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ORIGINAL ARTICLE



Multilocus sequence typing of *Campylobacter jejuni* and *Campylobacter coli* to identify potential sources of colonization in commercial turkey farms

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ABSTRACT

Poultry are the main reservoir for thermophilic *Campylobacter* spp., which is the most common causative agent of human bacterial gastroenteritis. The epidemiology of *Campylobacter* in poultry, particularly in turkeys, is not completely understood. This study aimed at identifying potential sources and transmission routes of thermophilic *Campylobacter* spp. in commercial turkey farms. *C. jejuni* and *C. coli* isolates from breeders ($n = 29$, 20 *C. jejuni* and 9 *C. coli*) and their progeny ($n = 51$, 18 *C. jejuni* and 33 *C. coli*) reared in two different farms for three sequential production cycles were analysed by multilocus sequence typing (MLST). Strains ($n = 88$, 42 *C. jejuni* and 46 *C. coli*) isolated from environmental (i.e. anteroom and in-house overshoes), water (i.e. drinkers and water line), and pest (i.e. flies, *Alphitobius diaperinus*, and mice) sources were also examined. MLST of *C. jejuni* and *C. coli* isolates resulted in 13 and 12 different sequence types (STs) belonging to six and one previously-described clonal complexes (CCs), respectively. Three novel STs were identified. Genetic similarities were detected between isolates from fattening turkeys and the considered environmental, water, and pest sources, and with the breeders to a lesser extent. Source attribution analysis estimated that environmental and water sources accounted for most (~75%) of fattening turkey isolates and were therefore identified as the most likely sources of flock colonization, followed by pests (~20%) and breeders (~5%). These sources may thus be targeted by control measures to mitigate the risk of *Campylobacter* colonization in commercial turkeys.

RESEARCH HIGHLIGHTS

- High occurrence of *C. jejuni* and *C. coli* in commercial turkey flocks.
- High genetic diversity of *C. jejuni* and *C. coli* in commercial turkey flocks.
- Horizontal transmission responsible for *Campylobacter* colonization of commercial turkey flocks.
- Environmental and water sources involved in *Campylobacter* colonization of commercial turkey flocks.
- Strategies for prevention and control of *Campylobacter* colonization of commercial turkey flocks are needed.

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Introduction

Thermophilic *Campylobacter* spp. are leading food-borne pathogens causing acute gastroenteritis in humans worldwide (Kaakoush *et al.*, 2015). In the European Union (EU), campylobacteriosis has been the most frequently reported zoonotic disease since 2005, with the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC) reporting 246,307 confirmed human campylobacteriosis cases in 2016 (EFSA & ECDC, 2017). Thermophilic *Campylobacter* spp., mainly *C. jejuni* and *C. coli*, colonize a wide range of domestic and wild animals (Humphrey *et al.*, 2007; Kaakoush *et al.*, 2015), with poultry being recognized

as the main reservoir and source of human *Campylobacter* infections (Skarp *et al.*, 2016; EFSA & ECDC, 2017). Indeed, it has been estimated that 50% to 80% of campylobacteriosis cases can be attributed to the chicken reservoir as a whole, while the handling and consumption of chicken meat can account for 20% to 30% of campylobacteriosis cases in the EU (EFSA, 2011). Chickens and turkeys can carry both *C. jejuni* and *C. coli* at high concentrations in their intestinal tract without showing clinical signs of disease (Cawthraw *et al.*, 1996; Wallace *et al.*, 1998). This poses a public health risk, as carcasses may become contaminated with *C. jejuni* and *C. coli* during slaughtering and processing (Keener *et al.*, 2004; Skarp *et al.*, 2016).

Campylobacter epidemiology in poultry production is complex, and either birds or their living environment play a crucial role in amplifying and disseminating *Campylobacter* in commercial farms. Consequently, *Campylobacter* prevention and control are challenging, even when strict biosecurity measures are implemented (Hermans *et al.*, 2011; Newell *et al.*, 2011; Sahin *et al.*, 2015). The introduction and spread of *Campylobacter* in poultry flocks have been attributed to both vertical and horizontal transmission. Whereas the importance of vertical transmission from breeders to their offspring is still a matter of debate, horizontal transmission from environmental sources has been recognized as an effective route of *Campylobacter* dissemination (Bull *et al.*, 2006; Callicot *et al.*, 2006; Hansson *et al.*, 2007; Cox *et al.*, 2012; Sahin *et al.*, 2015).

