Research Note: *Campylobacter* spp. control at field level two years after the implementation of European Regulation (EU) 2017/1495

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ABSTRACT Campylobacteriosis was the most frequently reported foodborne infection in humans in the European Union in the last years. *Campylobacter* spp. in broiler flocks from Spain was monitored at farm level during 12-month period (2020−2021). Feces samples were analyzed according to ISO (International Standard Organization) 10272-2:2018. From all samples collected, 54% were *Campylobacter* spp. positive. Regarding the age, *Campylobacter* spp. was isolated in 36% of the flocks during thinning and 64% in flocks at slaughter age. In addition, *Campylobacter* spp. counts increased with the age of the animals. On the other hand, the presence of *Campylobacter* showed statistical differences between the months of the year (P-value <0.05) in flocks at thinning age that exceeded the ≥1,000 CFU/g limit. The highest rates were found from June to December coinciding with the seasons of summer and autumn. In conclusion, our study shows the situation of *Campylobacter* spp. in broiler flocks in Spain considering age and season effects. This way, it was found higher rates and counts in broilers close to slaughter age and peaking during the summer to autumn period.

Key words: Campylobacter spp, broilers farms, foodborne pathogens, counts, seasonal variation

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INTRODUCTION

Campylobacteriosis, caused by thermotolerant *Campylobacter* species, was the most frequently reported foodborne gastrointestinal infection in humans in the European Union (**EU**) with a total of 120,946 confirmed cases in 2020 (EFSA, 2021). *Campylobacter* spp. can colonize the intestines of clinically healthy broilers and contaminate carcasses after slaughter; therefore, broiler meat has been considered one of the sources of foodborne *Campylobacter* spp. infection (EFSA, 2021).

The control of this pathogen in poultry and poultry meat is one of the main public health strategies in the prevention of campylobacteriosis in the EU (EFSA, 2021). For this reason, in 2017, Regulation (UE) 2017/1495 was established, which modifies Regulation (EC) No. 2073/2005 in relation to *Campylobacter* spp. surveillance in broiler carcasses where a process hygiene criterion of >1,000 CFU/g was set at slaughterhouse (UE, 2017).

Several authors have deeply studied *Campylobacter* spp. epidemiology in broilers flocks, trying to reduce the prevalence at farm level in Spain (Marin et al., 2015; Ingresa-Capaccioni et al., 2015). However, although several control options are available, there is no gold standard measure that could be successfully implemented across Europe, and control strategies are still being evaluated at farm level (Vidal et al., 2013). Systematic sampling of *Campylobacter* spp. in broiler flocks at a given time can be a valuable tool to assess the current presence of *Campylobacter* spp. with the aim of estimating its prevalence.

The overall aim of this study was to investigate the current situation of *Campylobacter* spp. in broilers flocks in the field, two years after the implementation of European regulation.

MATERIALS AND METHODS

Selection of Samples

Over 12 months (June 2020-June 2021), 2,074 commercial broiler flocks were sampled at farm level. All flocks were reared indoors under commercial production conditions. Samples were collected in two different moments during the production cycle: at thinning age (30−35 d of rearing) and at slaughter age (40−45 d of

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rearing). For this purpose, feces samples were taken aseptically from the bedding with sterile gloves (2 sterile pots with approx. 500 g of feces in each; Sandberg et al., 2006). From all samples collected, 691 collected at thinning age and 1,383 samples were collected at slaughter age. All collected samples were transported to the laboratory under refrigeration conditions at 0°C to 4°C.

Campylobacter spp. Isolation, Identification and Enumeration

Samples were analyzed according to ISO (International Standard Organization) 10272-2:2018, the horizontal method for the enumeration of *Campylobacter* spp. in food and feed stuffs (ISO, 2018). For detection purposes, serial dilutions were prepared in Buffered peptone water (**BPW**) (VWR, Leuven, Belgium) and a 0.1 mL drop from each inoculum was plated onto modified charcoal cefoperazone deoxycholate agar (**mCCDA**) (Oxoid, Dardilly, France). The samples were then incubated at 41.5°C ± 1°C in a microaerobic atmosphere with microaerophilia sachets (Campygen 2.5L, Oxoid Dardilly, France) for 44 ± 4 h. After incubation, 5 *Campylobacter* like colonies were plated onto Columbia blood agar (Oxoid, Dardilly, France) for further characterisation. Colony morphology and motility were evaluated under dark field microscopy. Confirmation of suspicious colonies was performed by oxidase and catalase tests and plating at different temperatures and atmospheres (41.5 °C under microaerophilic conditions and 25 °C under aerobic conditions) in Columbia blood agar (Oxoid, Dardilly, France).

Statistical Analysis

A generalized linear model was used to compare results between the percentages of *Campylobacter* between the different months of the year. A *P-value* of ≤0.05 was considered to indicate a statistically significant difference. Analyses were carried out using a commercially available software application (Statgraphics Centurion XVI 16.2.04 software package; Statgraphics Technologies, Inc. The Plains, Virginia, 2021).

