud S. Consumer preferences, internal color and reduction of shigatoxigenic Escherichia coli in cooked hamburgers. Meat Sci. 2014; 96:9. https://doi.org/10.1016/j.meatsci.2013.09.009 PMID: 24200560.

Launders N, Byrne L, Adams N, Glen K, Jenkins C, Tubin-Delich D, et al. Outbreak of Shiga toxin-producing E. coli O157 associated with consumption of watercress, United Kingdom, August to September 2013. Euro Surveill. 2013; 18(44):5. https://doi.org/10.2807/1560-7917.es2013.18.44.20624 PMID: 24183803.