Campylobacter spp. are ubiquitous in the environment where they indicate recent contamination with animal faeces, sewage effluent, and agricultural runoff. The relative importance of the animal sources of *C. jejuni* and *C. coli* strains in surface water vary over seasons, type of water body, and geographical location, with the relative contribution of poultry as source of *C. jejuni* and *C. coli* contamination in surface water being correlated with the magnitude of the local poultry industry (Mughini-Gras *et al.*, 2016). *Campylobacter* spp. can survive for long periods outside the host, thanks to their high genetic variability (Parkhill *et al.*, 2000; Lee & Newell, 2006). Many potential sources and vehicles of *Campylobacter* spp. – such as drinking water (Ogden *et al.*, 2007; Pérez-Boto *et al.*, 2010), insects (e.g. flies and lesser mealworms) (Bates *et al.*, 2004; Hald *et al.*, 2008), wild birds (Colles *et al.*, 2008), other animals on the farm (e.g. dogs, rodents, cattle, swine, and sheep) (Zweifel *et al.*, 2008; Ellis-Iversen *et al.*, 2012), farm personnel, and equipment (Huneau-Salaun *et al.*, 2007; Lyngstad *et al.*, 2008) – have been identified, but it is still difficult to define which is the most likely contributor to *Campylobacter* colonization in a poultry flock.

Despite the increased turkey meat production and consumption in recent years and the high *Campylobacter* prevalence rates (65.3%) detected in commercial meat turkeys in Europe (EFSA & ECDC, 2017), most research on *Campylobacter* epidemiology has focused primarily on broiler production (Adkin *et al.*, 2006; Agunos *et al.*, 2014; Skarp *et al.*, 2016). Studies investigating *Campylobacter* epidemiology in turkey farms are limited (Arsenault *et al.*, 2007; Kiess *et al.*, 2007; Wright *et al.*, 2008; Perko-Mäkelä *et al.*, 2009; El-Adawy *et al.*, 2012; Giacomelli *et al.*, 2012), particularly those employing multilocus sequence typing (MLST) as genotyping technique (El-Adawy *et al.*, 2013; Kashoma *et al.*, 2014). Moreover, the *Campylobacter* transmission routes in turkey farms remain largely unknown. Consequently, effective prevention and control strategies are not yet available. A better understanding of the sources

and routes of *Campylobacter* transmission in commercial turkey farms is therefore needed.

The aim of this study was to identify possible sources and transmission routes of thermophilic *Campylobacter* spp. in commercial turkey farms, using MLST to assess the genetic relatedness of *C. jejuni* and *C. coli* isolates from turkeys and those from their farm environment. Turkey farming is very different from broiler farming: turkeys are reared for longer periods, are more susceptible to infections, and are more often in need of antimicrobial treatments. As translating knowledge gained from broilers to turkeys may be misleading, the more specific information one can obtain from turkeys the better *Campylobacter* epidemiology will be understood.

Materials and methods

Experimental study design

A longitudinal sampling strategy was adopted over a year (March 2012 to June 2013). A screening was carried out to detect *Campylobacter*-positive turkey breeder flocks. Afterwards, two meat turkey farms (farms A and B), housing the progeny of the *Campylobacter*-positive breeders, were examined for three sequential production cycles. During the first production cycle, two flocks per farm were monitored: one flock (house 1) was composed of the progeny of the *Campylobacter*-positive breeders, whereas the other one (house 2) was composed of the progeny of breeders of unknown *Campylobacter* status. In the target house (house 1), monitoring also continued for the following two production cycles. In each flock, sampling was carried out during downtime before housing turkeys and then three times during the production cycle by collecting samples from fattening birds and potential environmental sources of *Campylobacter* spp.

Turkey flocks

Turkey farms were located in Veneto Region (North-Eastern Italy) and belonged to the same integrated company. Both farms A and B reared female birds (about 10,000 birds per farm), with a length of the production cycle of 13–15 weeks. Farm management was based on an “all in-all out” system and a downtime of ≥ 21 days was implemented between subsequent production cycles. Complete litter removal, cleaning, and disinfection of houses and equipment were carried out after each production cycle. Farms were supplied by chlorinated pond water and managed exclusively by the farmers and their families (no employees).