RESULTS AND DISCUSSION

The present research was carried out to update the situation of *Campylobacter* spp. in broiler flocks in Spain after the limits imposed by the EU in 2017 in slaughterhouses (EU, 2017). Control of *Campylobacter* spp. in primary broiler production is a key element of public health strategies to reduce the number of human campylobacteriosis cases (EFSA, 2021). In this study, *Campylobacter* was isolated from 1,128 of 2,074 samples processed (54%). These results are lower than those rates observed previously by Ingresa-Capaccioni et al. (2015) and Perez-Arnedo and Gonzalez-Fandos (2019) in Spain, who reported a 95.2% and 100% of *Campylobacter* spp. at field level, respectively. Similar rates to our research are observed Torralbo et al. (2014) with rates of 68.4% and 62.5% respectively in Spain. In various European countries, *Campylobacter* spp. also has been isolated from broiler flocks with widely prevalence rates between 3.5% and 71.9% (EFSA, 2021). The wide variability in Campylobacter rates found compared to other studies in Spain as well as at European level could be explained by the choice of a different type of sample, the seasonality of the increase in Campylobacter observed in Spain and the thinning performed.

Thinning, is a common practice in many European countries, based on reducing bird density within the broiler houses. During thinning, the doors of the poultry are opened and the catching crew and equipment enter to the poultry house (Newel et al., 2011). In addition, the stress that birds experience during thinning might assist the establishment and spread of pathogens through the flock (Koolman et al., 2014). Regarding the moment of sampling, during thinning samples collected were *Campylobacter* spp. positive in 36% of cases (248/691). In the other hand, flocks sampled at slaughter age were positive in 64% of samples collected (880/1128). According to those percentages observed, it seems that *Campylobacter* spp. counts increased with the age of the animals. These findings are in line with those of Ingresa-Capaccioni et al. (2015) and Perez-Arnedo and Gonzalez-Fandos (2019) who reported higher *Campylobacter* spp. detection rates as the age of the animals increased.

The results of *Campylobacter* spp. enumeration in this study were categorized as follows: 0 CFU/g, <1,000 CFU/g and ≥1,000 CFU/g and are shown in Table 1. Regarding thinning age flocks the % of counts with 0 CFU/g was 64%, the 9% were <1,000 CFU/g and 27% obtained counts of ≥1,000 CFU/g. However, in slaughter-age flocks, 36% obtained counts of 0 CFU/g, 4% were <1,000 CFU/g and 60% were up than 1,000 CFU/g.

Regarding the counts of *Campylobacter* spp. in flocks at slaughter age exceeded the ≥1,000 CFU/g limit, we found statistical differences between the different months of the year (*P*- alue <0.05). The counts obtained by months are shown in Table 2. The highest rates were found from June to December showed seasonal changes. The counts were higher in summer and autumn (except in September that presented lower rates). This finding agrees with other studies who reported seasonal variation in *Campylobacter* spp. in broilers, pointing out the peak in the warmer periods of the year (Wedderkopp et al, 2001; Reich et al., 2008).

Table 1. *Campylobacter* enumeration data obtained from feces in broiler flocks.

	Campylobacter counts (CFU/g)		
Age of sampling	0*	<1000	≥1000
Broiler flocks at thinning age	443 (64)	62 (9)	186 (27)
Broiler flocks at slaughter age	503 (36)	51 (4)	829 (60)

Number of samples (percentage).
*Negative detection and negative enumeration.

Table 2. Distribution of *Campylobacter* prevalence in broiler flocks at thinning age that exceed >1,000 CFU/g according to month of the year.

Month	N	% *Campylobacter* counts ≥1000 CFU/g
January	74	53[a]
February	199	58[ab]
March	363	54[a]
April	215	61[abc]
May	69	52[a]
June	41	76[cd]
July	20	85[d]
August	22	77[bcd]
September	50	50[a]
October	38	63[abcd]
November	194	66[bcd]
December	114	72[cd]

N, sample size; $\bar{x}$, mean.

For each month mean values with different superscript lowercase letters (a,b,c,d) in a row are significantly different (P value < 0.05).

Our study demonstrates the higher presence of *Campylobacter spp.* in broiler flocks close to slaughter age (40−45 d of life). Further studies are required to verify whether the high counts found at the farm level translate into later high counts at the slaughterhouse. The reduction at farm level may be one aspect for reducing the burden of *Campylobacter* spp. brought to the slaughterhouse and some authors point out that could be done by improved on-farm biosecurity (Reich et al., 2008; Torralbo et al., 2014). Thus, a combination of strict biosecurity measures and a high level of hygiene in the slaughterhouse would be crucial to reduce *Campylobacter* spp. in the food chain and therefore the risk of infection of consumers.

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DISCLOSURES

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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