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M.V. Zbrun, E. Rossler, A. Romero-Scharpen, L.P. Soto, A. Berisvil, J.A. Zimmermann, M.L. Fusari, M.L. Signorini, L.S. Frizzo

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Worldwide meta-analysis of the prevalence of *Campylobacter* in animal food products

Zbrun, M.V.[ab]; Rossler, E.[a]; Romero-Scharpen, A.[a]; Soto L.P.[ab]; Berisvil, A.[a]; Zimmermann, J.A.[a]; Fusari, M.L.[b]; Signorini, M.L.[bc*]; Frizzo, L.S.[ab]

[a] Laboratorio de Análisis de Alimentos, Instituto de Ciencias Veterinarias del Litoral (ICiVet Litoral), Universidad Nacional del Litoral; Consejo Nacional de Investigaciones Científicas y Técnicas (UNL/CONICET), Kreder 2805, (S3080HOF) Esperanza, Province of Santa Fe, Argentina.

[b] Departamento de Salud Pública. Facultad de Ciencias Veterinarias – Universidad Nacional del Litoral. Kreder 2805, (S3080HOF) Esperanza, Province of Santa Fe, Argentina.

Phone: +54 3496 420639/421037.

[c] Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto Nacional de Tecnología Agropecuaria - EEA Rafaela, Ruta 34 Km 227, (2300) Rafaela, Province of Santa Fe, Argentina. Phone: +54 3492 440121.

Phone: +54 3496 420639/421037.

*Corresponding author: María Virginia Zbrun. E-mail: virginiazbrun@yahoo.com.ar. Phone: +54 3496 420639.

ABSTRACT

The objective of this meta-analysis was to summarize available information on the prevalence of thermotolerant *Campylobacter* in different animal food products. A number of multilevel random-effect meta-analysis models were fitted to estimate mean prevalence of thermotolerant *Campylobacter* and to compare them among animal food products (cattle, pigs, broiler, hen, goat, sheep). The mean prevalence of *Campylobacter* spp. in animal food products was 29.6% (95% CI 27.6% - 31%), and the mean prevalence of *C. jejuni* and *C. coli* were 19.3% and 9.7%, respectively. The prevalence of *Campylobacter* spp. was higher in products whose sources were broiler meat (*p*-estimate= 47.8%; 95% CI 44.9% - 50.6%). *C. jejuni* was mainly observed in broiler meat where prevalence estimate (*p*-estimate) was 33.7% (95% CI 30.7% - 36.8%). On the other hand, *C. coli* was observed in broiler meat (*p*-estimate= 14.1%; 95% CI 12.3% - 16.1%) and sheep meat (*p*-estimate= 11.0%; 95% CI 3.6% - 29.1%). The animal food products with the lowest prevalence of *Campylobacter* spp. were milk and dairy products (*p*-estimate= 3.5%; 95% CI 1.8% - 6.5%), eggs (*p*-estimate= 4.0%; 95% CI 1.4% - 10.7%), sausage (*p*-estimate = 9.4%; 95% CI 3.3% - 24.0%), This meta-analysis concluding that *C. jejuni* is the most prevalent species worldwide and broiler meat is the main contamination source for human. The prevalence of *Campylobacter* species has public health importance and national authorities must monitor the situation in each country with the aim to establish the appropriate risk management measures.

Keywords: Thermotolerant *Campylobacter*; meta-analysis; prevalence; food-borne diseases; public health, zoonoses.

1. Introduction

Thermotolerant *Campylobacter* is a common foodborne pathogen of humans worldwide (Epps et al. 2013; Gillis et al., 2013, CDC, 2014; EFSA, 2019). The prevalence of campylobacteriosis has increased in recent years in many countries (EFSA 2016; 2019; WHO 2013). In addition, *Campylobacter* spp., and especially *C. jejuni* and *C. coli*, are the most important cause of acute gastroenteritis in people (Jorgensen et al. 2011; WHO, 2013; Kaakoush et al., 2015).

A previous study (meta-analysis research) indicated that the handling and consumption of chicken meat is a major risk factor for human campylobacteriosis (Domingues et al., 2012). According with this information, EFSA report suggested that 20–30% of campylobacteriosis cases are attributable to the handling, preparation, and consumption of chicken meat (EFSA, 2010). Also, the consumption of raw milk, raw red meat, fruits and vegetables has been identified as a possible risk factors (Mohammadpour et al., 2018, EFSA, 2019). However, several characteristic associated with this pathogen as asymptomatic nature of *Campylobacter* spp infection and the high genetic diversity, are aspects that difficult the epidemiological analysis of this pathogen throughout the agri-food chains (Zbrun et al. 2017).

Campylobacter spp. is commonly found in the intestinal tract of food-producing animals and their prevalence in cattle, swine and poultry have been found to exceeds 80% (Weitjens et al., 2003; Zbrun et al., 2013; Thépault et al., 2018). Also, *Campylobacter* is a commensal organism in broilers with colonization level up to 10^{10} colony-forming units (CFU) per gram of feces (Stas et al, 1999; Wassenaar et al., 1993; Sahin et al., 2002; Dhillon et al., 2006). Colonization of the animals with thermotolerant *Campylobacter* occur at farm level and contamination occur throughout the agri-food chain to the consumer (Signorini et al. 2013). In this sense, carcass contamination occurs especially during slaughter and processing (Zbrun et al. 2013). Broiler meat, milk, water consumption and direct contact with farm animals have been reported as the most important sources of human campylobacteriosis (Studahl and Andersson, 2000, EFSA, 2019).

To reduce the risk of human exposure to thermotolerant *Campylobacter*, it is essential to establish risk management measures to reduce contamination in food-producing animals. Therefore, it is essential to understand the epidemiology of thermotolerant *Campylobacter* in animals (Bull et al. 2006). In this sense, meta-analysis is a highly valuable statistical tool whose objective is to synthesize, integrate and contrast the results of a large number of primary studies that investigate the same questions. As a result, the meta-analysis generates a more accurate estimate of the effect size of a particular event with greater statistical power than using a single study (Borenstein et al. 2009).

The worldwide prevalence of thermotolerant *Campylobacter* in animal food products is an interesting topic to be summarized in a meta-analysis and, according to our knowledge there is no meta-analysis on this topic. The objective of this study was to quantitatively summarize and compare the prevalence of *Campylobacter* spp. in animal food products worldwide. This information may be used as a basis for risk management measures in public health.

2. Materials and methods

This systematic review was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Moher et al., 2015).

