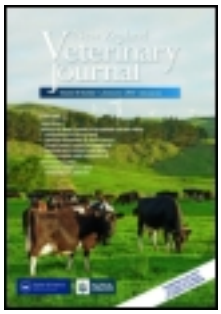

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Occurrence of thermotolerant Campylobacter spp. at different stages of the poultry meat supply chain in Argentina

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Scientific Article

Occurrence of thermotolerant *Campylobacter* spp. at different stages of the poultry meat supply chain in Argentina

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Abstract

AIMS: The objectives of this study were to investigate the occurrence and concentration of thermotolerant *Campylobacter* spp. at different stages of the poultry meat supply chain in Argentina.

METHODS: Three integrated poultry companies were sampled. Each supply chain was considered at different stages from the reproductive farm to chicken meat at a retail market. The stages sampled were: a) hens from breeder flocks, b) eggs in the incubator, c) broiler chickens in flocks (aged <1 week and >5 weeks), d) chickens at a slaughterhouse, and e) chicken meat at a retail market. The chickens sampled along each supply chain were in the same batch. Samples collected were: a) cloacal samples from hens and chickens on the farms, b) fertile eggs, c) feed, water and litter from flocks, d) chicken carcasses from the slaughterhouse and retail market, and e) caeca and livers from the slaughterhouse. Samples obtained were examined for *Campylobacter* spp. The isolates were biotyped and the genus and species identified by PCR. *Campylobacter* spp. on chicken carcasses at slaughterhouse and retail market were enumerated.

RESULTS: The highest proportions of *Campylobacter* positive samples were observed in carcasses at retail (25/30, 83.3%) and faecal samples from breeding hens (27/45, 60.0%). Only 3.3% (3/90) samples collected from broiler chickens aged <1 week were positive, but the percentage of positive samples had risen to 28.9% (26/90) by the end of the rearing period. The proportions of *Campylobacter* positive carcasses and caecal contents at the slaughterhouse were both 33.3% (10 of

30 samples each). The concentration of *Campylobacter* contamination observed on carcasses at retail markets ranged from no bacteria / carcass to 3.71 $\log_{10}$ cfu / carcass.

CONCLUSIONS: The data obtained provide essential information for future quantitative risk assessments aiming to estimate the probability of a person contracting campylobacteriosis following consumption of broiler meat in Argentina.

CLINICAL RELEVANCE: The proportions of *Campylobacter*-positive samples found in this preliminary study indicate that a large proportion of the cases of human gastroenteritis in Argentina may be due to this pathogen. The human cases of gastroenteritis should be studied in greater detail and measures should be developed to reduce the proportion of poultry products that are contaminated by *Campylobacter* species.

KEY WORDS: *Campylobacter spp., chickens, zoonosis, farm-to-fork, poultry production, food safety, biotype*

Introduction

Thermotolerant *Campylobacter* spp., especially *Campylobacter jejuni*, constitute the most frequent cause of human acute bacterial enteritis worldwide (Moore *et al.* 2005). These pathogens are frequently found in the intestinal tract of a wide variety of wild and domesticated animals, especially birds (Hansson *et al.* 2010).

Foods of animal origin, in particular poultry meat, are known to be a prominent source of human campylobacteriosis due to factors such as inadequate cooking and cross-contamination during food preparation (Anonymous 2010a). The reduction and / or elimination of thermotolerant *Campylobacter* spp. in the meat supply chains, particularly in chicken products, are important strategies to control this disease (Newell and Fearnley 2003).

The incidence of human infections caused by thermotolerant *Campylobacter* in the European Union (EU) has been continuously increasing in recent years (45.2 cases / 100,000), and these bacteria are still the most common gastrointestinal zoonotic pathogens reported in humans in the EU (Anonymous 2009; Hue *et al.* 2010). The epidemiological situation in the United States is less dramatic with an incidence rate of 13.02 / 100,000 inhabitants in 2009. Nevertheless, *Campylobacter* is still one of the most commonly reported bacterial causes of human enteritis in the USA (Anonymous 2010a).

