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Prevalence and levels of *Campylobacter* in broiler chicken batches and carcasses in Ireland in 2017–2018

Helen Lynch [a,b,*], Peter Franklin-Hayes [a], Leonard Koolman [c], John Egan [b], Montserrat Gutierrez [b], William Byrne [b], Olwen Golden [b], Declan Bolton [c], Paula Reid [c], Aidan Coffey [d], Brigid Lucey [d], Lisa O'Connor [e], Kilian Unger [f], Paul Whyte [a]

[a] UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland
[b] National Reference Laboratory Campylobacter, Backweston Campus, Celbridge, Ireland
[c] Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland
[d] Department of Biological Sciences, Munster Technological University, Cork, Ireland
[e] Food Safety Authority of Ireland, Dublin, Ireland
[f] Department of Agriculture, Food and the Marine, Kildare St, Dublin 2, Ireland

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ABSTRACT

In 2008, an EU wide baseline survey of broilers revealed a high *Campylobacter* prevalence. To assist with industry-wide controls, updated data were required. The primary objective of this study was to establish up-to-date data on *Campylobacter* carriage and carcass contamination in Irish broilers. Monthly samples were collected from the three largest broiler processing plants in Ireland over a twelve-month period. Samples were taken from both first and final thin birds (partial and full depopulation) from 358 batches of broilers. From each batch, a composite sample of 10 caecal contents ($n = 358$) and 5 neck skins ($n = 1790$) were collected and numbers of *Campylobacter* in each sample were determined. Of the 1790 neck skin samples tested, 53% were *Campylobacter* positive. *Campylobacter* was detected in the caecal contents of 66% of all batches tested. Depopulation and/or age had a significant effect on *Campylobacter* prevalence with 67% of final thin broilers yielding *Campylobacter*-positive neck skin samples in contrast to 38% of first thin broilers that yielded positive neck skin samples ($P \leq 0.002$). A significant seasonal variation was observed in the rate of *Campylobacter*-positive caecal samples with higher prevalence seen in July (85%) than the colder months of November (61%), December (50%), January (61%) March (57%) and April (59%). Neck skin samples were 7 times more likely to be *Campylobacter* positive if the caecal contents from the same batch were positive (odds ratio = 7.1; $P \leq 0.0001$). The decrease in *Campylobacter* prevalence observed in neck skin and caecal contents demonstrates the improvements and progress made in reducing prevalences of this important enteropathogen in the Irish poultry industry since the 2008 EU baseline survey. It also provides further supporting data on the impact of thinning, the processing environment and season on *Campylobacter* prevalence.

1. Introduction

Campylobacter has been the most commonly reported gastrointestinal bacterial pathogen in humans in the European Union (EU) since 2005. In 2017, 246,158 confirmed cases of human campylobacteriosis were reported in the EU with 37.4% of all cases linked to fresh meat from broilers (EFSA, 2018). *Campylobacter* present in the gastrointestinal tract of broilers can be readily transferred to carcasses during slaughter and processing which can result in extensive cross-contamination within the processing plant (Kottawatta et al., 2017; Meredith et al., 2013). According to EFSA, 20% - 30% of human cases of campylobacteriosis are attributed to the handling, preparation and consumption of broiler meat while the chicken reservoir as a whole may account for 50% - 80% of cases (EFSA, 2010).

In 2008, an EU wide baseline survey was coordinated by the European Food Safety Authority (EFSA) to estimate *Campylobacter* prevalence in broiler batches (caecal contents) and on carcasses at the slaughterhouse level. The results of this study performed in four Irish

* Corresponding author at: UCD School of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland.
E-mail address: helen.lynch@agriculture.gov.ie (H. Lynch).

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slaughterhouses revealed an 83.1% *Campylobacter* prevalence in broiler batches ($n = 394$) and 98.3% of carcasses ($n = 394$) being contaminated with *Campylobacter* at the end of the slaughter process (EFSA, 2010). In response to these results, the Food Safety Authority of Ireland (FSAI) requested its scientific committee to provide practical recommendations on a control programme for *Campylobacter* in the Irish broiler production and slaughter chain (FSAI, 2011).

It was estimated that a 50% reduction in public health risk from the consumption of broiler meat could be accomplished if all batches complied with microbiological criteria setting a critical limit of 1000 (10^3) CFU/g of neck skin (EFSA, 2011). Thus, the European Commission recently introduced a process hygiene criterion (PHC) for the broiler sector which aims at controlling *Campylobacter* contamination of carcasses during the slaughter process (Commission Regulation (EC) No 2073/2005, as amended by Commission Regulation (EU) 2017/1495). When the criterion was first introduced in January 2018, no more than 20 samples could exceed the limit of 1000 CFU/g within each 10 week period. In January 2020, the criterion became more stringent, allowing no more than 15 samples to exceed 1000 CFU/g within each ten-week period; this will change to no more than 10 samples in January 2025.