2.1. Data sources

Scopus, PubMed, and ScienceDirect databases were searched for scientific papers published in English, Spanish, Portuguese from 1980 to 2019. For each of these database the search terms included "prevalence" and "*Campylobacter*". The abstracts and titles were assessed and the articles that met the *a priori* inclusion criteria were selected. Two reviewers independently selected eligible primary studies, with disagreement resolved by consensus.

2.2. Criteria for study selection

Initially, it was evaluated whether the scientific articles fit the selection criteria. This was an initial stage of the analysis, looking for repeated articles, reviews, studies in humans or wild animals or validations of diagnostic techniques. Then, each scientific article was analyzed deeply looking for the statistical information necessary for the meta-analysis. Then, the references cited in these studies were reviewed to determine whether any other trials fit the selection criteria. Data were extracted by one author, and were independently validated by another investigator. Any conflicts were re-solved by consulting a third review.

The scientific papers included in the meta-analysis were selected based on the following criteria: observational study (prevalence studies) and published in peer reviewed journals between 1980 and 2019. When food product of different animal species has been included in one scientific paper, each animal food product was included separately in the meta-analysis as a particular "study". Similarly, when a scientific paper reported the results derived from different conditions (*i.e.*, country of origin, sample type, methodology used to confirm the presence of *Campylobacter* spp.), each condition was considered as an individual study. Therefore, one scientific paper may have been included in the analysis as several studies.

Studies must have reported the total number of samples studied (population) and the number of samples that were positive for the presence of thermotolerant *Campylobacter*. In the studies evaluated, the identification of the isolates of *Campylobacter* spp could be performed based on their typical morphology, biochemical confirmation or, in some studies, PCR detection. When the identification of *Campylobacter* species was available, this information was included in the analysis. Assorted reviews, duplicate reports, detection of *Campylobacter* in artificially contaminated samples, non-peer reviewed articles (i.e., thesis, opinion articles, and editor letters), non-food-producing animals, and randomized controlled trials were excluded of this meta-analysis.

2.3. Outcomes and definitions

Prevalence of thermotolerant *Campylobacter* and its species (*C. jejuni* and *C. coli*) was calculated from the number of positive samples over the total number of samples. Population of study was the type of animal food product investigated in each study.

2.4. Data extraction

Information on the study design, country, years considered, animal food products sampled, type of samples, origin of the samples, methodology to isolate and confirm the thermotolerant *Campylobacter* identity and the outcomes (number of animal food products samples positive to *Campylobacter* and total animal food products sampled), were extracted from each research paper. However, no scores were used to exclude studies (Lean et al. 2009).

2.5. Statistical analysis and subgroups analysis

Statistical analysis was performed used Comprehensive Meta-Analysis version 2.2 (2011). Due to the measured outcome was binary (*i.e.*, an animal food product tests either positive or negative for the pathogen) and was given only for single groups, the only possible parameter to measure effect size was the raw proportion *p* (with 95% confidence intervals –CIs-) using a random effects model (Borenstein et al. 2009). Heterogeneity among studies was assessed using the DerSimonian and the Laird test (Q-statistic). The degree of heterogeneity was quantified with the inconsistency index (I^2-statistic; Higgins and Thompson 2002). Sensitivity analyses have also been used to examine effects of studies identified as being aberrant or highly influential on the analysis outcome (Lean et al. 2009). This consisted of completing the same analysis, but dropping one study in each iteration. Additionally, a cumulative meta-analysis was performed to display how the outcomes shift as a function of the year of publication.

A priori subgroup analyses were planned depending on factors that could potentially influence the prevalence of thermotolerant *Campylobacter* in food products: (1) continents (geographic

distribution), (2) animal species, (3) type of sample (carcasses or part of carcasses, liver, sausages, milk and milk products, eggs, and other food product samples), (4) storage method (ambient, refrigerated, or freeze), and (5) methodology to isolate (agar with or without blood, the use or not of an enrichment broth, and different combination of broths and agar) and identify *Campylobacter* species. For period analyzed subgroup, we considered the year of publication instead of the year when the study was conducted. Normally, the year of publication of a scientific article is usually close (two or three years) to the year in which the study was conducted.

Additionally, a meta-regression analysis was performed to explore the sources of heterogeneity, evaluating the relationship between years of publication and the prevalence of thermotolerant *Campylobacter* using the method of moments. To test the impact of covariates for statistical significance, it is important to quantify the magnitude of their relationship with effect size. For this purpose, we can use an index based on the percent reduction in true variance, analogous to the R^2 index used with primary studies (Borenstein et al. 2009).

The presence of publication bias was investigated using funnel plots. An adjusted rank correlation test using the Egger method (Egger et al. 1997), the Begg's test (Begg and Mazumdar 1994) and the fail-safe N method were used to assess publication bias.

3. Results

3.1. Excluded studies

The literature yielded 8233 scientific papers using the terms "*Campylobacter*" and "Prevalence". Reviews, prevalence studies in humans or in wild animals or pets, randomized controlled experiments, prevalence in non-food-producing animals, studies about laboratory techniques, and studies without enough data to estimate the prevalence were excluded (n= 7701; Figure 1).

3.2. Overview of included studies

Two-hundred and fifty-four out of the 8233 screened scientific articles met all inclusion criteria to estimate *Campylobacter* spp. prevalence (with 667 prevalence studies), while 276 prevalence studies and 217 prevalence studies were included in the evaluation of *C. jejuni* and *C. coli* prevalence, respectively.

From all the studies which estimated the prevalence of *Campylobacter* spp., the most proportion was find in the last period analyzed after 2010 (Figure 2). In the same way studies for *Campylobacter* species were more after 2010 and *C. jejuni* had more number of studies than *C. coli*. The studies were conducted in 64 different countries from all the continents where Europe and North America had the most part of studies (Figure 2).

3.3. Thermotolerant Campylobacter prevalence

Out of the 254 scientific papers that met the inclusion criteria, 667 studies of *Campylobacter* spp. prevalence were identified (143,452 animal-food products analyzed). Based on these 667 studies, the pooled prevalence estimate of *Campylobacter* spp. was 29.6% (95% CI 27.6% - 31%). Across the 667 studies, a significant heterogeneity was observed (Q-statistic: $P< 0.0001$; I^2-statistic= 97.4%).

A total of 276 studies of prevalence of *C. jejuni* were identified (48,607 animal food products analyzed). The prevalence estimate of *C. jejuni* was 19.3% (95% CI 17.4% – 21.3%) and a significant heterogeneity was observed (Q-statistic: $P< 0.0001$; I^2-statistic= 95.94%).