In developing countries, information on food-borne disease is often scant due to inadequate data provided by the passive surveillance systems. Additionally, outbreak information is frequently unsubstantial because health authorities lack the capabilities or resources to detect diarrhoeal diseases (Zaidi *et al.* 2008). A recent study conducted in Argentina (Fuentes 2010) concluded that thermotolerant *Campylobacter* spp. are the most important gastrointestinal pathogens in humans, with a higher incidence rate than that of other common pathogens such as *Salmonella* spp., *Shigella* spp. and *Escherichia coli*. The percentage of *Campylobacter* isolations was 22.4% in children under 3 years and 13.6% in adults. However, no epidemiological studies in Argentina have previously assessed the prevalence of *Campylobacter* on the whole meat supply chain from farm-to-fork. This information is essential to establish a public health programme to control the disease.

In Argentina, the production of chicken meat has grown substantially during the last 7 years, reaching a total of over 615 million chickens processed in 2010, producing 1,779,000 tonnes of poultry meat, which is approximately 56.1% higher than the figures for 2005. The apparent *per capita* consumption of chicken meat has increased by 42% in the last 5 years, reaching 34.4 kg / inhabitant / year in 2010 (Anonymous 2010b).

Poultry production in Argentina is concentrated within 3,800 farms and there are 54 slaughterhouses in the country. In most cases, poultry production is carried out within integrated companies, and the production system is intensive, with birds reaching a final weight of 2.71 kg in less than 48 days.

The objectives of this study were to investigate the occurrence and concentration of thermotolerant *Campylobacter* spp. at different stages of the poultry meat supply chain in Argentina.

Materials and methods

Experimental design

Three different companies (Companies A, B, and C, which are referred to here as poultry meat supply chains) were sampled at five stages: a) hens from breeder flocks, b) eggs in the incubator, c) broiler chickens in flocks (aged <1 week and >5 weeks), d) chickens at the slaughterhouse, and e) chicken meat at the retail market. Within each company, the chickens sampled along each supply chain were from the same batch; where batch is defined as a group of chickens from the same flock, delivered at the same time to the same slaughterhouse, and sold together at the same retail market.

Sample collection

Breeder flocks

Faecal samples were randomly collected from the cloacae of 15 hens within each breeder flock that was supplying fertile hatching eggs to the broiler flocks. Samples were taken with sterile cotton swabs and then placed in capped plastic tubes containing 10 ml of Cary-Blair transport medium and transported to the laboratory under refrigeration, within 4 h of collection.

Fertile hatching eggs

Fifteen fertilised eggs were randomly selected from the cabinet egg incubator and transported to the laboratory in plastic bags. Under sterile conditions, the egg shell was separated from the egg content. These sub-samples were analysed separately.

Broiler farm

Three flocks per company were sampled. Cloacal samples were randomly collected from 10 individual chickens per flock (i.e. 30 broilers per company) at two different ages: <1 week, and >5 weeks (i.e. the second sample was collected just before the slaughter date for that batch). Samples were placed in capped plastic tubes containing 10 ml of Cary-Blair transport medium and transported to the laboratory under refrigeration, within 4 h of collection. Samples of chicken feed (500 g), drinking water (1 L) and litter (500 g) were also taken from each flock (four samples per flock in each age range, in total 8 samples per company). Inside the shed, feed was taken directly from the feeders, drinking water was taken from nipples and soiled litter was taken in houses containing birds.

Slaughterhouse

The three companies used the same slaughterhouse to slaughter their broilers. The flocks were transported by truck from the farms to the slaughterhouse. For each farm, 10 caecal and liver samples were randomly collected from the evisceration line by one of the researchers and placed in sterile plastic bags. One sample was taken every 10 minutes.

Ten broiler carcasses 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 quarter-strength Ringer's solution. Carcasses were rinsed by shaking for 60 seconds in each of two directions to ensure that the solution came into contact with all surfaces; the solution was recovered and transported to the lab in sterile plastic tubes (under refrigeration), within 4 hours.