Despite the substantial amount of research performed in the last 10 years since the EU baseline survey and on-going dialogue between stakeholders and the research community significant data gaps existed prior to the start of this study. The aim of the current study was to establish up-to-date data on *Campylobacter* carriage and carcass contamination in Irish broilers and examine the relationship between *Campylobacter* concentrations in the caecal contents and on processed carcasses. The data generated will be useful in measuring progress since the 2008 Baseline survey and in estimating the proportion of batches of birds (both first and final thin) that would comply with the new PHC.

2. Materials and methods

2.1. Survey design

This survey was conducted in Ireland between September 2017 and August 2018 and was conducted at the broiler-batch level (pooled caecal samples from broilers and neck skin samples from batches). Monthly samples were collected of birds entering the food chain from the three main broiler processing plants in the country. The total number of batches sampled was 358 with 178 batches from first thin birds and 180 from final thin birds. Samples were taken from first and final thin birds from both conventional and free-range production systems in order to examine the impact of partial depopulation at first thin on *Campylobacter* prevalence. Samples collected from first thin and final thin birds were not always collected from the same batches as this was logistically impossible in the processing plants. The number of batches sampled from free range broiler farms was 77 compared to 281 batches which originated from conventional production systems. Broilers sampled from a free range setting were between 56 and 58 days old and birds from a conventional farm were between 30 and 43 days old.

Examined broiler batches were selected as randomly as possible with regard to slaughterhouses, day of the week, age of broilers and production type (conventionally reared or free range). However, Fridays were excluded due to difficulties in dispatching the samples to the laboratory. Samples were processed at the National *Campylobacter* Reference Laboratory, Department of Agriculture, Food and the Marine, Backweston, Kildare.

2.2. Sample collection and transport

For each randomly selected broiler batch, intact caecal contents of 10 birds were collected aseptically at evisceration by official veterinarians and placed in a single sterile container for transport. Birds were sampled at random throughout the batch, avoiding the first part of the batch to be slaughtered and collecting samples from non-consecutive birds.

Moreover, from the same batch, 5 neck skins were collected from 5 slaughtered birds immediately after chilling for *Campylobacter* spp. enumeration. The samples collected were placed in 5 separate plastic bags, avoiding cross contamination. All relevant information was recorded for each batch of birds including flock age, mean weight, flock size, first/final thin status, on-farm house number, line speed and time of day. Samples from the same batch were packaged together in a large plastic bag with a cooling ice block, transported to the laboratory within 24 h and analysed as soon as possible. In cases where this was not possible, the samples were refrigerated and analysed no later than 72 h after sampling in line with the sampling to testing times performed in the 2008 EFSA Baseline survey (EFSA, 2010). A styrofoam layer was used to ensure the cooling block within the transport box did not come into direct contact with the samples as this could affect *Campylobacter* levels.

2.3. Enumeration of Campylobacter from neck skins and caecal contents

The enumeration of *Campylobacter* in each sample was undertaken as described in ISO 10272-2:2006 with a modification to include tazobactam in the modified charcoal cefoperazone deoxycholate agar (mCCDA) (Syntec, Dublin, Ireland), to inhibit interference from extended spectrum β-lactamase *E. coli*. This TmCCDA was prepared by adding 1 mg/l tazobactam to mCCDA (ISO, 2006; Smith et al., 2015). Ten grams ±0.5 g of neck skin was weighed avoiding any fat and placed into a stomacher bag before adding 90 ml ± 2 ml of buffered peptone water (BPW) (Lab M ltd., Bury, UK). If a 10 g test portion was not available, the weight of the neck skin was noted and an appropriate one in ten dilution with BPW was performed. This mixture was stomached for approximately 1 min and a 10-fold dilution series (from 10^{-1} to 10^{-6}) was prepared. Dilutions (100 μl) were spread plated in duplicate onto TmCCDA and incubated micro-aerobically at 41.5 ± 1 °C for 44 ± 4 h using sealed boxes and micro-aerobic atmosphere-generating sachets (GENbox, BioMerieux®, Lyon, France). Caecal samples were prepared by spraying the surface of each intact caecum with 70% ethanol (Hannson et al., 2010) and allowed to dry for 1–2 min before an incision was made aseptically and the caecal contents extracted. One gram of contents from each of the 10 caeca was extracted and mixed together using a sterile cotton swab. One gram ±0.1 g of this mixture was added to 9 ml of BPW. Pooled samples were mixed by vortexing and a 10-fold dilution series (from 10^{-1} to 10^{-6}) prepared which was then plated in duplicate onto TmCCDA and incubated as described for the neck skin samples to enumerate *Campylobacter* levels (ISO, 2006). Following incubation, plates were examined and all typical colonies were counted.