Finally, 217 studies of prevalence of *C. coli* were identified (39,487 animal food products analyzed) and its prevalence estimate was 9.7% (95% CI 8.5% – 11.0%). Significant heterogeneity was observed across the 217 studies (Q-statistic: $P< 0.0001$; I^2-statistic= 94.74%).

3.4. Analysis of subgroups

3.4.1. Prevalence of thermotolerant Campylobacter across continents and species/type of sample analyzed

Studies conducted in North America, African, and European countries showed the highest prevalence of *Campylobacter* spp. (*P*=0.015) whereas studies conducted in Asia showed the lowest prevalence (*p*-estimate= 24.0% 95% CI 20.4% – 27.9%) (Figure 3A). However, these differences may be a reflection of the animal food products sampled in each continent, or due to differences in the experience of *Campylobacter* isolation or difference in the isolation procedure, and not a true difference in the prevalence of this pathogen.

In this sense, the prevalence in swine meat was higher in European (*p*-estimate= 14.6% CI 95% 10.7%-19.6%) and Asian countries (*p*-estimate= 13.1% CI 95% 6.9%-23.4%) compared with Latin America (*p*-estimate= 7.9% CI 95% 2.3%-23.3%) and North America (*p*-estimate= 4.4% CI 95% 2.6%-7.4%) (*P*< 0.001).

In broiler meat, studies conducted in Oceania countries showed the highest prevalence (*p*-estimate= 86.5% CI 95% 74.3%-93.4%). However, these results have to be considered with caution because they are based on only three studies. Broiler meat samples from other continents had prevalence between 43.0% and 52.6%. The prevalence in food products derived from cattle was similar among all countries with values ranged from 0.9% to 8.6% (*P*= 0.177).

If we analyzed *C. jejuni* prevalence in continents, studies conducted in Oceania countries showed the highest prevalence of *C. jejuni* but the number of studies was extremely low (n= 2), so this result has to be considered with caution (Figure 3B). *C. jejuni* prevalence observed in the rest of the continents was relatively similar for broiler, porcine and cattle meat.

Studies conducted in African, Oceania, and European countries showed the highest prevalence of *C. coli* while studies conducted in Latin-America and North-America countries showed the lowest prevalence (*P*< 0.001) (Figure 3C). When animal food products were considered, the prevalence of

C. coli in cattle meat and swine meat were similar in the different continents (*P*= 0.389, *P*= 0.082, respectively). In contrast, the prevalence in broiler meat was the highest in African countries (*p*-estimate= 36.4% CI 95% 14.6%– 65.7%; *P*< 0.001).

3.4.2 Prevalence of thermotolerant Campylobacter in animal species

Laying hens, broiler, and other poultry samples, such as turkey and ducks, showed higher prevalence of *Campylobacter* spp. than cattle and swine food products (*P*< 0.001) (Figure 4A).

Food products derived from laying hens, broiler, and other poultry showed the most important prevalence of *C. jejuni* (*P*< 0.001) (with levels higher than 27.6%) in comparison with the rest of the animal food products (with prevalence below 8.8%) (Figure 4B).

The highest prevalence of *C. coli* in animal food products was observed in broiler and sheep (*p*-estimate= 14.1%, 95% CI 12.3% – 16.1%; and 11.3%, 95% CI 3.6 %– 29.1%, respectively) in comparison with the prevalence in the other animal food products which presented prevalence estimates lower than 10.0% (*P*< 0.001) (Figure 4C).

3.4.3. Prevalence of thermotolerant Campylobacter according to the type of sample and species analyzed

The highest prevalence of *Campylobacter* spp. was observed when the samples were taken from liver (*p*-estimate= 43.2%), and carcasses (*p*-estimate= 40.7%). On the other hand, the lowest prevalence of *Campylobacter* spp. was observed when the samples were taken from eggs (*p*-estimate= 4.0%), milk and milk products (*p*-estimate= 3.5%) (Figure 5A).

If we analyzed results according animal species and type of sample, the prevalence was higher in broiler carcasses (*p*-estimate= 52.3% CI 95% 48.3% – 56.3%) than in swine (*p*-estimate= 13.0% CI 95% 9.6%-17.4%) and cattle carcasses (*p*-estimate= 5.0% CI 95% 2.9% - 8.5%) (*P*< 0.001). When comparing liver samples, broiler showed the highest prevalence (*p*-estimate= 65.5% CI 95% 57.0%

– 73.1%) followed by sheep (*p*-estimate= 57.6% CI 95% 23.2% - 85.9%), cattle (*p*-estimate= 32.1% CI 95% 14.7% - 56.5%) and swine (*p*-estimate= 17.2% CI 95% 8.5% - 32.8%) (*P*< 0.001).

Regarding *C. jejuni* prevalence, eggs, sausages, milk, and milk products showed the lowest prevalence (Figure 5B) whereas carcasses presented the highest prevalence. About *C. coli* prevalence, samples of liver showed the highest prevalence of *C. coli*, whereas milk and milk products, eggs, and sausages presented the lowest prevalence (*P*< 0.001) (Figure 5C).

3.4.4. Prevalence of thermotolerant Campylobacter considering the storage method and type of samples

When animal food products samples were stored without using any refrigeration system (street food), the prevalence of *Campylobacter* spp. was the highest. On the contrary, when animal food products were stored refrigerated or frozen, the prevalence of *Campylobacter* spp. was lower (Figure 6A). However, when analyzed only broiler carcass stored at ambient temperature, the prevalence of *Campylobacter* spp. (*p*-estimate= 57.0% CI 95% 47.9% – 65.7%) was similar to carcasses stored refrigerated or frozen (*p*-estimate= 46.9% CI 95% 43.5% – 50.4%; *p*-estimate= 47.8% CI 95% 28.7% - 67.5%, respectively) (*P*= 0.136).

In contrast, the prevalence of *C. jejuni* in animal food products was different considering the storage conditions. Food products stored frozen showed the highest prevalence (*p*-estimate 38.3%) while food products stored at ambient temperature (street food) showed the lowest prevalence (*p*-estimate 12.9%) (*P*= 0.004) (Figure 6B). However, this results must be analyzed with caution because this meta-analysis could not identify if it is a true prevalence or the result is due to different aspects as: a- initial *Campylobacter* status of food products analyzed b- the microbiology analytic methods of this samples, c- experience of research team for analysis *Campylobacter* in food products.