Retail market

Chickens were packaged at the processing plant in the slaughterhouse and transported to the retail market. Ten whole chickens from the same flock were randomly sampled at the retail market, following the same procedure described for the broiler carcasses in the slaughterhouse.

Sample analysis

Campylobacter were recovered using the selective media Bolton Broth and Preston Agar (Bolton and Coates 1983).

The cotton swabs (with cloacal samples from hens and broilers) were immersed in 5 ml Bolton Broth and incubated for 24 h at 42ºC under microaerophilic conditions (5% O_2, 10% CO_2 and 85% H_2). About 20 µl (2 loopfuls) of Bolton Broth was streaked on Preston Campylobacter-Selective Agar, and incubated for 48 h at 42ºC under microaerophilic conditions. Identification of *Campylobacter* spp. was based on colony morphology, microscopic appearance and the following phenotypic characteristics (biotyping): production of oxidase and catalase, hippurate hydrolysis reaction, DNase test, and H_2S production (Lior 1984). The positive colonies were streaked on Columbia blood agar and a sweep of cells equal to half of a 10 µl loopful was removed and stored in glycerol broth (15% glycerol and 85% serum broth) at -80ºC (Terzolo *et al.* 1987).

The samples of water, feed, litter, and eggs (shells and contents) were incubated in Bolton Broth (25 g of each sample in 225 ml of broth). The same procedure described for cloacal samples was then followed.

The quarter-strength Ringer's solution recovered from the rinsed broiler carcasses was centrifuged at 5000 rpm for 5 minutes; the pellet was resuspended in 2 ml of the same solution, and then inoculated into Bolton Broth. The same procedure described for cloacal samples was then followed.

Samples of liver and caeca were obtained in aseptic conditions and homogenised with a Stomacher (Seward biomaster) in Bolton Broth (10 g of each sample in 90 ml of broth). The same procedure described for cloacal samples was then followed.

DNA preparation and species identification

DNA of all *Campylobacter* isolates was extracted using CTAB, following the protocol reported by Ausubel *et al.* (1991), and then purified and precipitated with chloroform-isoamyl alcohol and isopropanol, respectively.

For confirmation of the genus, the 16S rRNA gene (Linton *et al.* 1997) was amplified by PCR using the oligonucleotide primers C412F, 5'-GGATGACACTTTTCGGAGC- 3' and C1288R, 5''-CATTGTAGCACGTGTGTC-3' to yield a 816-bp-long fragment. PCR amplification was

performed in 25 µl volumes containing 25 ng genomic DNA; 20 mM Tris-HCl (pH 8.3); 50 mM KCl; 2.5 mM $MgCl_2$; 0.2 mM of each deoxyribonucleotide triphosphate; 0.41 µM of each primer; and 1 unit of *Taq* DNA polymerase (GoTaq, Promega). Samples were subjected to 25 cycles of amplification in a DNA thermal cycler with the following parameters: denaturation, 94°C 1 min; annealing, 55ºC 1 min; extension, 72°C 1 min. PCR products were analysed on 1.5% agarose gels and stained with GelRed (Biotium).

For confirmation of species (*C. jejuni* and *C. coli*), two genes were selected *(*Vandamme *et al.* 1997*)* and tested by PCR in all *Campylobacter* isolates. The primer sequences were COL 1 (5'-AGGCAAGGGAGCCTlTAATC-3') and COL 2 (5'-TATCCCTATCTACAAATTCGC-3') for *C. coli* and JUN 3 (5'-CATCTTCCCTAGTCAAGCCT-3') and JUN 4 (5'-AAGATATGGCTCTAGCAAGAC-3') for *C. jejuni*. The expected amplicon size was 773 bp for *C. jejuni* and 364 bp for *C. coli*. Samples were brought to a final volume of 50 µl for PCR with a solution containing 20 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, each deoxyribonucleotide triphosphate at a concentration of 0.2 mM and 20 pmol of each primer. The reaction mixture also contained 1 U of Taq polymerase (GoTaq, Promega) and was performed by using a touchdown protocol described previously (Vandamme *et al.* 1997*)*. PCR products were analyzed on 1.5% agarose gels and stained with GelRed (Biotium).