2.4. Campylobacter identification and speciation

From each sample, 5 random typical colonies were chosen for subculturing for the confirmatory tests. Each of the 5 colonies selected was streaked onto Columbia Blood agar (Syntec, Dublin) and incubated at 41.5 ± 1 °C for 44 ± 4 h in a microaerobic atmosphere. The same 5 colonies were also streaked onto another Columbia Blood agar plate and incubated microaerobically at 25 ± 1 °C for 44 ± 4 h. However no *Campylobacter* was identified from the 25 °C incubated Blood agar plates. Following incubation one colony from each blood agar plate incubated at 41.5 ± 1 °C was examined for morphology and "corkscrew" motility using a microscope and tested for oxidase production. Following confirmation, *Campylobacter* counts (CFU/g) were calculated for each sample. The 5 confirmed colonies were then speciated using a Bruker Microflex™ LRF Matrix-Assisted Laser Desorption Ionization Time-of-Flight mass spectrometer (MALDI-TOF) under Flex control software (v.3; Bruker, Massachusetts, USA). To do this, colonies were smeared onto the MALDI-TOF target plate (MSP 96 target Polished steel BC; Bruker, MA, USA) using a toothpick. Following this, 1 μl of crystalline, non-liquid matrix solution, α-cyano-4-hydroxycinnamic acid (HCCA) (Bruker, MA, USA) was pipetted over each smear. Each smear

was ionised through protonation and deprotonation and then entered the mass analyser component of the mass spectrometer. A mass spectrum was created for each smear and this was compared and matched to reference strains in the 'MBT Compass Explorer – Bio-Typer database' (Bruker, MA, USA) for identification. Each spectrum was calibrated using a bacterial test standard (BTS) (Bruker, MA, USA). For each *Campylobacter* positive sample identified, one strain (in the case of all confirmed isolates being one species) or two strains (in the case of a mixed sample) were stored frozen at −80 °C in defibrinated horse blood (Fannin, Leopardstown, Dublin).

2.5. Statistical analysis

Microbial counts were converted to $\log_{10}$ CFU/g with a limit of detection (LOD) of 1.7 $\log_{10}$ cfu/g with counts below the LOD considered *Campylobacter* negative. All statistical analysis was performed using SAS version 9.13 (Statistical Analysis Software; Cary, NC, USA). For a descriptive summary of the *Campylobacter* enumeration results, counts were converted to a logarithmic scale to approximate the results to normal distribution with values below the LOD assigned a 0.1 value. Descriptive analysis was performed by means of frequency (N%) of *Campylobacter* positive and negative carcasses and batches. Univariable logistic regression models were performed using the GENMOD procedure in SAS and were used to screen for potential risk factors (depopulation, age category, time of day of slaughter, processing plant, caecal colonisation, season). All tests of difference were at a statistical significance level $\alpha = 0.05$.

3. Results

During the 12 month period 358 slaughter batches were sampled from three processing plants in Ireland. Of these, 207 were slaughtered at Plant A, 115 at Plant B and 36 at Plant C. These batches originated from 194 Irish flocks, with 70% (249/358) of the sampled batches originating from flocks which were sampled more than once during first and subsequent final thin during the survey. The majority of the batches included were from conventionally reared flocks (78%, 281/358 batches) whereas 77 (22%) batches came from free-range flocks. The free range flocks were sampled at either first or final thin.

In total, 1790 neck skin samples were tested with 947 (53%) identified as *Campylobacter* positive. *Campylobacter* spp. were detected in 805 (68.2%) of 1180 neck skin samples from colonised broiler batches, relative to 142 (23.3%) of 610 neck skin samples from batches of broilers with *Campylobacter* negative caecal results (Table 1). Of the 805 neck skin positive samples from batches that were caecal positive for *Campylobacter*, 709 (88%) were less than the PHC limit of 1000 CFU/g. In addition, of the 142 neck skin positive samples from batches that were caecal negative for *Campylobacter*, 127 (89%) were less than the PHC limit of 1000 CFU/g. Depopulation/age had a significant effect on *Campylobacter* prevalence with 67% (605/900) of neck skins originating from final thin birds being *Campylobacter* positive compared to 38% (342/890) at first thin. Neck skin samples were significantly more likely to be *Campylobacter* positive if they originated from final thin birds compared to first thin; odds ratio = 3.3 (95% CI: 2.3–4.7 ($P \leq 0.0001$)) (Table 2). Neck skin samples were significantly more likely to have a positive contamination status if the caecal contents from the same batch were *Campylobacter* positive; odds ratio = 7.1 (95% CI: 4.7–10.7; $P \leq 0.0001$) (Table 1). Out of a total of 120 broiler batches for which caecal contents were *Campylobacter* free entering the processing plant the carcasses of 47 (39%) of these broiler batches were later contaminated during the slaughter process with levels of up to 3.75 Log_{10} CFU/g found on the neck skin samples (Table 1). The percentage of *Campylobacter* positive neck-skins amongst the processing plants is presented in Table 2.