When broiler carcass was stored at ambient temperature (street food), the prevalence of *C. jejuni* (*p*-estimate= 19.0% CI 95% 10.8% – 31.3%) was lower in comparison with carcasses stored refrigerated (*p*-estimate= 33.5% CI 95% 29.7% – 37.6%) or frozen (*p*-estimate= 38.3% CI 95% 23.7% - 55.4%) (*P*= 0.043).

On the other hand, animal food products preserved at room temperature had similar prevalence of *C. coli* (p-estimate 17.2%) than food products stored refrigerated (p-estimate 9.5%) or frozen (p-estimate 10.9%) (*P*= 0.062) (Figure 6C). This behavior was verified when food products of broiler (*P*= 0.170), swine (*P*= 0.085) and beef (*P*= 0.082) were analyzed separately.

3.4.5. Prevalence of thermotolerant Campylobacter considering isolation agar (with or without blood) and identification method for species

Additionally, we compared the *Campylobacter* spp. prevalence according to the use or not of blood in the composition of isolation agar base. Studies which used agar media with blood, reported similar prevalence of *Campylobacter* spp. (*p*-estimate= 29.7%; CI 95% 26.2% – 33.5%; n= 233) than studies which used agar media without blood (*p*-estimate= 29.2%; CI 95% 26.6% – 31.9%; n= 390) (*P*= 0.845). Similarly, there were not differences between the *C. jejuni* prevalence observed when the studies used the isolation agar with or without blood (*P*= 0.473). In contrast, studies which used agar media with blood, reported a prevalence of *C. coli* lower (*p*-estimate 5.9%; CI 95% 4.6% - 7.5%) than those which used agar media without blood (*p*-estimate 12.4%; CI 95% 10.6% – 14.6%) (*P*< 0.001). However, this effect was only observed when broiler meat (*P*= 0.006) was evaluated but the prevalence of *C. coli* was similar independently the use of agar with or without blood in swine (*P*= 0.209) and bovine meat (*P*= 0.386).

In order to evaluate the impact of different methodologies to isolate *Campylobacter* spp., we studied their impact in *Campylobacter* spp. prevalence. In this way, analyzing the prevalence of *Campylobacter* spp. on the food matrix taking account the use or not of an enrichment broth, results

showed non-differences in prevalence in avian meat (P = 0.162), swine (P= 0.983) or bovine (P= 0.349). Regarding the prevalence of *C. jejuni* or *C. coli* and the influence of the enrichment step for the isolation of the food matrix, non-differences were found between use or not of an enrichment medium (P= 0.196 and P= 0.232, respectively).

On the other hand, we analyzed different combinations of broths and agars used for the isolation of *Campylobacter* spp. We could identify 13 methodologies that grouped almost all the studies analyzed: Bolton broth + a) Campycefex agar (n= 10), b) Campyfood agar (n= 2), c) CCDA agar (n= 85), d) Karmali agar (n= 13), e) mCCDA agar (n= 113), f) Preston agar (n= 9), g) Skirrow agar (n= 2), h) other agars (n= 14); Preston broth + i) CCDA agar (n= 37), j) mCCDA agar (n= 77), k) other agars (n= 76); l) other broths + other agars (n= 82); m) only agars (n= 113). In 26 studies was not possible to identify the protocol used. Results demonstrated that when the analysis was stratified by type of meat matrix (avian, swine and bovine), the type of protocol used in the isolation of *Campylobacter* spp did not have effect in the prevalence (P> 0.05).

Analyzing the type of food matrix analyzed (poultry, swine and bovine meat), the prevalence of *C. jejuni* was similar (P> 0.05) comparing the use of different combinations of broth and agar (including more than 95% of the studies analyzed). Regarding to *C. coli* and the same stratified analysis, protocols employed in more than 70% of the studies evaluated showed similar prevalence for each of the matrices analyzed (P> 0.05).

3.5. Sensitivity analysis and Publication bias

No individual study had a particularly large influence (according with the sensitivity and cumulative analysis) on the summary prevalence of thermotoleant *Campylobacter* estimate.

As part of this study, Egger´s regression test, Begg and Mazumdar rank correlation test, and the fail-safe N method were used to detect publication bias in the studies included (Table 2). There was a general tendency of publication bias for most of the thermotolerant *Campylobacter*. However, the

large number of scientific articles included in this meta-analysis provide valid results beyond the potential bias.

4. Discussion

Thermotolerant *Campylobacter* species are the most frequently identified bacterial cause of human gastroenteritis in many developed and developing countries (EFSA, 2019; CDC, 2014; WHO 2013; Máckiw et al. 2012; Friedman et al. 2000). This human pathogen is part of the intestinal microbiota of a wide range of wild and domestic animals, especially poultry (Newell 2002; Whyte et al. 2004; Abulreesh et al. 2006; Young et al. 2007; Ogden et al. 2009; Jokinen et al. 2011). In this work, we analyze the presence of thermotolerant *Campylobacter* in different animal food products worldwide. According to this meta-analysis, approximately 30% of the animal food products analyzed have *Campylobacter*, regardless of animal species. This result is important because transmission along the food chain is generally accepted as the most frequent route used by the pathogen to generate human campylobacteriosis (Damjanova et al. 2011).

The endemic presence of *Campylobacter* in animal food products may be explained, at least partially, by livestock production systems. This tended to be more intensive during the last decades (Fraser 2008). Several factors support the hypothesis about the adverse effects of modern animal production systems on food safety. In this way, the incidence of *Campylobacter* has been reported to be higher in concentrate rather than forage-fed cattle (Grau, 1988; Bailey et al., 2003; Beach et al., 2002) possibly due to increased stocking densities, high frequency of shared access of cattle to community feed and water troughs and constant physical contact with feces from other animals during confinement (Horrocks et al., 2009).

There are many investigations where *Campylobacter* persistence and diffusion was reported for broiler (Peyrat et al. 2008; Damjanova et al. 2011; Zbrun et al. 2013) and swine (Jensen et al. 2006; Alter et al. 2005; Payot et al. 2004) meat production chains. In this sense, *Campylobacter* presence

in meat, regardless the animal species, is caused by exposure on farms, and can occur anytime during the slaughter, processing and until the meat is served on a plate (EFSA, 2019). This meta-analysis showed that poultry product (laying hens, broiler and turkey food products) are the most important *Campylobacter* source. It is generally accepted that chickens are a natural host for *C. jejuni* and for *Campylobacter* in general, and that colonized broiler chicks are the primary vector for transmitting this pathogen to humans (Hermans et al. 2012). On the other hand, the low prevalence found in cattle or swine food products is mainly due to slaughter process. While poultry intestines are frequently damaged during slaughtering and it contaminated carcasses with pathogens, slaughter process in cattle and swine is more controlled with the aim to prevent the intestines perforation.