Sample collection

Twenty cloacal swabs per flock were collected from three breeder flocks. At the hatchery, 36 samples

(six pools of six samples) of *meconium* per housed flock were collected from day-old chicks. At the farm, fattening turkeys were sampled at the beginning, middle, and end of the production cycle by collecting caecal faeces (36 swabs, six pools of six samples). At each sampling time, drinking water was also collected from the in-house collection tank (1 l), the water line (1 l), and the drinkers (10 swabs from 10 drinkers, two pools of five samples). Additionally, the following environmental samples were collected: dust (anteroom door and fans, one surface swab each), air (400 l), overshoes in the anteroom ($n=1$) and inside the house ($n=2$). When found, insects such as flies (*Musca domestica*), beetles (*Blatta orientalis*), and lesser mealworms (*Alphitobius diaperinus*), and mice were collected. During downtime, water and environmental samples were harvested. Swabs were placed in Amies transport medium with charcoal (Copan, Brescia, Italy) and the other samples into sterile plastic containers. Air samples were collected using the SAS Super 100TM instrument (International PBI S.r.l., Milano, Italy) mounting Karmali agar plates (OXOID, Basingstoke, UK), and transported inside the CampyGen[®] compact AGS bags (OXOID). All samples were stored in a cool box and immediately transported to the laboratory, where they were processed within 1–2 h.

Isolation and identification of thermophilic *Campylobacter* spp.

Isolation and identification of *Campylobacter* spp. were performed according to Giacomelli *et al.* (2012), with some minor modifications. Briefly, samples were inoculated into the selective enrichment Exeter Broth (Mast Diagnostics, Merseyside, UK) for 24 h at 41.5 °C under microaerobic conditions provided by the gas generating kit CampyGenTM (OXOID). Water samples were filtered through 0.2 µm membrane filters (Sartorius, Goettingen, Germany) using a vacuum pump before processing. Broth cultures were inoculated onto Karmali agar (OXOID) and incubated for 48 h at 41.5°C under microaerobic conditions. At least three suspected *Campylobacter* colonies per positive sample were selected, the DNA extracted by boiling for 20 min (one colony in 100 µl of sterile RNase/DNase-free water) and submitted to genus confirmation and species identification by multiplex PCR.

Multilocus sequence typing

Selected isolates representing each sampling and source of isolation were genotyped by MLST according to the seven loci scheme for *C. jejuni* and *C. coli* developed by Dingle *et al.* (2001, 2005). In total, 168 isolates were genotyped: 29 (20 *C. jejuni* and 9 *C. coli*)

from breeders, 51 (18 *C. jejuni* and 33 *C. coli*) from fattening turkeys, 33 (15 *C. jejuni* and 16 *C. coli*) from drinking water (water line, $n=2$; drinkers, $n=31$), 26 (8 *C. jejuni* and 18 *C. coli*) from overshoes (anteroom, $n=2$; in-house floor, $n=24$), 14 (6 *C. jejuni* and 8 *C. coli*) from flies, 13 (11 *C. jejuni* and 2 *C. coli*) from lesser mealworms, and 2 *C. jejuni* from a mouse. PCR and sequencing of DNA templates were performed using primers and reaction conditions suggested by the *Campylobacter* MLST database (<http://pubmlst.org/campylobacter/>). Sequencing was performed at MacroGen Europe (Amsterdam, The Netherlands). Allele number, sequence type (ST), and clonal complex (CC) assignment were obtained by interrogation of the *Campylobacter* MLST database. Novel alleles and STs were submitted to the *Campylobacter* MLST database curator for number assignment.

Data analysis

To show the distribution of *C. jejuni* and *C. coli* STs among breeders, fattening turkeys and the farm environment, phylogenetic trees of concatenated DNA sequences from the seven MLST loci were constructed. The maximum likelihood method by using the neighbour joining algorithm and the iTOL software (Letunic & Bork, 2016) to visualize phylogenetic data as circular cladograms were used. To determine the relationship between STs and their occurrence, PHYLOViZ 2.0 (Nascimento *et al.*, 2017) was used with the goeBURST option. The *Campylobacter* MLST database (last accessed on December 27th, 2017) was used to obtain information on *C. jejuni* and *C. coli* isolates from poultry (i.e. chickens and turkeys) and humans belonging to the 25 STs identified in this study. These data were added to the ST cladogram by the iTOL graphic option.

An MLST-based source attribution analysis was performed to infer the most likely origins of the *Campylobacter* isolates from fattening turkeys considering the general farm environment (i.e. isolates from overshoes samples), pests (i.e. isolates from flies, lesser mealworms and mice), drinking water, or the breeders (i.e. to allow for putative vertical transmission) as possible sources. Source attribution was performed using STRUCTURE, a Bayesian model-based clustering method that infers population structure and attributes probabilistically strains to populations using multilocus genotype data (Pritchard *et al.*, 2000). The analysis was carried out using the seven MLST loci and the “No Admixture” model with correlated allele frequencies; 5000 burn-in iterations were followed by 10,000 iterations for parameter estimation using source population information (USEPOPINFO) with target isolates differentiated from the other using the POPFLAG option.