Campylobacter was detected in the caecal contents of 66% (238/358) of all batches tested. Over half (52%; 93/178) of the pooled caecal contents originating from first thin batches were *Campylobacter* positive compared to 81% (145/180) which came from final thin batches. Batches were significantly more likely to be found colonised if they originated from final thin birds compared to first thin birds; odds ratio = 3.8 (95% CI: 2.4–6.1 ($P \leq 0.0001$) (Table 2).

Initial analysis using logistic regression modelling comparing conventional vs. free range was not statistically significant (odds ratio of 1.5 with confidence intervals of 1.0 to 2.2) so this data wasn't included in Table 2. As such, it was decided to group conventional and free range batches together in the analyses similar to the EFSA (2010) baseline study. Batches from a free-range setting had a higher prevalence of *Campylobacter* with 78% (60/77) of all batches *Campylobacter* positive compared to 63% (178/281) reared in a conventional system.

The age of the broilers at slaughter had a significant effect on the contamination status of the neck skin samples. (Table 2). Significant differences were observed when comparing age groups 35–39 with 40–44 (odds ratio = 0.4 ($P = 0.0004$)) 40–44 with ≤35 (odds ratio = 3.6 ($P \leq 0.0001$) and 40–44 with ≥45 (odds ratio = 3.3 ($P \leq 0.0001$).

Neck skin samples from broilers slaughtered in the afternoon/evening (65%; 160/245) had a significantly higher *Campylobacter* prevalence than those slaughtered in the early morning/mid-morning (51%; 787/1545); odds ratio = 1.8 (95% CI: 1.1–3.1) ($P = 0.02$)) (Table 2).

The monthly percentage prevalence of *Campylobacter* colonised batches and on carcasses by processing plant is shown in Table 3. The processing plant with the highest *Campylobacter* prevalence was Plant C with 81% of batches colonised; followed by Plant B with 70% and finally Plant A with 62%. Plant C had significantly higher percentage of *Campylobacter* colonised batches when compared to Plant A ($P = 0.03$) but not when compared to Plant B ($P = 0.23$). Plant C also had a significantly higher percentage of *Campylobacter* contaminated neck skin samples when compared to Plant A ($P = 0.003$) and Plant B ($P \leq 0.0001$).

A seasonal variation was observed in the caecal samples with higher prevalence seen in July (85%) than the colder months of November (61%), December (50%), January (61%) March (57%) and April (59%) (Table 3).

Of 947 *Campylobacter*-positive neck skin samples, the percentage of neck skin samples with *Campylobacter* enumeration results between 1.00 and 1.99Log_{10} CFU/g, 2.00–2.99Log_{10} CFU/g and > 3.00Log_{10} CFU/g were 33% (316/947), 54% (514/947) and 13% (117/947), respectively (Table 4). Of the 342 *Campylobacter* positive carcasses which originated from first thin broilers, 7% had counts which exceeded the critical limit of the PHC, compared to 15% from final thin broilers. The *Campylobacter* enumeration counts did not differ significantly between the three

Table 1
Frequency of caecal contents x neck skin *Campylobacter* status and neck skin x caecal contents *Campylobacter* status.

	Caecal contents		
Neck skin samples	Positive	Negative	Total
Positive	805 (45%)[a]	142 (8%)	947 (53%)
Negative	375 (21%)	468 (26%)	843 (47%)
Total	1180 (66%)	610 (34%)	1790 (100%)

	Neck skin samples		
Caecal contents	Positive	Negative	Total
Positive	213 (59%)	25 (7%)	238 (66%)
Negative	47 (14%)	73 (20%)	120 (34%)
Total	260 (73%)	98 (27%)	358 (100%)
Unadjusted OR	7.1		
95% CI	4.7–10.7		
P-value	0.0001		

[a] Figures in parentheses refer to the percentage of total neck skin samples or caecal contents.

Table 2
Univariable analysis: Association between exposure variables and *Campylobacter* status in broiler carcasses ($n = 1790$) and broiler batches ($n = 358$) at slaughter.