According to meta-analysis results, *C. jejuni* was more prevalent in poultry products. The proportion of each *Campylobacter* species observed in poultry is influenced by the productive systems and the age of slaughter of the chickens (Hoepers et al., 2016). On the other hand, the geographical origin of isolates, *C. coli* was more prevalent in Spain, Italy and Bulgaria (EFSA 2010), while *C. jejuni* was more prevalent in South and North America (Zbrun et al. 2013; Newell and Fearnley 2003; Stern et al. 2005).

On the other hand, transmission of *Campylobacter* infections to humans by another *via* different to meat were describe. Consumption of raw milk has been reported in numerous *Campylobacter* outbreaks (Newkirk et al. 2011; Heuvelink et al. 2009; Whyte et al. 2004). The presence of thermotolerant *Campylobacter* in raw milk is commonly the result of faecal contamination during the milking process (Haghi et al. 2015). It is possible that raw milk is one of the links between seasonal trend in cattle faecal shedding and seasonal trend in human campylobacteriosis, especially in warmer months (Bertasi et al. 2016). In this meta-analysis, the prevalence of thermotolerant *Campylobacter* in raw milk and dairy products was low, and it would not appear to be a significant source of contamination in comparison with other sources, such as poultry food product. In the same way, sausages and eggs follow the same pattern of low prevalence and would have a poor

participation in the *Campylobacter* epidemiology. Although these products came from the same animals, the environment in which they are obtained and stored, could have a direct influence on the low prevalence found for *Campylobacter*.

Other sources of *Campylobacter* as offal were described previously. Numerous studies have provided data that suggest that *Campylobacter* can be isolated from offal (especially from liver) from a variety of food animals (Teixeira da Silva et al. 2016; Lazou et al. 2014; Atanassova et al. 2007; Paulsen et al. 2005). It could be explained by cross-contamination between meat and offal during slaughter process. Additionally, thermotolerant *Campylobacter* species have the ability to translocate to the internal environment and reach the livers. Reported prevalence are typically in the range of 50–75% in a number of offal, including pig, ox and lamb livers (Bolton et al. 2002; Kramer et al. 2000). In the present meta-analysis, we found that the average prevalence on liver was 41.8% (considering broiler, porcine, ovine and cattle) and this support Bolton results (2002) being *C. coli* the most prevalent species founded in this animal food product.

On the other hand, another point analyzed in this meta-analysis was the influence on the storage temperature in *Campylobacter* prevalence. Storing under low temperature is a recognized measure for food conservation and it can control the microorganism concentration (Fennema, 2003). In the meta-analysis we observed higher prevalence of *C. jejuni* in frozen samples than in samples stored at room temperature (it is relevant to note that in many countries meat is sold without refrigeration as street food). However, this study could not define if it is due to temperature resistance of *C. jejuni* or if it is due to differences in the sample microbiological status analyzed or the methodology to isolate the pathogen used. Different authors (Ritz et al. 2007; Georgsson et al. 2006; Sampers et al. 2010) reported this result previously. Apparently, chicken skin might provide a protective microenvironment for *C. jejuni,* which can explain its high prevalence in carcasses stored at refrigeration or freezing temperature (Bhaduri and Cottrell 2004). A cross-protection between the cold-shock response and oxidative-stress responses has been reported as an additional explanation

for this situation (Garénaux et al. 2008). *C. jejuni* present in broiler meat stored at room temperature had a stronger oxidative stress than *C. jejuni* present in broiler meat stored at low temperature. On the other hand, *C. jejuni* has been shown to be more resistant to cold and another stress factors than *C. coli* (Madden et al. 2000). This type of stress factor may negatively influence the survivability of *C. coli* through processing measures such as chilling or air exposure. The apparent ability of *C. jejuni* to survive commercial meat storage conditions, suggest that the current methods of meat preservation would not add a significant margin of safety (Balamurugan et al. 2011; Moorhead and Dyes 2002). This should encourage researchers and the industry to generate new conservation processes to decrease the prevalence of thermotolerant *Campylobacter* in chicken carcasses and thus, avoid dissemination to the food chain.

Also, this meta-analysis allowed compare prevalence according the use of blood in culture medium and *Campylobacter* spp identification by biochemical tests or PCR. There are many protocols for the isolation of *Campylobacter* spp. from food samples. However, there is no global consensus on the methodology used to isolate *Campylobacter*. The Horizontal method for detection and enumeration of *Campylobacter* spp. (ISO 10272:2017, part 1 and part 2) has been described for food samples and it is used in most laboratories.

The enrichment step plays an essential role facilitating the growth of *Campylobacter* when there is low number of *Campylobacter* in the sample. Different studies tried to reduce the enrichment time from 48 to 24 hours to avoid competitive bacterial growth, but the results were not successful (Oyarzabal et al., 2007; Liu et al, 2009). On the other hand, the current methodology used in the United States by the Food Inspection and Safety Service of the United States Department of Agriculture, does not include an enrichment step, and only the results of the direct plate are recorded (Food Safety and Inspection Service, 2014). In this sense, this meta-analysis did not find differences in prevalence related to the use or not of the enrichment step for *Campylobacter* isolated for the food matrix.

Few media have been developed and marketed in the last 10 years. However, contradictory results are around the use of these media and the recovery of *Campylobacter* in an artificial inoculated matrix or in natural conditions (Habib 2011). In this way, the most common plate media used to isolate *C. jejuni* and *C. coli* from foods media can be divided into blood-based, charcoal based and others. In this way, Charcoal cefoperazone deoxycholate (CCDA) is the most commonly used selective plate medium worldwide (Oyarzabal and Fernández, 2016, Oyarzabal and Carrillo 2017). However, as a matter of fact, *C. jejuni and C. coli* will grow well on agar media without blood if all the other growth conditions are met (Oyarzabal et al., 2005). Results of this meta-analysis showed that the use of agar medium with or without the addition of blood did not appear to significantly influence the prevalence of *Campylobacter* obtained independently the food matrix analyzed.

While the use of biochemical tests in the identification of *Campylobacter* species could underestimate the prevalence of this pathogen in domestic animals, results of this meta-analysis showed the confirmation of the bacterial isolate was not more precise when PCR methods was applied.

