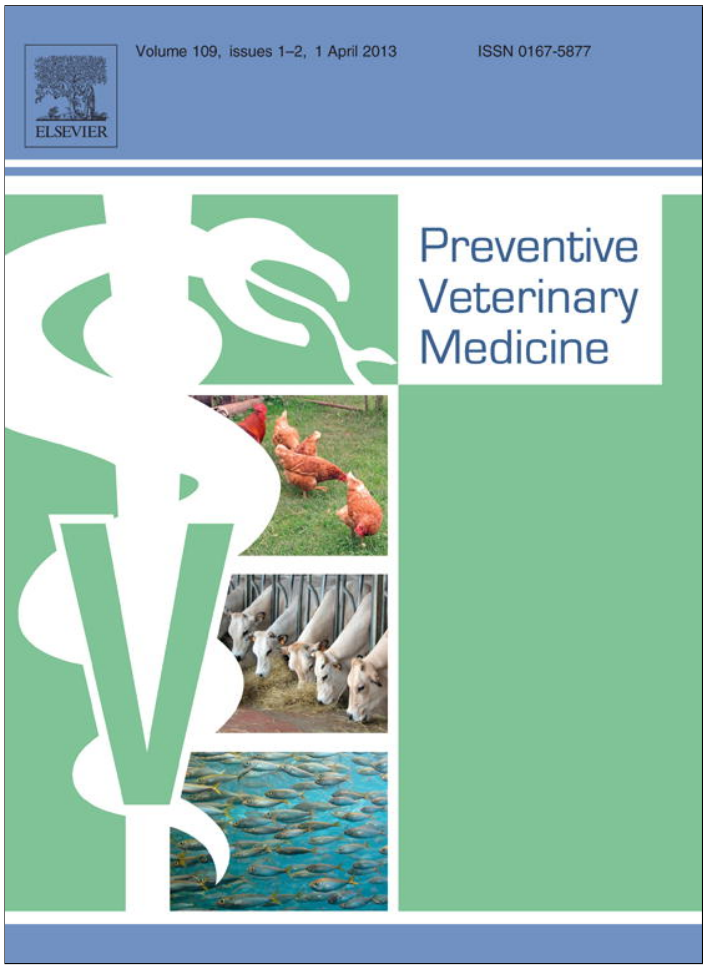

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Quantitative risk assessment of human campylobacteriosis by consumption of salad cross-contaminated with thermophilic *Campylobacter* spp. from broiler meat in Argentina

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ABSTRACT

Here, we developed a quantitative risk assessment for thermophilic *Campylobacter* spp. related to the consumption of salad prepared alongside broiler meat. The assessment considered initial contamination levels, cross-contamination and decontamination events during the broiler slaughter process and distribution, and storage and consumption patterns in Argentina and other Latin American countries. The model predicted an infection risk of 3.32×10^{-4} per serving. This estimation was variable according to the dose–response model used. Considering the number of chickens slaughtered annually in Argentina, the estimated number of people who could suffer campylobacteriosis related to poultry meat consumption was, on average, 484,304. The risk of human campylobacteriosis was most sensitive to the probability of infection from a *Campylobacter* ($r = 0.72$), the number of *Campylobacter* spp. per serving ($r = 0.40$), the frequency of washing the cutting board ($r = -0.31$), the preparation of raw poultry before salad using the same cutting board ($r = 0.14$), and the frequency of hand washing ($r = -0.14$). The most sensitive stages of the process identified through the risk assessment can be used as a basis for measures of risk management. Public campaigns on hygiene habits during food preparation at home should focus on the importance of washing the cutting board before preparing raw and ready-to-eat foods and of washing the hands during food preparation.

1. Introduction

Thermophilic *Campylobacter* spp., specifically *Campylobacter jejuni* and *Campylobacter coli*, are a leading cause of zoonotic enteric infection in most developed and developing nations (CDC, 2010; ECDC, 2010). Thermophilic *Campylobacter* spp. are frequently found in broiler chickens and broiler chicken meat (FAO-WHO, 2009).

Humans can thus be exposed to *Campylobacter* when consuming improperly heated broiler meat. However, the most important route of infection is related to the consumption of foods which are cross-contaminated with *Campylobacter* during food preparation of a meal with broiler meat (Nauta et al., 2006).

In developing countries, information on food-borne disease is scant due to the inadequate data provided by

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the passive surveillance systems. Additionally, outbreak information is frequently unsubstantial because health authorities lack the capabilities or resources for detection of diarrheal diseases (Zaidi et al., 2008). Recent studies (Fuentes, 2010) have shown that thermophilic *Campylobacter* spp. are the most important gastrointestinal pathogens in humans in Argentina. Their incidence rate (22.4% in children <3 years and 13.6% in adults) is higher than that of other common pathogens such as *Salmonella* spp., *Shigella* spp. and *Escherichia coli*. However, there are no epidemiologic studies in Argentina conducted with the aim to assess the prevalence of *Campylobacter* in the different stages of the food chain and their genotypic relationship. This information is essential to establish a public health program to control the disease.

Risk analysis is now widely accepted as the preferred means to assess possible links between hazards in the food chain and actual risks to human health (FAO-WHO, 2006). The quantitative risk assessments of acquiring thermophilic *Campylobacter* spp. associated with broiler meat consumption published thus far (Brynestad et al., 2008; Calistri and Giovannini, 2008; Fazil et al., 1999; Hartnett et al., 2001; Lake et al., 2007; Lindqvist and Lindblad, 2008; Nauta et al., 2006; Rosenquist et al., 2003; Uyttendaele et al., 2006) have been conducted for developed countries. There are not models for developing countries that considered their storage conditions, or their distribution and consumption patterns. Thus, they have not been useful to the risk managers in developing countries for the identification or implementation of appropriate measures to control the risk of acquiring campylobacteriosis.

The objective of this study was to develop a quantitative risk assessment for thermophilic *Campylobacter* spp. related to the consumption of salad cross-contaminated from broiler meat (whole carcasses) in Argentina. This risk assessment provides information to the Argentinean risk managers to define strategies to reduce the risk of human campylobacteriosis.