Campylobacter enumeration

Bacteria present on whole chicken carcasses from the slaughterhouse and retail market were enumerated. Aliquots of 1 ml from each ten-fold dilution (10^{-1} through 10^{-4}) were pipetted into three MPN-tubes containing 9 ml of Bolton Broth. The MPN-tubes were incubated under microaerophilic conditions (5% O_2, 10% CO_2 and 85% H_2) at 42ºC for 24 h. Then, 10 µl from each tube was streaked onto Preston Agar and after incubation for 48 h at 42ºC under microaerophilic conditions, five colonies per MPN plate were biochemically confirmed. Every positive plate was biochemically confirmed.

After biochemical confirmation of these colonies, the most probable number (MPN) was calculated on the basis of positive plates according to the standard ISO 7218 (International Organisation for Standardisation, 1996).

Statistical analysis

The log odds of samples being *Campylobacter*-positive were calculated as a function of the explanatory variables (company of origin and stage of production) using a multivariable, logistic, regression model. Breeding hens and Company A were used as reference levels for stage of

production and company of origin, respectively. The other stages and companies were compared to the reference levels using the Wald test.

Distributions of *Campylobacter* counts on poultry carcasses from the slaughterhouse and retail market for each of the 3 companies studied were diagrammed as box plots. A non-parametric Kruskal-Wallis test and the Mann-Whitney test were conducted to determine if there were significant differences in the *Campylobacter* counts of the three companies.

Concordance between species identification of *Campylobacter* by biotyping and PCR was measured using the kappa statistic (κ). All statistical analyses were conducted using Infostat software (Universidad Nacional de Córdoba, Córdoba city, Argentina, 2009).

Results

Thermotolerant *Campylobacter* were found in 103 (27.5%) of the 375 samples collected at the different stages of the poultry meat supply chain (hens, eggs and broilers on farms; carcasses, caecal and liver samples at the slaughterhouse; and carcasses at the retail market). The proportions of *Campylobacter*-positive samples at the different stages of the poultry meat supply chain are shown in Figure 1. The highest proportions of *Campylobacter*-positive samples were obtained from retail chickens (25/30, 83%) and breeding hens (27/45, 60%) (Table 1). None of the samples from eggs were *Campylobacter*-positive. Using the proportion of *Campylobacter*-positive samples in breeding hens as a reference level, broilers aged <1week showed a low proportion of *Campylobacter*-positive samples (3/90, 3%) ($p<0.001$). However, this proportion increased at the end of the rearing period (26/90, 29%), but was significantly lower than that observed in the reference level (breeding hens) ($p=0.001$). The proportion of *Campylobacter* in broiler carcasses and in the caecal content in the slaughterhouse was the same (10/30, 33 %) and significantly lower than in breeding hens ($p=0.03$), while a low translocation of *Campylobacter* to the poultry liver was observed (2/30, 7%). Chicken carcasses in the retail market showed a higher proportion of *Campylobacter*-positive samples than breeding hens ($p=0.04$). There were no differences in the proportion of *Campylobacter*-positive samples among the 3 companies sampled ($p=0.71$ and 0.58 for Companies B and C, respectively) considering all the stages (Table 1).

Only 2 of the samples collected from water (n= 23), litter (n= 23) or feed (n= 23) from the farms were culture-positive for species of thermotolerant *Campylobacter* - one from water and the other from feed. One sample of water, litter and feed were not collected.

The relative proportions of *Campylobacter jejuni* and *C. coli* that were detected from the positive samples are also shown in Figure 1. Of the total collection of positive samples, *C. jejuni* was

isolated from 57.3% (59/103), *C. coli* from 19.4% (20/103), and in 23% of positive samples (24/103) both species were detected. However, Figure 1 demonstrates that the proportions of each species varied according to the stages of the supply chain. The proportions of *C. coli* and *C. jejuni* positive samples were approximately equal in the samples collected from breeding hens, but by the end of the rearing stage (broilers aged >5 weeks) *C. jejuni* represented the majority of the isolates recovered, and this was also reflected at the slaughterhouse where *C. jejuni* represented 100% of the *Campylobacter* species isolated from poultry carcasses and caecal contents. However, *C. coli* isolates were identified in 20% of samples collected from meat on sale at the retail market. Species identification by PCR was not possible on 5 occasions (2 isolates from broilers aged <1 week from Company A and C, and 3 from broilers aged >5 weeks, 2 from Company B and the other from Company C).