5. Conclusions

This meta-analysis showed the high prevalence of thermotolerant *Campylobacter* in animal food products, especially in liver, carcasses and part of carcasses of different animal species. Milk and milk products, eggs and processed meat foods such as sausages would not be important sources of thermotolerant *Campylobacter*. In contrast, poultry meat was the raw food with the highest prevalence of thermotolerant *Campylobacter*. *C. jejuni* was more prevalent in poultry meat, whereas *C. coli* was more prevalent in poultry and lamb meat. In addition, while low temperature is a recognized methodology for food preservation, it does not appear to be a measure that alone has a significant effect on reducing the prevalence of *C. jejuni* in food animal products. Also, the use of agar media with or without the addition of blood did not influence the prevalence of *Campylobacter* obtained. The prevalence of thermotolerant *Campylobacter* in animal food products requires the

implementation of risk measures. Intensive systems of animal production, poor hygienic measures in slaughterhouses and food processing plants, and storage conditions are important points to review with the aim to control this pathogen in animal food products.

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Figure 1: Flow diagram of selected studies included in the meta-analysis

Figure 2: Studies included for *Campylobacter* spp. in the meta-analysis according to year of publication and continent.

Figure 3: Subgroup analysis comparing the prevalence of thermotolerant *Campylobacter* across the continents.

References: A) *Campylobacter* spp.; B) *Campylobacter jejuni*; C) *Campylobacter coli*; point estimate= prevalence

Figure 4: Subgroup analysis comparing the prevalence of thermotolerant *Campylobacter* across the animal species.

References: A) *Campylobacter* spp.; B) *Campylobacter jejuni*; C) *Campylobacter coli*; point estimate= prevalence

Figure 5: Subgroup analysis comparing the prevalence of thermotolerant *Campylobacter* according to the type of sample.

References: A) *Campylobacter* spp.; B) *Campylobacter jejuni*; C) *Campylobacter coli*; point estimate= prevalence

Figure 6: Subgroup analysis comparing the prevalence of thermotolerant *Campylobacter* considering the storage method.

References: A) *Campylobacter* spp.; B) *Campylobacter jejuni*; C) *Campylobacter coli*; point estimate= prevalence

Table 1: Results of publication bias detection.

Table 1 (Supplemental material): List of studies included in the meta-analysis.

References: [1] n= number of samples; [2] n(+) number of samples positive to *Campylobacter*.

Table 1. Results of publication bias detection.

Response variable	Fail-safe N[a]	Begg and Mazumdar Test	Egger´s regression test	
			Intercept	*P*-value
Campylobacter spp.	0	<0.001	0.4235	0.226
C. jejuni	0	<0.001	-2.5499	<0.001
C. coli	0	<0.001	-3.385	0.003

References: [a] Number of studies required to reverse the effects are calculated on the condition of *P*= 0.05.

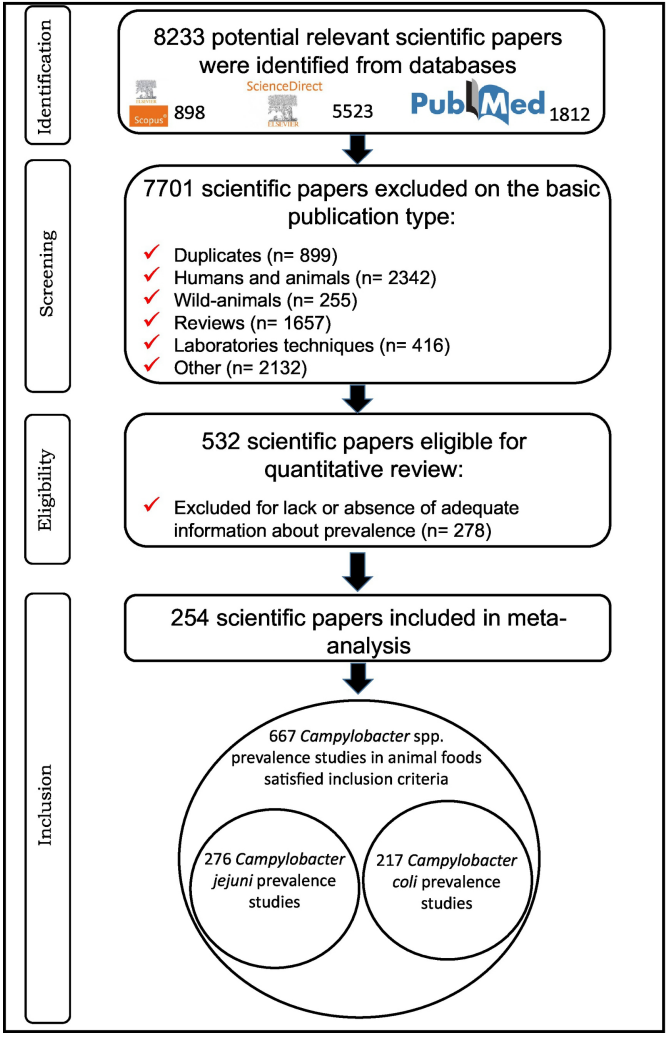

Identification
8233 potential relevant scientific papers were identified from databases
Scopus 898
ScienceDirect 5523
PubMed 1812
Screening
7701 scientific papers excluded on the basic publication type:
✓ Duplicates (n= 899)
✓ Humans and animals (n= 2342)
✓ Wild-animals (n= 255)
✓ Reviews (n= 1657)
✓ Laboratories techniques (n= 416)
✓ Other (n= 2132)
Eligibility
532 scientific papers eligible for quantitative review:
✓ Excluded for lack or absence of adequate information about prevalence (n= 278)
Inclusion
254 scientific papers included in meta-analysis
667 Campylobacter spp. prevalence studies in animal foods satisfied inclusion criteria
276 Campylobacter jejuni prevalence studies
217 Campylobacter coli prevalence studies