2. Materials and methods

2.1. Model development

The prevalence and counts of thermophilic *Campylobacter* spp. were modeled at various stages along the broiler food chain. The conceptual model upon which the mathematical model was based is depicted in Fig. 1. The model was created in Microsoft Excel 2007 with the add-on package @Risk (version 4.0, Palisade Corporation, New York, USA). The main output of the model was the probability of food-borne gastrointestinal disease following consumption of salad prepared alongside broiler meat contaminated with thermophilic *Campylobacter* spp. This probability was estimated through 5000 iterations with Latin hypercube sampling. The number of iterations provided adequate convergence of the simulation statistics (<1%).

Essentially, the model was developed using inputs derived from data obtained directly by our group in Argentina (prevalence and concentration of thermophilic *Campylobacter* spp. at slaughterhouses and retail, and consumer food handling activities). With the aim to obtain

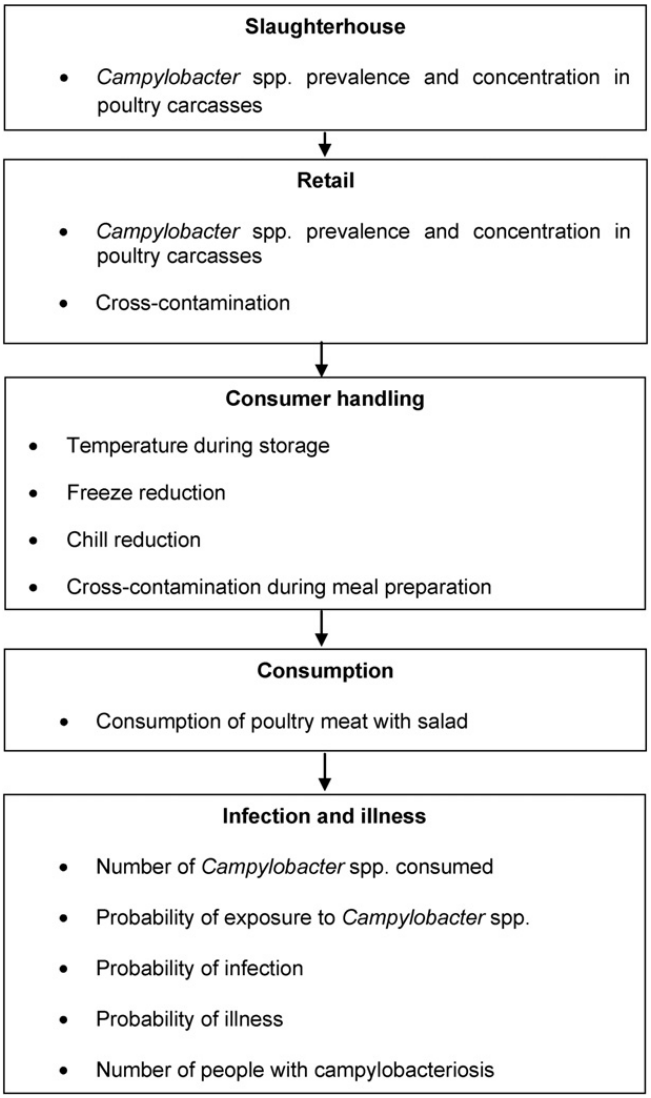


Fig. 1. Flow diagram of the mathematical model of exposure assessment and the dose–response model for *Campylobacter* spp. in poultry meat.

information about consumer food handling activities (*e.g.* consumer preferences, food storage, food handling and consumption of poultry meat), a survey was performed by means of personal interviews using a structured questionnaire in homes of Esperanza city (Argentina) ($N = 83$) during 2011 (Astesana et al., 2011). However, where Argentinean-specific data were not available (*e.g.* dose–response model), international data and scientific literature were consulted to improve the basis for the model.

2.2. Exposure assessment

The potential exposure to thermophilic *Campylobacter* spp. in a single-meal serving was estimated to assess the risk to human health associated with the consumption of salad prepared alongside broiler meat (whole carcasses). Consumers in Argentina eat chicken meat principally as whole carcass preparations, and only 13% of the chicken meat is consumed into smaller pieces (MinAgri, 2011a). The exposure model covers the food chain starting from the chilled carcasses at the end of the slaughter

process to their consumption in the home. The model was restricted to the exposure to salads cross-contaminated during food preparation (in the domestic environment) with raw chicken meat, either fresh or frozen. The exposure model was divided into three modules: slaughter and processing, retail and preparation, and consumption. Each module yielded one or more output distributions that served either as inputs to the next module or as final outputs of the estimation of the probability and number of viable organisms present in a single-meal serving at the time of consumption.

During 2010, Argentina produced 1,598,000 tons of poultry meat, 217,000 of which were exported. On the other hand 18,000 tons of poultry meat were imported. Considering these data from the total amount of chicken meat consumed in Argentina, the imported meat represents only 1.3%, and, for that reason, the present risk assessment model considered only the domestic origin (MinAgri, 2011b).

The level of detail of each module depended on the relevance of the process and the availability of data to model the stage.