Ethical statement

Cloacal swabs from turkey breeders were collected by veterinarians of the private company as part of the routine monitoring of the flock health status and conducted in compliance with good veterinary practices.

Results

Occurrence and distribution of *C. jejuni* and *C. coli*

All samples from the three breeder flocks tested positive for thermophilic *Campylobacter* spp., both *C. jejuni* (67.7%) and *C. coli* (32.3%). Except for day-old chicks (all *meconium* samples tested negative), *C. jejuni* and *C. coli* were isolated from fattening turkeys during each production cycle in both farms, with *C. coli* being predominant over *C. jejuni* (50% to 100% of isolates) (Table 1). Either *C. jejuni* or *C. coli* were isolated during all production cycles in both farms from the in-house overshoes samples (73.9%) and the water from the drinkers (69.6%). During the three production cycles, *C. jejuni* and *C. coli* positive flies (66.7%) and lesser mealworms (44.4%) were found in both farms. Anteroom overshoes (8.7%), water from the line (4.3%), and a mouse tested positive for *C. coli* and *C. jejuni* once per each farm. Dust from the anteroom door and fans, air from inside the house, water from the in-house collection tank, and beetles always tested negative. No *Campylobacter* spp. were ever isolated during downtime.

MLST of *C. jejuni* and *C. coli* isolates

MLST analysis showed high genetic diversity among both *C. jejuni* and *C. coli* isolates (Table S1, presented in supplementary data). Twenty-five different STs, 13 for *C. jejuni* (STs 51, 104, 223, 257, 2116, 2274, 2361, 2850, 2863, 3102, 4242, 6941, and 8347), and 12 for *C. coli* (STs 825, 829, 832, 1170, 1586, 1628, 1764, 2912, 5150, 5401, 6689, and 6940) were identified, and three of them were novel (STs 6941 and 8347 for *C. jejuni* and ST 6940 for *C. coli*). Ten *C. jejuni* STs were assigned to six previously described CCs (CCs 21, 257, 353, 354, 443, and 446), whereas three (the new STs 6941 and 8347, and ST 2274) belonged to undefined CCs. For *C. coli*, nine STs were assigned to a single previously described CC 828, while three (STs 1764, 5401, and 5150) belonged to undefined CCs. The distribution of *C. jejuni* and *C. coli* STs among breeders, fattening turkeys, and the farm environment is shown in Figure 1.

In Figure 2, the genetic relatedness and the frequency of *C. jejuni* and *C. coli* STs is represented. The degree of genetic diversity of *C. jejuni* isolates was higher than that of *C. coli* isolates. For *C. jejuni*, a cluster including two STs (i.e. ST 51 and 2361)

differing by one allele was identified, whereas a cluster including the founder ST (i.e. ST 829) and five STs (i.e. ST 825, 832, 1586, 2912, and 6940) differing by 1 or 2 alleles was identified for *C. coli*. Among *C. jejuni* and *C. coli* isolates, the most common STs were ST 2863 (18.8% of isolates) and ST 829 (27.3% of isolates), respectively. These STs were found in breeders, fattening turkeys and in the farm environment. However, the predominant STs were ST 2116 (6.7% of isolates) and ST 104 (15% of isolates) among *C. jejuni* isolates from fattening turkeys and the farm environment, respectively, and ST 832 (10.1% and 17.7% of isolates, respectively) among *C. coli* isolates. A total of four different STs were found among breeder strains: two for *C. jejuni* (STs 2863 and 6941) and two for *C. coli* (STs 829 and 6940) isolates. Two of these STs (ST 2863 for *C. jejuni* and ST 829 for *C. coli*) were also found among isolates from fattening turkeys and environmental sources (i.e. anteroom and in-house overshoes, drinking water and lesser mealworms) in both farms. Except for four *C. jejuni* STs (STs 223, 257, 4242, and 8347) and one *C. coli* ST (ST 5401) identified only once, all the remaining STs were shared between strains from fattening turkeys and environmental samples (i.e. anteroom and in-house overshoes, drinking water, lesser mealworms, flies, and the mouse) isolated from the same flock (*C. jejuni* STs 104, 51, 2850, and 2116; *C. coli* STs 829, 2912, 832, 1764, 1586, 1628, and 6689), from different flocks of the same farm (*C. jejuni* STs 2863, 104, 51, 2274, 2850, 2116, 2361, and 3102; *C. coli* STs 829, 2912, 832, 825, and 1170), from sequential flocks of the same farm (*C. jejuni* ST 2274; *C. coli* STs 832, 825, and 1170) and from different farms (*C. jejuni* STs 2863, 2274, 2850, and 2116; *C. coli* STs 829 and 832).