We were able to biotype 103 strains. The hippurate test has been used to differentiate *C. jejuni* (hippurate +) from *C. coli* (hippurate −). Fifty-seven strains were hippurate negative, but of these only 31 were confirmed as *C. coli* by PCR, the remaining 26 strains were PCR positive for *C. jejuni*. Therefore, the hippurate test had a concordance (kappa index) of 0.502 (95% CI 0.334 – 0.671) with the PCR assay, which may be considered moderate agreement.

The *C. jejuni* biotyping test showed that biotype I was the most prevalent along the broiler meat supply chain (56/78, 71.8%). The remaining strains belonged to biotypes II (9/78, 11.5%) and III (13/78, 16.7%). *C. jejuni* biotype I was commonly isolated from hens (12/16) and broilers aged >5 weeks (18/24); however, no *C. jejuni* biotype I isolates were obtained from broilers aged <1 week (0/2).

Biotype I was the most prevalent biotype of *C. coli* (27/32, 84.4%), especially in breeder hens and on chicken carcasses at the retail market. The remaining biotype identified was *C. coli* II, which was isolated in low numbers from hens (1/15), broilers aged >5 weeks (2/3), carcasses at the slaughterhouse (1/2), and carcasses at the retail market (1/10).

Considering the carcasses in the slaughterhouse, the contamination found ranged from no bacteria / carcass to 4.25 $\log_{10}$ cfu / carcass. The contamination observed in carcasses at the retail market ranged from no bacteria / carcass to 3.71 $\log_{10}$ cfu / carcass (Figure 2). Poultry produced by Company A showed a lower concentration of *Campylobacter* both in the slaughterhouse (p= 0.02) and the retail market (P= 0.01). Moreover, there were no differences in the *Campylobacter* counts between chicken carcasses from Companies B and C at the slaughterhouse (p=0.71) and the retail market (p=0.70).

Discussion

This study provides the first data in Argentina about the occurrence and concentration of *Campylobacter* at different stages in the poultry meat supply chain. The results show that thermotolerant *Campylobacter* were present at all stages (except in eggs) of the supply chain in this country. The transmission of thermotolerant *Campylobacter* along poultry meat supply chains has been previously recognised as a major source of human campylobacteriosis in many countries (Anonymous 2010ab). The results of this study suggest that poultry products also represent an important risk to public health in Argentina. Therefore, there is a need to improve the understanding of the epidemiology of *Campylobacter* in poultry in Argentina, so that appropriate intervention strategies can be developed to prevent flock colonisation and reduce poultry carcass contamination (Bull *et al.* 2006).

The proportion of *Campylobacter* positive samples from breeder flocks observed in this study was similar to that reported from previous studies in other countries (Petersen *et al.* 2001; Callicott *et al.* 2006). Some flocks of breeder hens have been shown to be colonised by multiple strains of *Campylobacter* that can be recovered from various sections of the reproductive tract (Hiett *et al.* 2002). The presence of *Campylobacter* spp. in the oviduct could result in the colonisation of chicken embryos; however, only a few studies have reported the isolation of *Campylobacter* species from eggs, with a prevalence of approximately 1% reported in one study (Doyle 1984). In fact, experimental work has suggested that natural infection of egg contents is mainly due to faecal contamination of the external surface and penetration via shell cracks (Newell and Fearnley 2003). Similarly, in the present study we did not isolate any *Campylobacter* from intact eggs; however, we did isolate *Campylobacter* in broiler birds that were less than one week of age. Humphrey *et al.* (1993) reported that the mean time for within-flock prevalence to reach 5% was 9 days, with a range of 6−18 days. Colonisation of young birds may be due either to vertical transmission from parent flocks or to rapid transmission of the agent from the environment. Different studies (Hiett *et al.* 2002; Callicott *et al.* 2006) have suggested that vertical transmission is rare and that the most useful approach to control *Campylobacter* on farms is to control the horizontal sources that present more obvious risk. With respect to hatchery environments, Petersen *et al.* (2001) did not succeed in isolating a single *Campylobacter* from hatchery samples, suggesting that horizontal transmission via the hatchery is less likely.