2.2.1. Slaughter and processing

The prevalence and concentration of thermophilic *Campylobacter* spp. were analyzed in samples taken from broiler carcasses in the slaughterhouse (Zbrun, unpublished results). Slaughterhouse is automatic, with national habilitation and federal traffic, which slaughter approximately 33,000 animals per day (3300 chickens/h). The chickens sampled were in the same batch (defined as a group of chickens from the same flock delivered at the same time to the same slaughterhouse and sold together in the same retail store) trying to mimic the process in the reality. Three flocks were sampled and ten carcasses from each flock were taken from the processing line after chilling, using a clean pair of latex gloves and put into a sterile bag with 200 ml of Ringer 1/4 solution. Carcasses were rinsed by hand-shaking for 60 s in each of two directions to ensure that the solution came into contact with all surfaces and the solution was recovered and transported to the lab in sterile plastic tubes. *Campylobacter* was recovered using the selective media Bolton broth (Oxoid, UK) and Preston agar (Oxoid, UK) (Bolton and Coates, 1983). The Ringer 1/4 solution recovered from the rinsed broiler carcass was centrifuged at 5000 rpm for 5 min, and the pellet was resuspended in 2 ml of the same solution, which was inoculated on Bolton broth and incubated for 24 h at 42 °C in gas jars with a microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2; INDURA^{TM}). About 20 μl (two loopfuls) of Bolton broth was streaked on Preston agar, which was incubated for 48 h at 42 °C in gas jars with a microaerophilic conditions. Identification of *Campylobacter* was based on colony morphology, microscopic appearance and the following phenotypic characteristics: production of oxidase and catalase, hippurate hydrolysis reaction, DNase test and SH_2 production (Lior, 1984). The positive colonies were streaked on Columbia blood agar (Oxoid, UK) and one colony from each positive sample was stored in glycerol broth (15% glycerol and 85% serum broth) at −80 °C (Terzolo et al., 1987) for further molecular typing.

The prevalence of thermophilic *Campylobacter* spp. (P_s) was 33.33% (10 positive samples out of 30 total samples taken) after chilling of the carcasses in the slaughterhouse (Zbrun, unpublished results). The experimental data were used to estimate the theoretical frequency distribution of contamination, assuming that the prevalence could be characterized with a Beta distribution (Vose, 1996) (Table 1).

2.2.2. Retail

The prevalence of thermophilic *Campylobacter* spp. was analyzed in samples taken from broiler carcasses at retail. The chickens sampled were in the same batch. The same sampling procedure and sample analysis described for broiler carcasses in the slaughterhouses were followed. The prevalence of thermophilic *Campylobacter* spp. at retail (P_r) was 83.33% (25 positive samples out of 30 total samples taken) (Zbrun, unpublished results) and was modeled using a Beta distribution.

To quantify the number of thermophilic *Campylobacter* spp. in chickens ($n = 40$), we conducted the most probable number (MPN) technique described in the *Bacteriological Analytical Manual* (FDA, 1998) using three dilution series (Scherer et al., 2006). Aliquots of 1 ml from each rinse fluid dilution (10^{-1} until 10^{-4}) were brought into three MPN-tubes containing 9 ml Bolton broth. The MPN-tubes were incubated in gas jars with a microaerophilic conditions at 42 °C for 24 h. Then, 10 μl from each tube was streaked onto Preston agar and, after an incubation time of 48 h at 42 °C in gas jars with a microaerophilic conditions, plates were screened for presumptive colonies. After biochemical confirmation (oxidase and catalase), the number of positive tubes at each dilution was determined and the thermophilic *Campylobacter* spp. counts present in the rinse fluid were computed from the statistical MPN table of de Man (1983). Of the 40 samples, 13 (32.5%) had from 1 to 36 organisms/carcass, 6 from 37 to 200 MPN/carcass, 7 from 201 to 500 MPN/carcass, 2 from 501 to 1000 MPN/carcass, 6 from 1001 to 5000 MPN/carcass, 5 from 5001 to 10,000 MPN/carcass and 1 sample from 10,001 to 18,000 MPN/carcass. The level of thermophilic *Campylobacter* spp. on carcasses in retail (C_r) was described, taking into account the data, by a Cumulative distribution of the logarithms of the MPN per carcass (Table 1).

Prevalence and concentration of *Campylobacter* spp. on carcasses in slaughterhouse and retail were modeled based on a limited number of flocks, so it is assumed the presence of uncertainty in these variables.

The cross-contamination from carcasses surfaces during the storage and transportation from slaughterhouses to retail is a process which is considered unavoidable. Information presented previously was used to model the cross-contamination factor (F_{cc}) combining the two Beta distributions for *Campylobacter* prevalence in carcasses at slaughterhouses (P_s) and in carcasses at retail (P_r) (Cummins et al., 2008) (Table 1). The probability of a carcass being contaminated (P_c) is thus given by the equation:

$$P_c = \frac{F_{cc} \times P_s}{1 - P_s + F_{cc} \times P_s}$$

Table 1
Model input parameters.