Figure 1

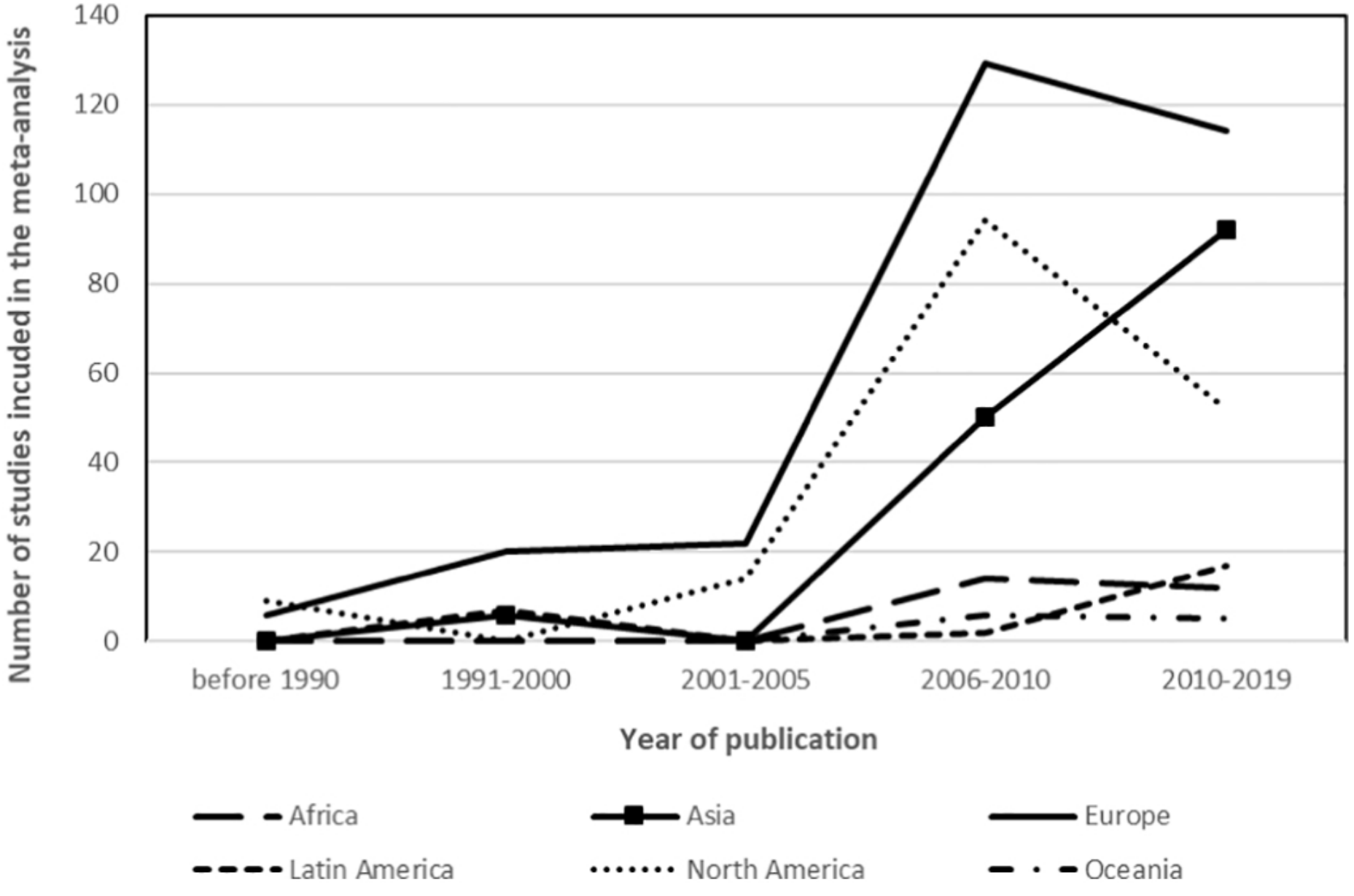
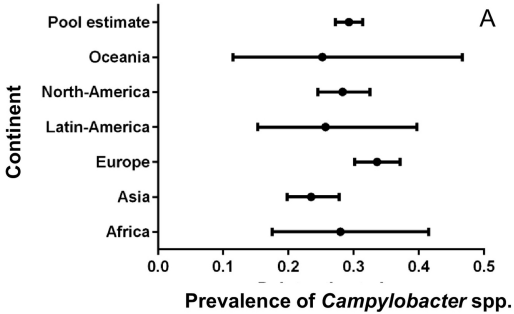
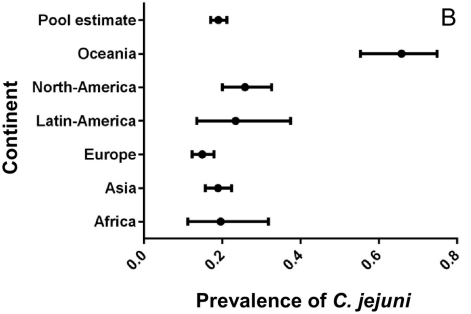

Number of studies incuded in the meta-analysis
140
120
100
80
60
40
20
0
before 1990
1991-2000
2001-2005
2006-2010
2010-2019
Year of publication
Africa
Asia
Europe
Latin America
North America
Oceania

Figure 2


A
Pool estimate
Oceania
North-America
Latin-America
Europe
Asia
Africa
Continent
0.0
0.1
0.2
0.3
0.4
0.5
Prevalence of Campylobacter spp.


B
Pool estimate
Oceania
North-America
Latin-America
Europe
Asia
Africa
Continent
0.0
0.2
0.4
0.6
0.8
Prevalence of C. jejuni

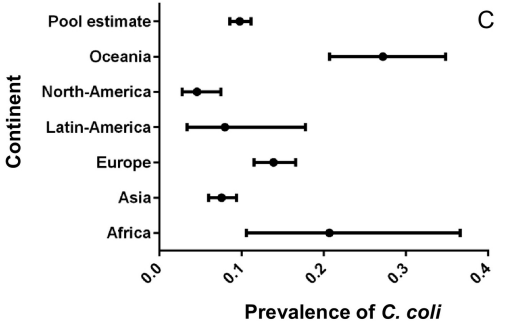
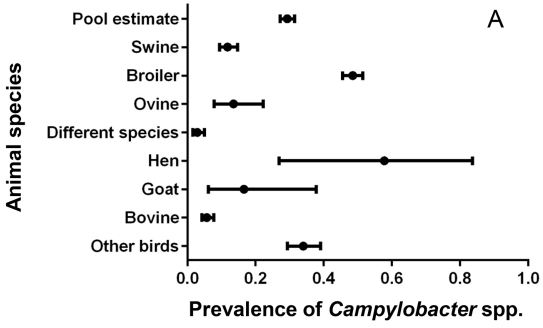
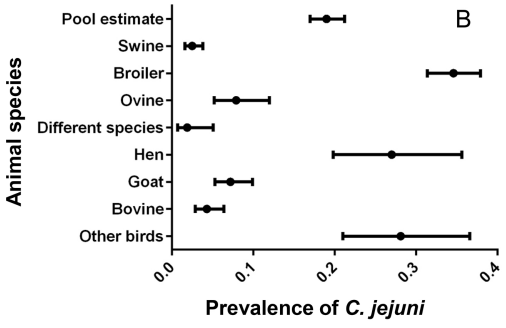

C
Pool estimate
Oceania
North-America
Latin-America
Europe
Asia
Africa
Continent
0.0
0.1
0.2
0.3
0.4
Prevalence of C. coli

Figure 3


A
Pool estimate
Swine
Broiler
Ovine
Different species
Hen
Goat
Bovine
Other birds
Animal species
0.0
0.2
0.4
0.6
0.8
1.0
Prevalence of Campylobacter spp.