In the study reported here, only one sample of water and one of feed were *Campylobacter*-positive. Although feed has been identified as a potential source of *Campylobacter* (Berndtson *et al*. 1996), the dry conditions of commercial feed may be considered lethal to *C. jejuni* (Humphrey *et al*. 1993; Altekruse *et al.* 1999). Water has been implicated as an important environmental source of colonisation of chickens with *Campylobacter* spp. (Stern *et al.* 2002), and the ability of *C. jejuni* to

survive in water under experimental conditions is well recognised (Newell and Fearnley 2003). The water supplied to the chickens in this study was chlorinated. However, Stern *et al*. (2002) concluded that chlorinated drinking water does not decrease the colonisation of chickens with *Campylobacter* spp. Other researchers (Berndtson *et al*. 1996) have concluded that water does not seem to be a significant source of infection for chickens. Consequently, in this study drinking water was unlikely to have been the source of *Campylobacter* and water contamination could be a result of faecal contamination of drinking nipples within the broiler sheds. Further studies should be conducted to confirm or reject this hypothesis.

In the present study, the proportions of carcasses and caecal contents at the slaughterhouse that were positive for *Campylobacter* were similar. Isolation of *Campylobacter* from poultry caeca has previously been identified as a risk factor for the presence of the pathogen on carcasses. In a study conducted in France, Hue *et al.* (2010) observed prevalence's of *Campylobacter* in caecal contents and poultry carcasses of 77.2% and 87.5%, respectively and it has been noted that cross-contamination may occur during the slaughtering process (Zorman *et al.* 2006).

The proportion of *Campylobacter*-positive carcasses at retail found in this study is comparable to prevalence reports for retail chicken carcasses from other countries. However, the concentration of *Campylobacter* on carcasses varies between countries (Anonymous 2010b), which may be due to differences in the sampling schemes, the ages of the birds sampled, and the production methods. Figueroa *et al.* (2009) focussed their sampling at different points within the slaughterhouse and reported a prevalence of *Campylobacter* on poultry carcasses of 54% in Chile, where *C. jejuni* was the species most frequently isolated (97%) and *C. coli* accounted for only 3% of the strains collected.

In our study, a significant number of samples had both thermophilic *Campylobacter* species detected by PCR. The isolation methodology used has some limitations and cells corresponding to more than one species may have been stored. This is due to the tendency for bacterial colonies to spread and merge on the isolation plate, which makes it impossible to select a pure, individual colony. The presence of both PCR products from a single faecal sample has already been observed by Denis et al. (1999).

In the present study, carcass contamination ranged from no bacteria to 4.26 $\log_{10}$ cfu / carcass, but almost half of the carcasses showed less than 10 *Campylobacter* cfu / g. The counts of *Campylobacter* on broiler carcasses also vary widely between countries. In general, there is a tendency for high counts in countries with a high prevalence of *Campylobacter*. Hue *et al.* (2010) reported that in France, poultry meat had, on average, 2.39 $\log_{10}$ cfu / g (range 1.0 to 4.39 $\log_{10}$ cfu /

g); while in Chile, Figueroa *et al.* (2009) showed that *Campylobacter* contamination ranged from 3.3 to 7.7 $\log_{10}$ cfu / carcass. Additionally, our results showed wide between-company variation in *Campylobacter* counts on chicken carcasses at the slaughterhouse and at retail within a single country. This is important information, with respect to the aim of using this data to develop a quantitative risk assessment model, because it implies that more companies should be sampled to ensure that the model is representative of the true Argentinean situation.