Variable	Description	Units	Distribution/Model
P_s	Prevalence of *Campylobacter* spp. in poultry carcasses at slaughterhouses		Beta(10+1, 30-10+1) Fitted from Zbrun (unpublished results)
P_r	Prevalence of *Campylobacter* spp. in poultry carcasses at retail		Beta(25+1, 30-25+1) based on Zbrun (unpublished results)
C_r	Number of *Campylobacter* spp. in poultry meat at retail	Log MPN/carcass	Cumulative(−2.079, 4.255 {1.556; 2.301; 2.699; 3; 3.699; 4}, {0.325; 0.475; 0.65; 0.7; 0.85; 0.975}) based on Zbrun (unpublished results)
F_{cc}	Cross-contamination factor		P_r/P_s (Cummins et al., 2008)
P_c	Probability of infected carcass		$F_{cc} \times P_s/(1 - P_s + F_{cc} \times P_s)$ (Cummins et al., 2008)
S_c	Storage condition		Binomial(1;(Uniform(0,1))) Uncertainty distribution Refrigeration = 1 Freeze = 0
R_r	Refrigeration reduction	$\log_{10}$	R_{rmin}: Normal(0.31;0.13) R_{rmax}: Normal(0.81;0.24) R_r: Uniform(R_{rmin}; R_{rmax}) (Bhaduri and Cottrell, 2004)
R_f	Freeze reduction	$\log_{10}$	Uniform(0.6;2.87) Georgsson et al. (2006)
C_r	Number of *Campylobacter* spp. in poultry carcasses stored under refrigeration	MPN/carcass	$10^{(C_r - R_r)}$
C_f	Number of *Campylobacter* spp. in poultry carcasses stored under freeze	MPN/carcass	$10^{(C_r - R_f)}$
N_s	Number of servings per poultry carcass		4
C_{cs}	Number of *Campylobacter* spp. per serving	MPN	Poisson(C_r/N_s) or Poisson(C_f/N_s)
Cross-contamination module according with Chen et al. (2001), Luber et al. (2006), Montville and Schaffner (2003), and Signorini and Frizzo (2009)			
$P_{s\text{-}p}$	Probability of eating salad with poultry		Binomial(1;(Beta(63+1;83-63+1))) Astesana et al. (2011)
$P_{p\text{-}s}$	Probability of preparing poultry before salad		Binomial(1;(Beta(55+1;63-55+1))) Astesana et al. (2011)
P_{wh}	Probability of washing hands		Binomial(1;(Beta(1;1))) Uncertainty distribution
R_{wh}	Reduction by washing hands	%	RiskExpon(3.2396, RiskShift(−0.10699), RiskTruncate(0.107, 100))
P_{wcb}	Probability of washing the cutting board		Binomial(1;(Beta(27+1; 63-27+1))) Astesana et al. (2011)
T_{ph}	*Campylobacter* spp. transfer from poultry to hands	%	Lognormal(7.7933; 7.864; Shift(0.091172)
C_{nwh}	Number of *Campylobacter* spp. in unwashed hands	MPN	$(C_{cs} \times T_{ph})/100$
C_{wh}	Number of *Campylobacter* spp. in washed hands	MPN	$(C_{nwh} \times R_{wh})/100$
T_{hf}	*Campylobacter* spp. transfer from hands to faucet	%	Lognormal (0.67538; 3.3581; Shift (0.0014066)
C_t	Number of *Campylobacter* spp. in faucet	MPN	$(C_{nwh} \times T_{ht})/100$
T_{th}	*Campylobacter* spp. transfer from faucet to hands	%	Exponential (5.1095; Shift (0.04793)
C_{twh}	Number of *Campylobacter* spp. in washed hands	MPN	$((T_{th} \times C_t)/100) + C_{nwh}$
T_{hs}	*Campylobacter* spp. transfer from hands to salad	%	Lognormal (5.2679; 5.823; Shift (−0.37878)
C_{sal1}	Number of *Campylobacter* spp. in salad	cfu	In washed hands = $(C_{twh} \times T_{hs})/100$ In unwashed hands = $(C_{nwh} \times T_{hs})/100$
$T_{p\text{-}cb}$	*Campylobacter* spp. transfer from poultry to cutting board	%	Lognormal (12.7; 7.0087; Shift (0.54332)
C_{cb}	Number of *Campylobacter* spp. in cutting board	MPN	$(C_{cp} \times T_{p\text{-}cb})/100$
$T_{cb\text{-}s}$	*Campylobacter* spp. transfer from cutting board to salad	%	Loglogistic (−0.42502; 11.002; 3.8223)
C_{sal2}	Number of *Campylobater* spp. in salad	MPN	$(C_{cb} \times T_{cb\text{-}s})/100$
C_{sal}	Total number of *Campylobacter* spp. in salad	MPN	Poisson ($C_{sal1} + C_{sal2}$)

Contamination (prevalence and concentration) in the slaughterhouse and cross-contamination during the storage and transportation from slaughterhouses to retail was modelled because in a next stage this model will be used to evaluate the effect of slaughter-level interventions to reduce cross-contamination (scenario analysis).

2.2.3. Preparation and consumption

This module describes the transfer and survival of thermophilic *Campylobacter* spp. from the purchase of the chicken meat product at the retail to the exposure due to the consumption of a meal containing chicken meat.

We assumed that thermophilic *Campylobacter* spp. do not grow below 30 °C (ICMSF, 1996), and that during storage of chicken meat (and occasional temperature abuse) no growth is expected. The initial levels of *Campylobacter* on the carcass change during storage depending on whether chickens are stored refrigerated or frozen. Although microbial growth is arrested during refrigeration or freezing storage, a proportion of *Campylobacter* may survive (Bhaduri and Cottrell, 2004). In Argentina, data about storage temperature for chicken meat applied by consumers are very scarce. To consider the uncertainty about the storage condition (S_c), we considered that approximately 50% of the production is sold at the retail and stored at home as frozen and that 50% is sold as fresh chicken. S_c was modeled using a Binomial distribution (Table 1).

Studies on the inactivation of *Campyobacter* spp. in chicken stored at refrigeration temperatures have yielded varying results. According to Bhaduri and Cottrell (2004),

Table 2
Dose–response assessment.

Variable	Description	Distribution/Model
Beta-Poisson Model according to FAO/WHO (2002)		
$P_{inf(1)}$	Probability of infection from one *Campylobacter*	Beta(0.21; 59.95)
P_{inf}	Probability of infection/serving of chicken + salad	$1-(1-P_{inf(1)})C_{sal}$
$P_{ill\|inf}$	Conditional probability of illness	Beta(30,61) Black et al. (1988)
P_{ill}	Probability of illness	$P_{inf} \times P_{ill\|inf}$
N_{pc}	Number of poultry carcasses produced in Argentina	495,143,444
N_{ill}	Number of human cases/year	Binomial($(N_{pc} \times N_s \times N_{s\text{-}p})$; P_{ill})

refrigerated storage alone results in small decreases in viable counts after 7 days. The minimum (R_{rmin}) and maximum (R_{rmax}) reduction in viable counts of *Campylobacter* spp. were 0.31 (±0.13) log cfu/g and 0.81 (±0.24) log cfu/g, respectively after 7 days under refrigeration. Therefore, the effect of this assumption was investigated by also running the model under the assumption that inactivation during refrigeration (R_r) is variable and described by a Uniform distribution with uncertain minimum and maximum values (using Normal distributions according to the results reported by Bhaduri and Cottrell, 2004) (Table 1).

The inactivation of *Campylobacter* spp. in chicken during freezing also shows variable inactivation rates. According to that reported by Georgsson et al. (2006), the levels of *Campylobacter* on broiler carcasses are reduced by log mean values ranging from 0.6 to 2.87 after 31 days of freezing storage. The process (R_f) was considered variable and modeled following a Uniform distribution (Table 1).

Studies have shown that the main factors responsible for outbreaks of food poisoning are inappropriate storage, inadequate cooking or reheating, and, especially, cross-contamination (Uyttendaele et al., 2006). Considering that *Campylobacter* is a heat-sensitive microorganism, proper heat treatment of chicken meat preparation eliminates all *Campylobacter* organisms. The model used in the present study assumed that no *Campylobacter* spp. would survive the cooking of the chicken.