B
Pool estimate
Swine
Broiler
Ovine
Different species
Hen
Goat
Bovine
Other birds
Animal species
0.0
0.1
0.2
0.3
0.4
Prevalence of C. jejuni

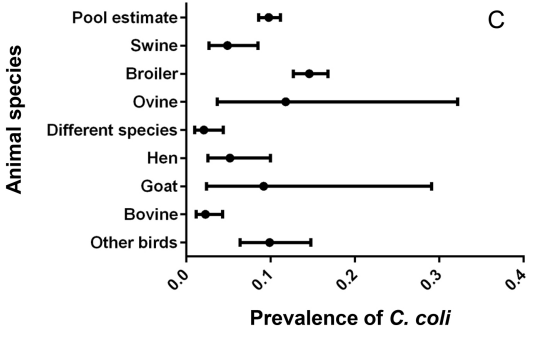
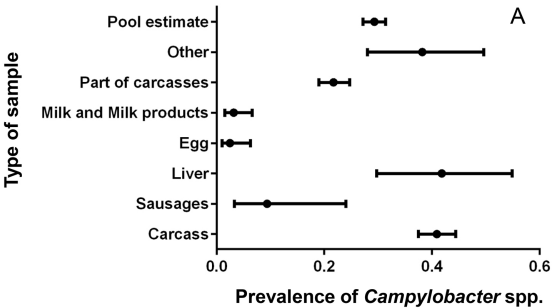
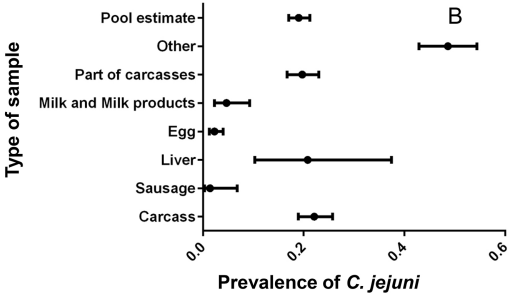

C
Pool estimate
Swine
Broiler
Ovine
Different species
Hen
Goat
Bovine
Other birds
Animal species
0.0
0.1
0.2
0.3
0.4
Prevalence of C. coli

Figure 4


A
Type of sample
Pool estimate
Other
Part of carcasses
Milk and Milk products
Egg
Liver
Sausages
Carcass
0.0
0.2
0.4
0.6
Prevalence of Campylobacter spp.


B
Type of sample
Pool estimate
Other
Part of carcasses
Milk and Milk products
Egg
Liver
Sausage
Carcass
0.0
0.2
0.4
0.6
Prevalence of C. jejuni

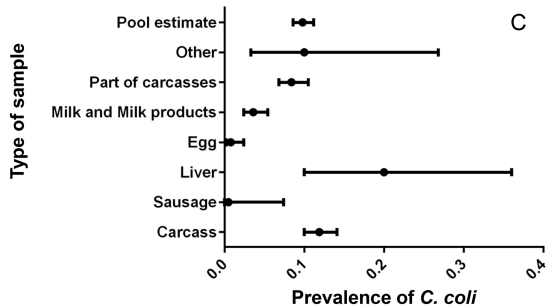
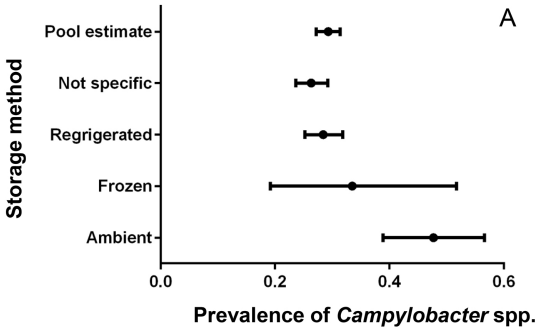
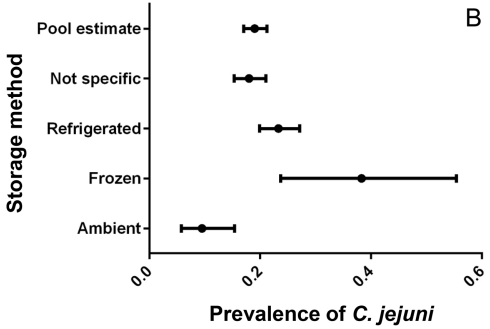

C
Type of sample
Pool estimate
Other
Part of carcasses
Milk and Milk products
Egg
Liver
Sausage
Carcass
0.0
0.1
0.2
0.3
0.4
Prevalence of C. coli

Figure 5


A
Pool estimate
Not specific
Regrigerated
Frozen
Ambient
Storage method
0.0
0.2
0.4
0.6
Prevalence of *Campylobacter* spp.


B
Pool estimate
Not specific
Refrigerated
Frozen
Ambient
Storage method
0.0
0.2
0.4
0.6
Prevalence of *C. jejuni*

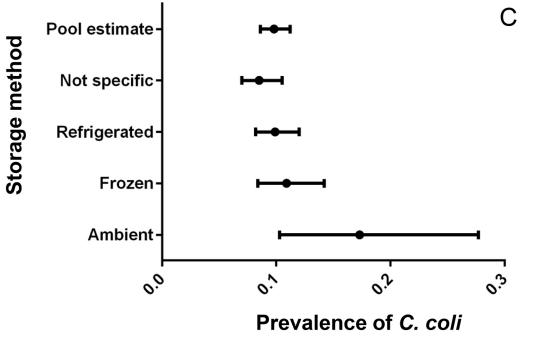

C
Pool estimate
Not specific
Refrigerated
Frozen
Ambient
Storage method
0.0
0.1
0.2
0.3
Prevalence of *C. coli*

Figure 6