Quantifying potential sources of *Campylobacter* colonization

The results of the source attribution analysis of the 168 *Campylobacter* isolates from fattening turkeys are reported in Table 2. Overall, 40.3% of these isolates were attributed to the farm environment as a possible source of horizontal transmission, followed by water sources (34.7%) and pests (20.3%), as well as potential vertical transmission from breeders to a minor extent (4.7%).

Discussion

The occurrence of thermophilic *Campylobacter* spp. in breeders and fattening turkeys detected in the present study was very high. Both *C. jejuni* and *C. coli* were isolated, although *C. coli* predominated over *C. jejuni* in fattening turkeys, which is in agreement with previous studies (Smith *et al.*, 2004; Lee *et al.*, 2005; Luangtongkum *et al.*, 2006; Alter *et al.*, 2011; Giacomelli *et al.*,

Table 1. *C. jejuni* and *C. coli* from fattening turkeys and the farm environment.

Farm	House/Cycle	Sampling time	Anteroom overshoes	Caecal faeces	In-house overshoes	Water		Insects		Mice
						Water line	Drinkers	<i>Alphitobius diaperinus</i>	Flies	
Farm A	House 1 1 st cycle	5 wks	<i>C. coli</i> (6)	<i>C. coli</i> (17) and <i>C. jejuni</i> (1)	–	–	<i>C. coli</i> (4)	–	–	
		10 wks	–	<i>C. coli</i> (14) and <i>C. jejuni</i> (3)	<i>C. coli</i> (3)	–	<i>C. jejuni</i> (2)	–	<i>C. jejuni</i> (6)	
		13 wks	–	<i>C. coli</i> (12) and <i>C. jejuni</i> (3)	<i>C. coli</i> (1) and <i>C. jejuni</i> (3)	–	<i>C. jejuni</i> (3)	<i>C. jejuni</i> (6)	<i>C. jejuni</i> (6)	
	2 nd cycle	5 wks	–	<i>C. coli</i> (12)	<i>C. coli</i> (3)	–	–	<i>C. coli</i> (6)		<i>C. jejuni</i> (6)
		10 wks	–	<i>C. coli</i> (9)	<i>C. coli</i> (6)	–	<i>C. coli</i> (3) and <i>C. jejuni</i> (4)	–		
	3 rd cycle	5 wks	–	–	–	–	–			
		10 wks	–	–	–	–	–		–	
		14 wks	–	<i>C. coli</i> (6)	<i>C. coli</i> (6)	–	<i>C. coli</i> (6)			
	House 2 1 st cycle	5 wks	–	<i>C. coli</i> (11)	–	–	<i>C. coli</i> (2) and <i>C. jejuni</i> (3)	<i>C. jejuni</i> (6)		
		10 wks	–	<i>C. coli</i> (15) and <i>C. jejuni</i> (3)	<i>C. coli</i> (3)	–	<i>C. coli</i> (5) and <i>C. jejuni</i> (1)	<i>C. jejuni</i> (6)	<i>C. jejuni</i> (6)	
		13 wks	–	<i>C. coli</i> (13) and <i>C. jejuni</i> (3)	<i>C. coli</i> (2) and <i>C. jejuni</i> (4)	–	–	<i>C. jejuni</i> (6)	–	–
Farm B	House 1 1 st cycle	5 wks		<i>C. coli</i> (3) and <i>C. jejuni</i> (3)	<i>C. coli</i> (5)	–	<i>C. coli</i> (3)	<i>C. jejuni</i> (6)		
		10 wks		<i>C. coli</i> (14) and <i>C. jejuni</i> (1)	<i>C. coli</i> (6)	–	<i>C. jejuni</i> (6)	–	–	
		14 wks		<i>C. coli</i> (7) and <i>C. jejuni</i> (3)	<i>C. coli</i> (5)	–	–	<i>C. jejuni</i> (6)	<i>C. coli</i> (2)	
	2 nd cycle	5 wks		<i>C. coli</i> (7)	<i>C. coli</i> (1)	–	<i>C. coli</i> (3) and <i>C. jejuni</i> (3)		<i>C. coli</i> (6)	
		10 wks		<i>C. coli</i> (10) and <i>C. jejuni</i> (2)	<i>C. coli</i> (6)	–	<i>C. coli</i> (7)			
		15 wks		<i>C. coli</i> (4)	–	–	<i>C. coli</i> (9)			
	3 rd cycle	5 wks		–	<i>C. jejuni</i> (12)	–	<i>C. jejuni</i> (12)	–		
		10 wks		–	<i>C. coli</i> (7)	–	<i>C. coli</i> (12)	–	–	
		15 wks		<i>C. coli</i> (3)	–	–	<i>C. coli</i> (6)	<i>C. coli</i> (4)	<i>C. coli</i> (4)	
	House 2 1 st cycle	5 wks	<i>C. jejuni</i> (6)	<i>C. coli</i> (13)	<i>C. coli</i> (3)	<i>C. coli</i> (6)	–	–		
		10 wks	–	<i>C. coli</i> (8) and <i>C. jejuni</i> (1)	<i>C. coli</i> (6)	–	<i>C. jejuni</i> (6)	–	<i>C. coli</i> (6)	
		14 wks	–	–	<i>C. jejuni</i> (1)	–	–	–	<i>C. coli</i> (1)	