For consumer preparation this quantitative risk assessment developed a model for cross-contamination during food preparation based on Signorini and Frizzo (2009). It describes cross-contamination from raw poultry meat to hands, cutting board, faucet and salad considering the data presented by Chen et al. (2001), Luber et al. (2006), and Montville and Schaffner (2003). The model included transfer rates of thermophilic *Campylobacter* spp. from (1) raw chicken meat to the kitchen equipment (cutting board ($T_{p\text{-}cb}$) and faucet ($T_{h\text{-}f}$)) or to hands (T_{ph}) and (2) from the kitchen equipment ($T_{cb\text{-}s}$) or hands (T_{hs}) (washed or unwashed hands) to salad which was considered the only relevant route. These transfer rates (which express the probability for each *Campylobacter* to be transferred) and washing effects were incorporated in the model. The number of ingested thermophilic *Campylobacter* spp. depends on the frequency of chicken consumption (handling of raw chicken) when the conditions for cross-contamination were present (frequency of eating salad together with a poultry meal ($P_{s\text{-}p}$)). Then, $P_{s\text{-}p}$, the probability of washing hands (P_{wh}), the probability of preparing poultry before salad (using the same cutting board) ($P_{p\text{-}s}$), and the probability of washing the cutting board before salad preparation (P_{wcb}) were modeled considering data from an Argentinean survey (Astesana et al., 2011). Since there were no data to model the probability that people wash their hands (P_{wh}) during meal preparation, this variable was modeled considering the maximum uncertainty (~Uniform(0,1)) (Table 1).

The preparation and consumption of chicken or chicken/salad were linked to the family composition. In this quantitative risk assessment, we considered that a broiler was consumed by four people (N_s) (Table 1). The number of *Campylobacter* spp. per serving of salad (C_{salad}) was modeled using a Poisson distribution.

2.3. Dose–response

The dose–response is the relation between the dose of thermophilic *Campylobacter* spp. ingested and the probability of consequent human campylobacteriosis (Nauta et al., 2009). Since only limited data are available on the infective dose of thermophilic *Campylobacter* spp., in this model the Beta Poisson Model developed by the Joint FAO/WHO activities on Risk Assessment of Microbiological hazards in food (FAO/WHO, 2002), was applied. A Beta-Poisson model was fit to the data reported by Black et al. (1988) for two *C. jejuni* strains. The form of the Beta-Poisson model was adjusted to estimate the probability of infection ($P_{inf(1)}$) for an individual consuming a meal with a specific dose (C_{salad}). The uncertainty in the $P_{inf(1)}$ parameter was considered applying a Beta distribution with parameters $\alpha = 0.21$ and $\beta = 59.95$ (Table 2):

$$P_{\text{inf}} = 1 - (1 - P_{\text{inf}(1)})C_{salad}$$

The conditional probability of illness (P_{ill}) given infection was considered independent. The uncertainty on the probability of illness ($P_{ill|inf}$) was described by a Beta distribution (with parameters $\alpha = 30$ and $\beta = 61$), based on the fact that 29 out of 89 individuals that were infected became ill, according with the data reported by Black et al. (1988) (Table 2).

$$P_{ill} = P_{\text{inf}} \times P_{ill|\text{inf}}$$

2.4. Risk characterization

Risk characterization is the estimation of the probability of occurrence and severity of adverse health effects in a given population based on dose–response and exposure

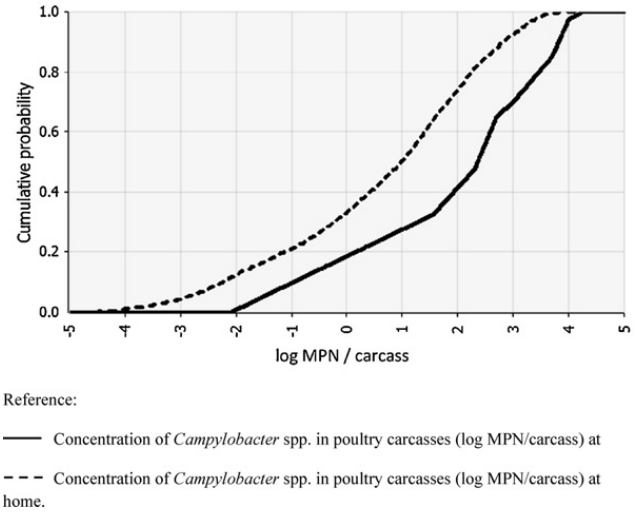


Fig. 2. Predicted *Campylobacter* spp. concentration (log MPN) in carcass at retail and at home.

assessment. The number of thermophilic *Campylobacter* spp. in a meal with chicken meat was estimated using the predictions of the exposure assessment, and was the input for the dose–response model. Thus, the probability of illness was the product of the probability of non-zero exposure and the probability of illness from the output of the dose–response model. Additionally, the number of human cases of campylobacteriosis per year due to poultry meat consumption (N_{ill}) was estimated considering the number of poultry carcasses produced in Argentina (N_{pc}) (MinAgri, 2011b), the number of servings (N_s), the proportion of meals with salad ($P_{s\text{-}p}$), and the probability of illness (P_{ill}).

2.5. Sensitivity analysis

To determine the impact of each input variable and the uncertain variables on the output variable (probability of illness), a sensitivity analysis was conducted, using Pearson's correlation coefficient to determine the degree of association. The sensitivity analysis was performed using the @Risk® version 4.5 software (Palisade, New York).