Neck skin samples[a]						Caecal content samples (flocks)[a]				
Variable	No. of positives/ No. of samples (%)	Comparison	UOR	95% CI	P-value	No. of positives/No. of samples (%)	Comparison	UOR	95% CI	P-value
Processing Plant										
Plant A	544/1035 (53%)	Plant A v Plant C	0.5	0.4–0.7	0.003	128/207 (62%)	Plant A v Plant C	0.4	0.2–0.9	0.0348
Plant B	282/575 (49%)	Plant A v Plant B	1.1	0.9–1.4	0.1762	81/115 (70%)	Plant A v Plant B	0.7	0.4–1.1	0.1223
Plant C	121/180 (67%)	Plant C v Plant B	2.1	1.5–3.0	≤0.0001	29/36 (81%)	Plant C v Plant B	1.7	0.7–4.3	0.2372
Depopulation										
First Thin	342/890 (38%)					93/178 (52%)				
Final Thin	605/900 (67%)	Final v First Thin	3.3	2.3–4.7	≤0.0001	145/180 (81%)	Final v First Thin	3.8	2.4–6.1	≤0.0001
Age Category (Days)[b]		35–39 v 40–44	0.4	0.2–0.6	0.0004		35–39 v 40–44	0.3	0.1–0.7	0.0024
<35	222/505 (44%)	35–39 v < 35	1.4	0.9–2.2	0.1708	49/101 (49%)	35–39 v < 35	1.8	1.0–3.2	0.0366
35–39	269/515 (52%)	35–39 v ≥ 45	1.3	0.8–2.0	0.2993	65/103 (63%)	35–39 v ≥ 45	0.5	0.252–0.965	0.0390
40–44	281/380 (74%)	40–44 v < 35	3.6	2.1–6.2	≤0.0001	64/76 (84%)	40–44 v < 35	5.7	2.7–11.7	≤0.0001
≥45	175/380 (46%)	40–44 v ≥ 45	3.3	1.9–5.7	≤0.0001	59/76 (78%)	40–44 v ≥ 45	1.5	0.7–3.5	0.3040
		<35 v ≥ 45	0.9	0.6–1.5	0.7324		<35 v ≥ 45	0.3	0.1–0.5	0.0001
Time of Day										
Early/Mid Morn	787/1545(51%)					n/a				
Afternoon/ Evening	160/245 (65%)	Afternoon/Evening v Early/Mid Morn	1.8	1.1–3.1	0.0285	n/a	n/a	n/a	n/a	n/a

[a] No. - Number; UOR - Unadjusted Odds ratio; CI - Confidence interval.
[b] The age of two broiler batches could not be specified and were omitted from the analysis (neck skin $n = 1780$; caecal contents $n = 356$).

Table 3
Total monthly percentage of *Campylobacter* contaminated broiler carcasses and batches from September 2017 to August 2018.

		Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Plant A	*NS*	63% (44/70)	47% (35/75)	58% (52/90)	33% (25/75)	49% (49/100)	47% (35/75)	40% (34/85)	48% (41/85)	56% (56/100)	56% (53/95)	79% (79/100)	48% (41/85)
	Caeca	71% (10/14)	67% (10/15)	61% (11/18)	47% (7/15)	55% (11/20)	53% (8/15)	47% (8/17)	53% (9/17)	60% (12/20)	63% (12/19)	90% (18/20)	71% (12/17)
Plant B	*NS*	68% (34/50)	60% (33/55)	52% (26/50)	38% (15/40)	40% (20/50)	66% (23/35)	40% (20/50)	24% (11/45)	56% (28/50)	76% (38/50)	18% (9/50)	50% (25/50)
	Caeca	80% (8/10)	64% (7/11)	60% (6/10)	63% (5/8)	70% (7/10)	100% (7/7)	60% (6/10)	67% (6/9)	70% (7/10)	80% (8/10)	70% (7/10)	70% (7/10)
Plant C	*NS*	33% (5/15)	100% (15/15)	67% (10/15)	47% (7/15)	67% (10/15)	87% (13/15)	60% (9/15)	47% (7/15)	67% (10/15)	80% (12/15)	80% (12/15)	73% (11/15)
	Caeca	66% (2/3)	100% (3/3)	67% (2/3)	33% (1/3)	67% (2/3)	100% (3/3)	100% (3/3)	67% (2/3)	67% (2/3)	100% (3/3)	100% (3/3)	100% (3/3)
Total	*NS*	61% (83/135)	57% (83/145)	57% (88/155)	36% (47/130)	48% (79/165)	57% (71/125)	42% (63/150)	41% (59/145)	57% (94/165)	64% (103/160)	61% (100/165)	51% (77/150)
	Caeca	74% (20/27)	69% (20/29)	61%[a] (19/31)	50%[a] (13/26)	61%[a] (20/33)	72% (18/25)	57%[a] (17/30)	59%[a] (17/29)	64% (21/33)	71% (23/32)	85%[b] (28/33)	73% (22/30)