Notes: Results of downtime sampling, *meconium*, dust from the anteroom door and fans, air from inside the house, water from the in-house collection tank and beetles are not reported in the table, since they always tested negative; cycle 2 of farm A lasted 10 weeks for commercial reasons. () = no. of *Campylobacter* isolates; – = negative; blank = no sample.

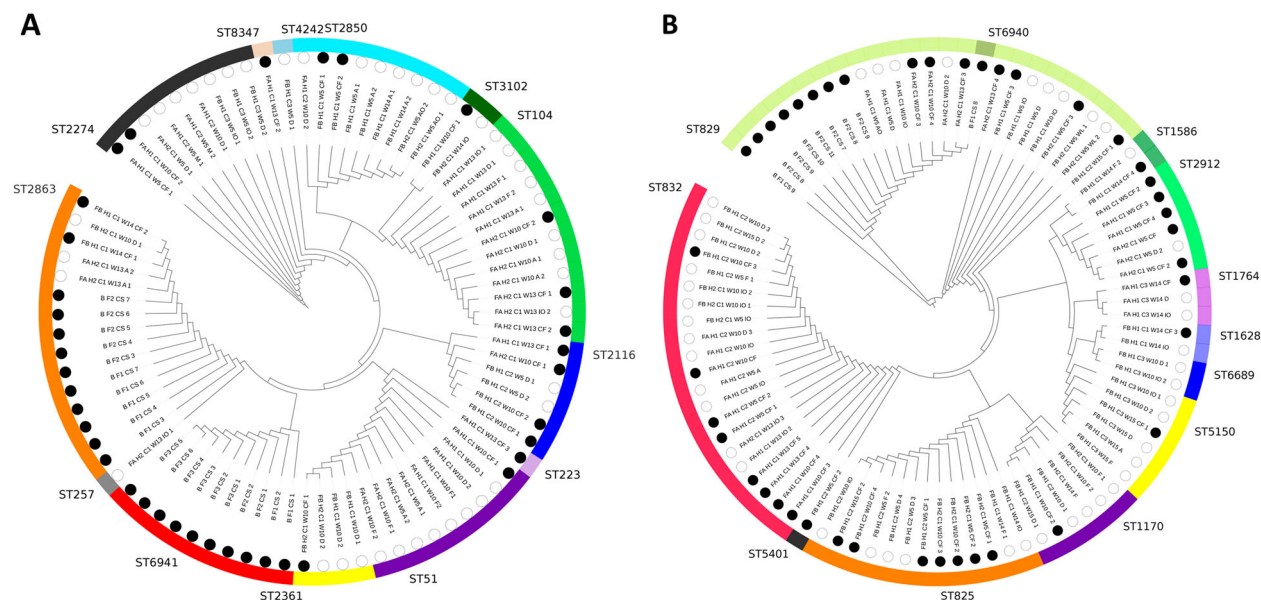


Figure 1. Circular cladogram representing the distribution of *C. jejuni* (A) and *C. coli* (B) STs among breeders, fattening turkeys, and the farm environment. Black circles: isolates from turkeys; white circles: isolates from the environment. STs are encircled by different colours/shading. B = breeders, F = flock, CS = cloacal swabs; FA/FB = farm A/farm B; H1/H2 = house 1/house 2; C1/C2/C3 = cycle 1/cycle 2/cycle 3; W5/W10/W13/W14/W15 = week 5/week 10/week 13/week 14/week 15; CF = caecal faeces; AO = anteroom over-shoes; IO = in-house overshoes; D = drinkers; WL = water line; F = flies; A = *Alphitobius diaperinus*; M = mouse.