3. Results

The amount of *Campylobacter* spp. to which a consumer was exposed in a single serving of poultry meat with salad was a function of the original number of *Campylobacter* spp. in the poultry carcass at retail and the subsequent effects of storage and handling (Fig. 2). In poultry carcasses at retail, the average concentration of the pathogen per carcass was 1.03 log MPN (95%CI −1.58 to 3.95 log MPN). The number of bacteria was reduced during distribution and home storage, reaching a microbial concentration of −0.21 log MPN/carcass (95%IC −2.89 to 3.18 log MPN/carcass) prior to cooking. Pathogen doses ingested from salad due to cross-contamination during the home preparation of the meal was, in most of the cases, 1 or 2 MPN/serving of salad. Less than 1.5% of the exposure are to doses > 10 MPN/salad serving. The model predicted the prevalence of salad contaminated with the pathogen to be 32.9%.

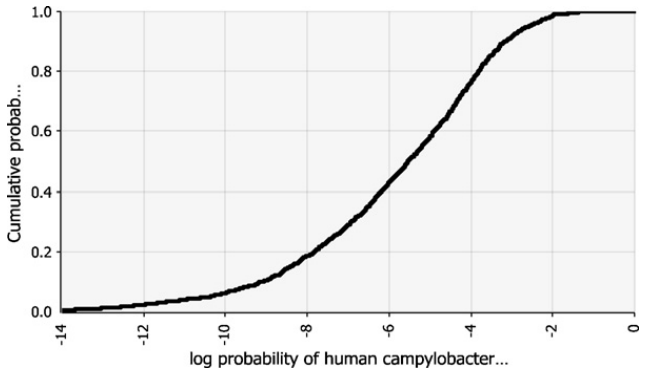


Fig. 3. Cumulative probability distribution for the risk of human campylobacteriosis due to chicken meat consumption.

Each iteration predicted the probability of illness for a single meal of chicken with salad (Fig. 3). The average probability of campylobacteriosis using the Beta-Poisson model according with FAO/WHO (2002) was 3.32×10^{-4} (95%CI 1.69×10^{-12} to 8.31×10^{-3}). The log probability of illness was chosen as a convenient representation for the probability of risk, because it was so concentrated near zero that it was not useful to display on a linear scale (Fig. 3).

Considering the number of chickens slaughtered and domestically consumed annually in Argentina (approximately 496 millions) (MinAgri, 2011b), we estimated the number of people who could suffer from campylobacteriosis related to poultry meat consumption. For the Beta-Poisson model according with FAO/WHO (2002), the number of human campylobacteriosis cases was, on average, 484,304.

The sensitivity analysis indicated that the risk of human campylobacteriosis was most sensitive to the probability of infection from a *Campylobacter* ($r = 0.72$), the number of *Campylobacter* spp. per single meal ($r = 0.40$), the frequency of washing the cutting board ($r = -0.31$), the preparation of raw poultry before salad using the same cutting board ($r = 0.14$), and the frequency of hand washing ($r = -0.14$) (Fig. 4).

Campylobacter spp. per single meal (C_{cs}) was an important factor that affected the probability of human campylobacteriosis. A reduction of 2 log MPN/serving (*e.g.* from a level of 100 NMP/serving to 1 NMP/serving) resulted

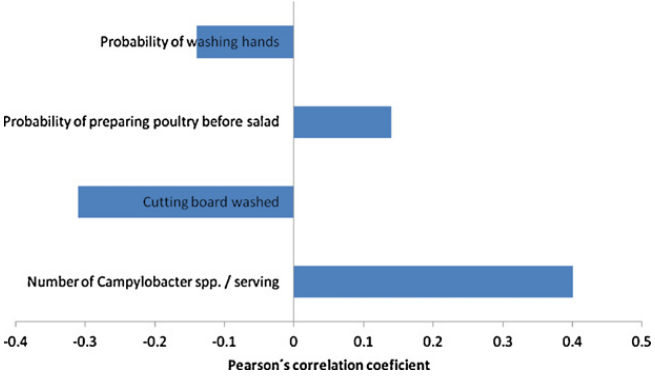


Fig. 4. Pearson's correlation coefficient between the estimated probability of human campylobacteriosis and the most important predictive factors.

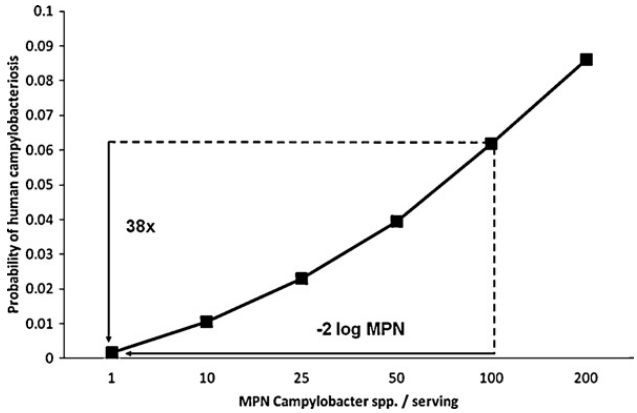


Fig. 5. Relationship between the number of *Campylobacter* per serving and the probability of human campylobacteriosis.

in a reduction of the probability of human campylobacteriosis of approximately a factor of 38 (Fig. 5).

This risk assessment included the cross-contamination during food preparation at home. The model showed that an improvement of the hygiene level in private kitchens could reduce the number of human campylobacteriosis (Fig. 6A). When the cutting board was washed immediately after handling raw chicken, the estimated risk of human campylobacteriosis was 5.19×10^{-5}. In contrast, if the cutting board was not washed after raw poultry manipulation, the estimated risk increased to 5.47×10^{-4}. The salad prepared after manipulation of raw poultry meat in the same unwashed cutting board had 10.54-fold greater than that prepared on a washed and previously used cutting board. If 95% of consumers wash the cutting board after handling raw chicken, it would be possible to expect 715,000 cases of human campylobacteriosis less than if only 5% of consumers adopting this practice.

The sequence of meal preparation was another important factor that influenced the risk of human campylobacteriosis. When the same cutting board is used to prepare the raw poultry meat and salad, it is essential to prepare first the ready-to-eat food (*e.g.* salad) and then the raw foods. When the salad was prepared before the raw poultry, the risk of human campylobacteriosis was 5.19×10^{-5}. In contrast, when the raw poultry was prepared first, the estimated risk increased to 3.47×10^{-4}. Raw poultry prepared before salad had 6.69-fold greater risk of generating human campylobacteriosis than poultry prepared after salad (Fig. 6B).