Our results suggest that the proportion of poultry carcasses that are colonised with *Campylobacter* at retail in Argentina could be high, and the development of an epidemiologically designed wide-scale survey of chicken meat to test this hypothesis is justified. In addition, this study has shown that some broiler carcasses in Argentina are contaminated with high numbers of *Campylobacter*. Such carcasses represent a potential health risk for consumers due to cross-contamination of other foodstuffs and surfaces during meal preparation (for example hands of food handlers, utensils and food contact surfaces) or by consumption of undercooked poultry meat (Luber 2009). It is important to point out that these data on the occurrence of *Campylobacter* spp. and concentrations at the different stages of the poultry meat supply chain studied were limited to 1 flock in each of 3 companies. The number of feed, water and litter samples taken per flock was also limited and the representativeness of these samples can not be guaranteed.

Nonetheless, the results of this study indicate that a larger study, sampling a more representative number of integrated companies, would be justified. Even considering these limitations, the proportion of *Campylobacter*-positive carcasses found at the retail market was comparable to prevalence reports for chicken carcasses in the European Union (Anonymous 2010b), Ireland (Madden *et al*. 2011), Chile (Figueroa *et al*. 2009), and the United States (Zhao *et al*. 2001). In conclusion, the consumers of poultry meat in Argentina may be exposed to a high proportion of *Campylobacter*-positive poultry carcasses, which would constitute a serious hazard for public health. Multiple biotypes of *C. jejuni* and *C. coli* may be spreading through the poultry meat supply chain, but the source of this heterogeneity was not defined in the present study. The data generated by this study are being used to assist with the development of a quantitative risk assessment model for thermotolerant *Campylobacter* spp. related to the consumption of chicken meat in Argentina. The model aims to provide information to the Argentinean risk managers to define strategies to reduce the risk of human campylobacteriosis.

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Table 1. Logistic regression analyses of the relationship between independent variables (stage of production and company) and *Campylobacter*-positive status of samples collected from 3 poultry meat supply chains in Argentina during 2011.

Variable	Category	Beta ± SE	Unadjusted odds ratio (OR)	95% CI	p-value[a]
Intercept		0.52 ± 0.37			0.16
Stage of production	Breeding hens		REF		<0.001
	Eggs	-4.19 ± 1.05	0.02	0.001–0.02	<0.001
	Broilers <1wk	-3.78 ± 0.66	0.02	0.01-0.08	<0.001
	Broilers >5wk	-1.31 ± 0.38	0.27	0.13–0.57	0.001
	Chicken carcasses at slaughterhouse	-1.10 ± 0.49	0.33	0.13–0.87	0.03
	Chicken carcasses at retail market	1.21 ± 0.58	3.34	1.08–10.35	0.04
Companies	A		REF		0.85
	B	-0.14 ± 0.37	0.87	0.42–1.81	0.71
	C	-0.21 ± 0.37	0.81	0.39–1.69	0.58

[a] Significance of Likelihood ratio test statistic
REF = reference category

Figure 1. The proportions of samples that were culture-positive for thermotolerant *Campylobacter* spp. (line) and the cumulative percentage of samples belonging to two species (*C. coli* = grey, *C. jejuni* = white, *C. coli* + *C.jejuni* = black bars) at various stages along the poultry meat supply chain in Argentina. Samples were obtained from 3 independent poultry companies during 2011.

Figure 2. Box plots of the *Campylobacter* spp. concentrations on chicken carcasses (log cfu/carcass) at a slaughterhouse (n= 30 carcasses) and at a retail market (n= 30 carcasses) for each of 3 independent poultry production companies (A = white, B = grey, C = black) in Argentina during 2011. The boxes represent the interquartile range; the whiskers represent the distance from the end of the box to the largest and smallest values observed; outlier values of two levels are shown, the double asterisk represents values between 1.5 and 3 times the length of the interquartile range from the end of a box, whilst the single asterisk represents extreme values that are more than 3 interquartile ranges from the end of a box.