2012). Over the entire sampling period and within each production cycle, fattening turkeys were commonly found to be positive for *Campylobacter* spp., with the exception of day-old chicks, which always tested negative. This finding is supported by previous investigations (Wallace *et al.*, 1998; Smith *et al.*, 2004; Lee *et al.*, 2005; El-Adawy *et al.*, 2012) showing that day-old chicks are usually free of *Campylobacter*, and gastrointestinal colonization typically occurs after the first week of life. On the other hand, the occurrence of the same STs (i.e. ST 2863 for *C. jejuni* and ST 829 for *C. coli*) between breeders and their progeny may suggest vertical transmission. However, these STs were detected not only in the progeny flocks, but also in turkeys of different origin reared in the same farm, as well as in environmental sources (e.g. anteroom and in-house overshoes, water from the line and the drinkers, and lesser mealworms), indicating a possible different origin of these genotypes. According to other authors (Smith *et al.*, 2004; El-Adawy *et al.*, 2012), our results suggest that vertical transmission would play a very minor role as a possible source of *Campylobacter* spp. and that horizontal transmission (mainly from environmental and water sources) is

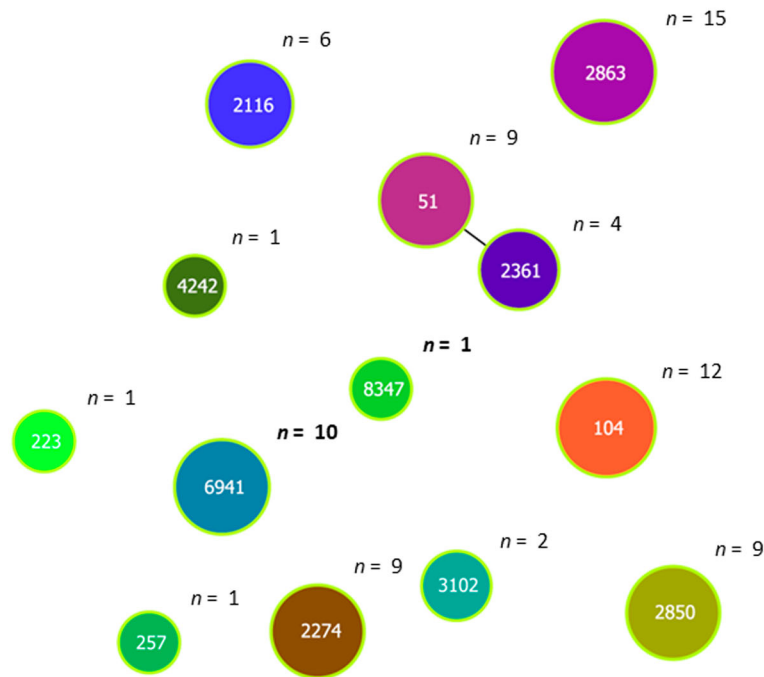
much more likely to be responsible for *Campylobacter* introduction into turkey flocks.

Among the environmental sources of *Campylobacter* contamination investigated in this study, the highest isolation rates were obtained from the overshoes (a proxy for contamination of the overall farm environment) and drinkers, which together (i.e. environment and drinking water) accounted for an estimated 75% of all isolates from turkeys. These sources have been frequently indicated as being important for *Campylobacter* introduction and transmission into turkey farms (Wallace *et al.*, 1998; Smith *et al.*, 2004; Kiess *et al.*, 2007; Ahmed *et al.*, 2016). El-Adawy *et al.* (2012) detected the first positive PCR results of drinking water samples when turkey chicks were six days-old, whereas colonization of birds occurred between the second and third week of age, suggesting that drinking water could have been the source of *Campylobacter*. In the same study, the environmental samples, including drinking water and feed, were found positive for *Campylobacter* by the fourth week of age onwards (El-Adawy *et al.*, 2012). Although in the present study it was not possible to ascertain the exact timing of colonization because of the sampling design, both the

Table 2. Percentages of *Campylobacter* isolates from fattening turkeys attributed to the farm environment, breeders, pests, or water sources. Numbers within brackets indicate 95% confidence intervals.