Hand washing is a fundamental activity during food preparation at home. When this practice was carried out, the estimated risk of human campylobacteriosis was 2.47×10^{-4}, whereas when it was not, the risk increased to 3.65×10^{-4}. Unwashed hands during food preparation had 1.47-fold greater risk of human campylobacteriosis than washed hands (Fig. 6C).

Our model had two input variables which were modeled based on scarce data: probability of frozen storage and probability of hand washing during poultry meat preparation at home. The impact of hand washing on the output was previously presented. To model the probability of frozen storage, we used a uniform distribution with minimum and maximum values of 0% and 100%, respectively. Considering these extreme values, the risk of human campylobacteriosis was 4.60×10^{-4} and 8.10×10^{-5}, respectively. Assuming that 100% of the chickens were stored frozen, the human campylobacteriosis risk would be 4.10 times lower than if none of the chickens were stored frozen. The Pearson's correlation coefficient between the risk of human campylobacteriosis/serving and the probability of frozen storage was <0.01.

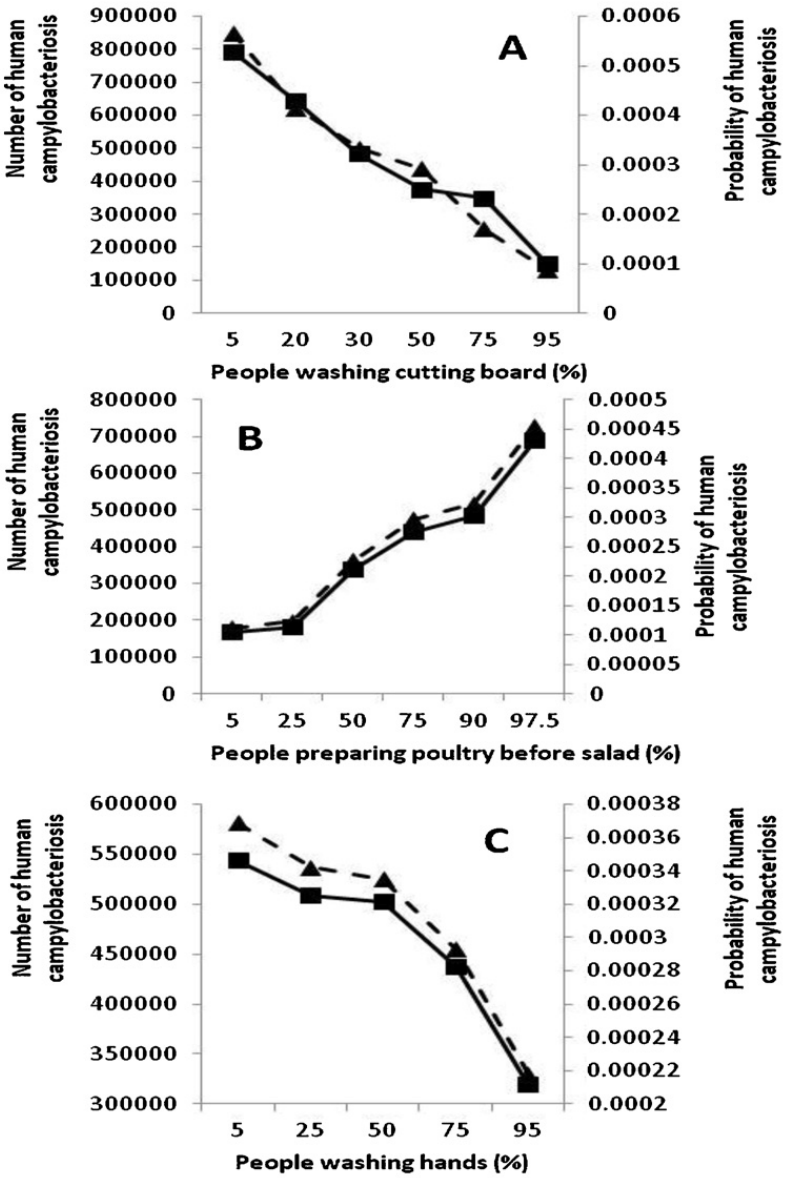
4. Discussion

The quantitative risk assessments for *Campylobacter* spp. infection associated with the consumption of chicken meat that have been published to date have not taken into account the storage conditions, and the distribution and consumption patterns in developing countries. The most important advantage of this model is that it allows evaluating the impact of the different inputs on the human campylobacteriosis risk, which could help in the selection of potential interventions in the poultry meat chain to reduce this risk.

In Argentina, the production of chicken meat has grown substantially during the last 7 years, reaching a slaughter of over 615 million of chickens during 2010, approximately 56.1% higher than that in 2005. The apparent *per capita* consumption of chicken meat has increased 42% in the last five years, reaching 34.4 kg/inhabitant/year. One of the reasons that may explain the higher consumption of this product in Argentina is its low price in comparison to other kinds of meat (MinAgri, 2011b).

There are only few studies conducted in Argentina about the prevalence of human infections by thermophilic *Campylobacter* (Fuentes, 2010). In Argentina, the National Health Surveillance System of the Ministry of Health does not discriminate diarrhea cases according to the etiological agent. In 2011, the entity "diarrhea" presented an incidence rate of 2588.2/100,000 people, a value relatively similar to that observed in the previous two years (SNVS, 2012). However, there is considerable underascertainment and underreporting, and the true number of cases of illness is likely to be 10–100 times higher than that reported (EFSA, 2010). A recent study conducted in Argentina (Fuentes, 2010) has shown that *Campylobacter* was the most prevalent agent of gastrointestinal infections, with a prevalence of 13.6% in adults.