NS – Neck skin; () - Number of samples positive/number of samples tested.
[a,b,] Within the Total row values not sharing a common superscript are significantly different ($p \leq 0.05$).

processing plants ($P \leq 0.65$). The percentage of *Campylobacter* positive neck skins tested with levels >3.00 Log_{10} was 13% in Plant A; 10% in Plant B and 21% in Plant C (Table 4). Table 5 reports the prevalence of *Campylobacter* contamination from the neck skins sampled within each batch. This contamination seemed random without any obvious pattern occurring. Table 6 depicts the *Campylobacter* species identified from the neck skin samples and the pooled caecal samples. No other species were identified in the study and there were no unidentified isolates.

4. Discussion

This study examined the levels of *Campylobacter* in caecal contents of Irish broiler batches and on corresponding carcasses over a 12 month period in the three main processing plants in Ireland. The main objective of the study was to provide updated current data on *Campylobacter* prevalence and levels in Ireland continuing on from the last known baseline study in 2008 (EFSA, 2010). The results suggest that the prevalence of *Campylobacter* in broilers in Ireland has declined since then with a 45% point decrease (98.3% down to 53%) in contaminated carcasses and a 17% point reduction (83.1% down to 66%) in colonised batches. However, there are some differences in the studies design between the two surveys; 384 batches were analysed in 2008 compared to 358 in this study although only one neck skin sample was collected per batch in the 2008 study compared to five per batch in the current study. Nevertheless, the reduction may be due to a concerted effort by the various stakeholders, informed by active research and monitoring programmes (Battersby et al., 2016; FSAI, 2011; Koolman et al., 2014). In more recent years, these activities have been driven by a *Campylobacter* Stakeholder Group, a government initiative that includes representatives of farmers, processors and retailers with the common aim to share

Table 4
Campylobacter count ranges on positive carcasses and in positive caecal contents from each processing plant.

Campylobacter counts (Log_{10} CFU/g)	Neck skins			
	Plant A	Plant B	Plant C	Total
1.00–1.99	146/544 (27%)	120/282 (42%)	19/121 (16%)	316/947 (33%)
2.00–2.99	258/544 (47.5%)	134/282 (48%)	76/121 (63%)	514/947 (54%)
≥3.00	59/544 (11%)	28/282 (10%)	26/121 (21%)	117/947 (12%)
	Caecal Contents			
4.00–5.99	6/128 (5%)	2/81 (2%)	1/29 (3%)	9/238 (4%)
6.00–7.99	78/128 (61%)	58/81 (72%)	17/29 (59%)	153/238 (64%)
≥ 8.00	44/128 (34%)	21/81 (26%)	11/29 (38%)	76/238 (32%)

Table 5
Prevalence of *Campylobacter* contamination on neck skin samples within the 358 broiler batches analysed.

No. of neck skin samples per batch positive or negative for *Campylobacter*[a]	Prevalence (no of batches/%)
0	100 (28%)
1	43 (12%)
2	28 (8%)
3	32 (9%)
4	30 (8%)
5	125 (35%)
Total	358 (100%)

[a] $n = 5$ neck skin samples analysed per batch.

Table 6
Campylobacter species characterised from positive neck skin and pooled caecal content samples[a].

	C. jejuni	*C. coli*	*C. jejuni/C. coli mix*
Neck skin	757/947 (80%)	76/947 (8%)	114/947 (12%)
Caecal contents	176/238 (74%)	44/238 (18%)	18/238 (8%)

[a] data based on 5 typical colonies per positive sample. In the event that all 5 colonies were confirmed as either *C. jejuni* or *C. coli* then these would be counted as one isolate.

knowledge and implement continuous improvements to reduce the incidence of *Campylobacter* in food (Campylobacter Stakeholder Group, 2017). The reduction encountered since 2008 could also be attributed to increased farmer information and training and improved biosecurity at farm which has been shown to be effective in decreasing the levels of *Campylobacter* on raw chicken (Sibanda et al., 2018).

Neck skin positivity of 23% of broilers from batches with *Campylobacter*-negative caecal contents provided further evidence that chicken carcasses produced from *Campylobacter*-negative broilers were predominantly (77%) *Campylobacter*-negative. Whereas carcasses produced from colonised broilers were predominantly *Campylobacter*-positive (68% of caecal-colonised batches). This correlation between *Campylobacter* status of chicken carcasses and intestinal colonisation, of respective broiler batches of origin, is evidence of the food safety benefit occurring as a result of on-farm production of *Campylobacter*-free broilers. Good farm biosecurity and hygiene are important measures to prevent and control *Campylobacter* contamination.