The results from different attribution approaches, as applied in different countries, confirm previous epidemiological investigations that poultry is a major, if not the largest, source of human infection. The proportion of outbreaks attributed to poultry meat has been estimated to be around 25.9% (EFSA, 2010). Considering the previous data (with a value for the underreporting variable between 10 and 50 times), it is possible to estimate that the annual number of cases of human campylobacteriosis in Argentina would be approximately 1,108,194 (95%CI 406,200–1,809,000), which corresponds with an annual incidence rate of 2.77%. Comparing these data with the number of human campylobacteriosis cases arising from eating chicken estimated by our model, our results seem to be realistic. Thus, the model is valid to compare the effect of



References: Relationship between food handling practices (A= washing cutting board, B= preparing poultry before salad, C= washing hands) and number of human campylobacteriosis (—■—) or probability of human campylobacteriosis (--▲--).

Fig. 6. Relationship between food handling practices and the probability of human campylobacteriosis and number of human campylobacteriosis.

different intervention strategies to reduce the incidence of *Campylobacter* spp. Taking into account that there are other routes of infection than chicken, our model estimated that the cross-contamination during poultry meat preparation at home seems to be the most important route of exposure to thermophilic *Campylobacter*.

Rosenquist et al. (2003) developed a quantitative risk assessment of human campylobacteriosis associated with *Campylobacter* in chickens in Denmark and estimated that the number of cases was one case out of 14,300 chicken servings (a likelihood of 6.99×10^{-5}). This estimation was lower than the risk estimated in our risk assessment (approximately 4.75-fold lower). The model developed by Rosenquist et al. (2003) has been restricted only to focus on the effect of not washing the cutting board. Our model estimated the probability of human campylobacteriosis considering different practices during food handling (*e.g.* washing hands).

The probability of human campylobacteriosis calculated by our risk assessment was in agreement with the probability reported by Uyttendaele et al. (2006) in Belgium, which was estimated in 7.84×10^{-4}. However, the consumption of chicken meals in Belgium was estimated as 0.9 kg/year/inhabitant, approximately 33 kg *per capita* less than the consumption in Argentina. This may explain the difference in the estimated annual incidence rate of human campylobacteriosis between Belgium (0.71%) and Argentina (2.77%).

Since *Campylobacter* is heat-sensitive, it poorly survives the heat treatment of food before consumption. Therefore, cross-contamination remains as the main kitchen route by which humans are exposed to the pathogen (Mylius et al., 2007). Our model included a section about sources of contamination during food preparation at home, including the most important routes of cross-contamination in private kitchens. In agreement with that found by Rosenquist et al. (2003), not washing the cutting board was an important source of contamination. However, the habits of handling raw chicken before preparing the salad (or any other ready-to-eat food) and not washing the hands during food preparation were additional factors which impact on the risk of human campylobacteriosis. Our results were in agreement with the information reported by Mylius et al. (2007), who identified that the risk of infection is proportional to the probability of preparing chicken before salad and negatively related to the probability of washing (hands and cutting board). Our survey on consumer habits (Astesana et al., 2011) showed that many consumers prepare their foods in a way which supports the transfer of microorganisms from raw chickens to ready-to-eat foods. This information should be considered when designing public campaigns to improve food hygiene and to put more effort in stimulating the washing of the cutting board and the washing of hands and emphasizing the importance of not preparing raw meat before ready-to-eat foods.

The quantitative risk assessments are models that provide a simplified representation of a complex reality, based on several assumptions and hypotheses (Nauta et al., 2009). However, they are representations of the most likely practices carried out throughout the poultry meat chain. Two input variables had the highest degree of uncertainty in this model: probability of hand washing and probability of frozen storage. Hand washing is an important hygienic habit during food preparation at home and it had a great impact on the probability of human campylobacteriosis. The uncertainty present in the probability of frozen storage had influence on the probability of human campylobacteriosis in spite of the Pearson's correlation coefficient was <0.01. Future studies should be conducted to reduce the uncertainty about these habits and reduce the variability across the whole model.

The ingested doses due to cross-contaminated salad consumption were very low (generally 1 or 2 *Campylobacter*), which is in agreement with the findings reported by Nauta et al. (2006). These low doses complicate the risk estimation because the dose–response models are inferred from higher doses (Nauta et al., 2006) and it has a dramatic influence on the final estimation of the model. The predicted risk could be affected by the uncertainty associated with the dose–response relationship and it could affect the identification of the most important factors which impact on the probability of human campylobacteriosis.

Although the results seem reasonable, there is still room for improving the model since different assumptions and simplifications were made: (a) data on the *Campylobacter* spp. prevalence and concentration in raw poultry was limited. However, this study provided the first data in Argentina (and preliminary) about the prevalence and concentration of *Campylobacter* in the poultry meat chain, and the first evidence of the seriousness of the problem. Furthermore, prevalence of *Campylobacter*-positive carcasses at retail found in this study was comparable to prevalence reports for chicken carcasses from European Union (EFSA, 2010), United States (Zhao et al., 2001), Chile (Figueroa et al., 2009) and Republic of Ireland (Madden et al., 2011). Taking into account that concentration of *Campylobacter* spp. per single meal was the most important factor that affected the probability of human campylobacteriosis, further studies should be conducted to improve the estimation of prevalence and concentration of *Campylobacter* spp. in raw chicken and, in any case, review this model to adjust the risk estimated. (b) Data about consumer habits, especially those related to home storage and other practices during food preparation at home, had an important degree of uncertainty. Therefore, this quantitative risk assessment has to be considered as a preliminary approach and it should be improved when more data are available. However, this model may be useful for risk managers as a scientific basis to establish different mitigation or intervention strategies.

5. Conclusions

The number of *Campylobacter* spp. present in the salad due to cross-contamination with row poultry was the most important factor impacting on the risk of human campylobacteriosis. The incidence of campylobacteriosis due to the consumption of poultry meals could be reduced 38 times by the reduction of 2 logs of the number of thermophilic *Campylobacter* on the chicken carcasses. Manipulation of raw poultry before ready-to-eat food, and not washing the hands and/or the cutting board during handling of foods were the main routes of cross-contamination. Although important sources of uncertainty were identified, this model can be used as a scientific basis by risk managers in deciding strategies to reduce the risk of human campylobacteriosis. Especially, public campaigns on hygiene habits should focus on stimulating the importance of washing the cutting board before preparing raw and ready-to-eat foods and of hand washing during food preparation.

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