The practice of thinning, also referred to as partial depopulation, is routinely performed in some EU countries, including Ireland, and involves batches of birds been removed from houses in two stages over time and been sent for slaughter. From a commercial perspective it increases productivity and profitability as it allows higher initial stocking densities to be used (FSAI, 2011). However, thinning and/or age has previously been identified as a risk factor for colonisation of broilers by *Campylobacter*. (Koolman et al., 2014; Patriarchi et al., 2011; Smith et al., 2016). In the present study thinning had a significant influence on *Campylobacter* prevalence compared to first thin birds. *Campylobacter* prevalence in broiler batches was found to increase by 29% at final depopulation which in turn increased the risk of contamination of carcasses during processing leading to an increase in prevalence of 29% on carcasses. Birds were more likely to be colonised if they were from the final thin (OR = 3.8), and their neck-skins were more than three times as likely to be contaminated (OR = 3.3).

Variability between processors needs to be considered as this can impact reliable *Campylobacter* monitoring. The differences observed between the processing plants could be due to several factors during the slaughtering process. Improper cleaning and hygiene practices in the abattoirs during the slaughter process can lead to increased *Campylobacter* contamination. Carcasses in the present study were at a higher risk of being contaminated by *Campylobacter* later in the working day (afternoon/evening) than earlier (early/mid-morning). This could possibly be explained by the occurrence of *Campylobacter* contamination of the machinery from contaminated batches slaughtered earlier in the same day (Johannessen et al., 2007). This risk factor was also reported in the analysis of the EU baseline survey in 2008 where carcasses were more likely to be contaminated with *Campylobacter* as the processing day progressed (EFSA, 2010). In addition, inadequate monitoring and inadequate adjustment of slaughtering equipment to the size of broilers before processing can cause contamination of carcasses following defeathering and evisceration (FSAI, 2011; Umaraw et al., 2017). During evisceration, the intestinal tract of broilers can rupture or leak faecal material. These contents can harbour a large number of *Campylobacter* and can lead to the contamination of carcasses from *Campylobacter*-free flocks by previously slaughtered colonised flocks (Berrang et al., 2001: FSAI, 2011; Keener et al., 2004: Umaraw et al., 2017) which was observed in the present study. This study demonstrated that carcasses were over seven times more likely to be *Campylobacter* positive if the caecal contents of the same batch were positive. This suggests that if the prevalence of *Campylobacter* in broiler batches was reduced on farm that this could lead to a reduction in *Campylobacter* entering the processing plant and consequently a reduction of contamination during processing. Carcasses with a low *Campylobacter* load as a result of cross contamination would present a limited risk to the consumers as opposed to the highly contaminated carcasses from *Campylobacter* positive flocks (Elvers et al., 2011; Nauta et al., 2007).

Of the 947 *Campylobacter* positive carcasses, 12% had counts greater than 1000 CFU/g on neck skin which is a reduction compared to equivalent data from Ireland in the 2008 EU baseline study (42%). This is a substantial improvement for the industry and signifies the progress made in reducing *Campylobacter* in the last decade. This reduction has unfortunately not resulted in a decrease in the number of notified cases in humans in the last decade. These lower *Campylobacter* levels indicate that the majority of carcasses tested complied with the 1000 CFU/g limit in the Process Hygiene Criteria (Commission Regulation (EC) No 2073/2005, as amended by Commission Regulation (EU) 2017/1495). This is a positive development as EFSA has previously reported that if all broiler batches complied with the critical limit of ≤1000 CFU/g of neck skin that a public health risk reduction of 50% would be achieved (EFSA, 2011). Only 7% of the 342 *Campylobacter* positive carcasses which came from first thin broilers had levels of *Campylobacter* that exceeded the PHC critical limit, compared to 15% of carcasses from final thin birds further supporting the impact of thinning.

Plant C had the highest percentage (21%) of carcasses with *Campylobacter* levels greater than the 1000 CFU/g limit amongst the processing plants; however this percentage was not significantly higher than those observed in Plant A (13%) or Plant B (10%). As seen in previous studies, (Allen et al., 2003; Gormley et al., 2014; Vinueza-

Burgos et al., 2018) high levels of *Campylobacter* contamination were detected in the caecal contents of the broilers examined. Of the 238 positive batches, over 96% had *Campylobacter* levels greater than 6.0 Log_{10} CFU/g with maximum levels of caecal carriage of 8.9 Log_{10} CFU/g. These high counts can be problematic as it provides a potential source of bacteria to be spread to the carcasses of these and other broilers, which in turn can lead to a considerable increased risk of *Campylobacter* infection in humans (Rosenquist et al., 2003). Berrang and colleagues demonstrated that contamination with as little as 5 mg of caecal material lead to a significant increase in the levels of *Campylobacter* on carcasses (Berrang et al., 2001).

In agreement with other studies, (Jørgensen et al., 2011; Meldrum et al., 2005; Näther et al., 2009; Reich et al., 2008; Zendehbad et al., 2015) a seasonal peak in *Campylobacter* prevalence was demonstrated in the present study with highest colonisation rates in the warmer Summer when compared to the colder months of November, December, January, March and April.

The risk of flock contamination increased in batches from free-range farms in the present study which has previously been described (EFSA, 2010; Fernández and Hitschfeld, 2009; Heuer et al., 2001; Näther et al., 2009). This could be attributed to increased environmental exposure from the outdoor access provided to free-range birds and/or the use of hygiene measures in conventional production types which may reduce the risk of infection (EFSA, 2010; Heuer et al., 2001). The higher slaughter age of the free-range birds (>56 days) could be a possible explanation for the observed difference in *Campylobacter* colonisation of batches between production types. The higher slaughter age of the broilers has been previously associated with increased risk of flock colonisation (Berndtson et al., 1996; Evans and Sayers, 2000; Heuer et al., 2001; McDowell et al., 2008). However, in the present study birds slaughtered at >40 days had a marked increase in *Campylobacter* prevalence when compared to those slaughtered earlier but no effect was encountered on *Campylobacter* colonisation. The distribution of *Campylobacter* contamination within individual broiler batches didn't show any discernible patterns of contamination at slaughter. This indicates that there may be more variables that impact contamination levels besides slaughter operation including, for example, the health and conditions of the birds pre-slaughter (Reich et al., 2018).

The most frequently isolated *Campylobacter* species on both carcasses and in caecal contents was *C. jejuni* and this has been reported in previous studies (Berndtson et al., 1996; EFSA, 2010; Evans and Sayers, 2000; Gonsalves et al., 2016; Madden et al., 2011). In the current study, co-colonisation with *C. jejuni* and *C. coli* was also demonstrated. The *Campylobacter* speciation in the present study might be considered more robust than others including the 2008 EU baseline survey in that 5 isolates per sample were speciated instead of just one which is necessary for samples with mixed colonisation (EFSA, 2010).

In conclusion, the decrease in *Campylobacter* prevalence observed demonstrates the improvements and progress made in reducing prevalences of this important enteropathogen in the Irish poultry industry since the 2008 EU baseline survey. The present study provided indications and supporting data of several factors on the *Campylobacter* contamination rates. However, these factors were screened using univariable logistic regression models, therefore some factors can be quite confounding including thinning, age at slaughter etc. The risk of contamination was seven times higher for carcasses which came from *Campylobacter* colonised batches; moreover, the carcasses of 47 *Campylobacter*-free batches were later contaminated during the slaughter process. This highlights the need for improvements both in reducing the levels of *Campylobacter* in the broiler intestines on farm and also in decreasing the prevalence and levels on broilers to address cross-contamination in the processing plant. Although progress has been made in the last decade, human cases of *Campylobacter* have not yet decreased (1752 in 2008 to over 3000 confirmed cases in 2018) and poultry meat remains a significant source of *Campylobacter* in Ireland. No single intervention will resolve this problem but rather a multifactorial approach by all stakeholders is necessary to continue to improve the *Campylobacter* issue.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT authorship contribution statement

Lynch: Methodology, Formal analysis, Investigation, Data curation, Writing – Original draft, Writing – Review & Editing. **Franklin-Hayes**: Formal analysis, Investigation, Writing – Review & Editing. **Koolman**: Formal analysis, Writing – Original draft, Writing – Review & Editing. **Egan**: Conceptualization, Funding acquisition, Review & Editing. **Gutierrez**: Methodology, Data curation, Supervision, Funding acquisition, Review & Editing. **Byrne**: Formal analysis, Project administration, Supervision, Review & Editing. **Golden**: Supervision, Project administration, Review & Editing. **Bolton**: Conceptualization, Data curation, Supervision, Project administration, Funding acquisition, Writing – Review & Editing. **Reid**: Formal analysis, Review and Editing. **Coffey**: Supervision, Project administration, Funding acquisition, Data curation, Review & Editing. **Lucey**: Supervision, Project administration, Funding acquisition, Review & Editing. **O′ Connor**: Funding acquisition, Project administration, Review & Editing. **Unger**: Funding acquisition, Project administration, Review & Editing. **Whyte**: Supervision, Funding acquisition, Data curation, Conceptualization, Methodology, Project administration, Writing – Original draft, Review & Editing.

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