	Environment (overshoes)	Breeders (ancestry)	Pests (flies, lesser mealworm, mice)	Drinkers and water line
Farm A/House 1	43.72% (28.89–58.54%)	0.09% (0.04–0.14%)	18.91% (7.78–30.03%)	37.28% (26.63–47.92%)
Farm A/House 2	34.77% (18.41–51.13%)	2.02% (0.49–3.55%)	29.55% (7.64–51.46%)	33.64% (16.39–50.89%)
Farm B/House 1	37.22% (24.31–50.14%)	11.79% (0.00–26.10%)	17.98% (7.07–28.90%)	33.01% (21.24–44.79%)
Farm B/House 2	49.25% (31.52–66.98%)	1.05% (0.00–2.56%)	15.82% (4.96–26.67%)	33.90% (26.29–41.51%)
Overall	40.32% (32.15–48.49%)	4.71% (0.00–10.07%)	20.30% (13.08–27.53%)	34.66% (28.39–40.93%)

A



B

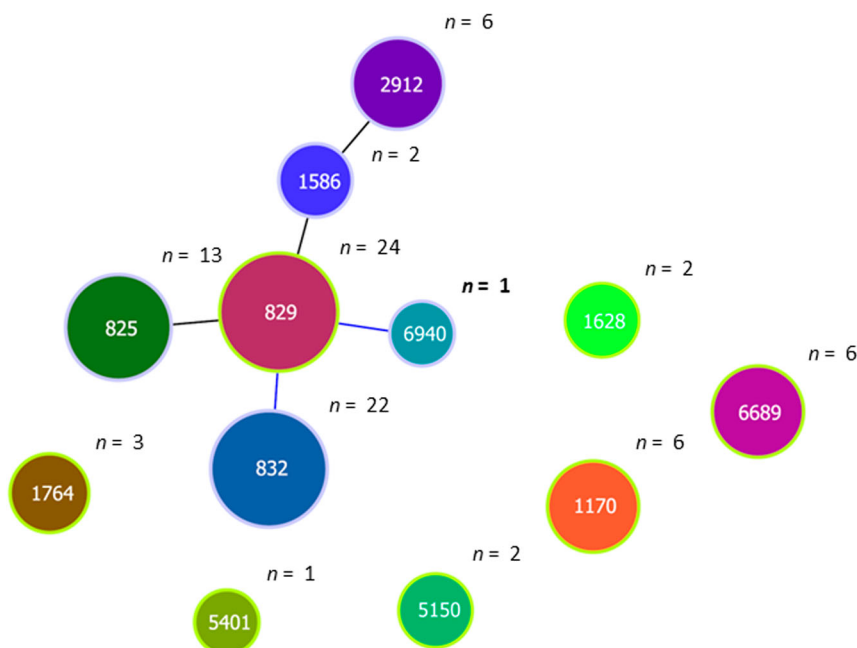


Figure 2. GoeBURST representation of the genetic relatedness and the frequency of *C. jejuni* (A) and *C. coli* (B) STs isolated in this study. Each circle corresponds to an ST (whose identifying numbers are reported in the circles) and the size of the circle is proportional to the number of isolates comprising that ST (indicated outside the circles). Circles not linked by a line differ by >3 alleles.

overshoes and the drinkers were found positive when turkeys became positive. Several STs (i.e. STs 51, 104, and 2361 for *C. jejuni* and STs 825, 829, 832, 1764, 2912, 5150, and 6689 for *C. coli*) were shared between isolates from drinkers and those from fattening turkeys. Similarly, overlapping STs (i.e. ST 104 for *C. jejuni* and STs 825, 829, 832, 1628, and 1764 for

C. coli) among *C. jejuni* and *C. coli* isolated from the in-house overshoes and from turkeys were also observed. The role of water as a potential source of colonization is highlighted by *C. coli* strains belonging to ST 829 that were concurrently isolated from the water line and the flock receiving that drinking water. Moreover, the role of contaminated litter as a potential

source of colonization is evidenced by strains belonging to the same STs (ST 2850 for *C. jejuni* and ST 829 for *C. coli*) that were concurrently isolated from the anteroom overshoes of a turkey house and the flock reared in another house. However, we suppose that drinking water and litter contamination detected in our study was related to the strict contact with turkey faeces containing high loads of *Campylobacter* (Wallace *et al.*, 1998), and thus these environmental sources are more likely to act as vehicles for *Campylobacter* dissemination within the turkey house rather than being sources of flock contamination per se.

Other sources from the farm environment (i.e. flies, lesser mealworms, and a mouse) were found positive for *C. jejuni* and *C. coli*, accounting for an estimated 20.3% of all turkey isolates. Relatively few studies investigating these environmental sources as potential vectors of *Campylobacter* spp. colonization of turkey flocks are currently available (Lee *et al.*, 2005; Ahmed *et al.*, 2016), whereas they have been frequently reported as being responsible for chicken flock colonization (Bates *et al.*, 2004; Meerburg and Kijlstra, 2007; Hald *et al.*, 2008; Newell *et al.*, 2011; Agunos *et al.*, 2014). In our study, ST 51 (*C. jejuni*) and ST 832 (*C. coli*) matched between isolates from flies captured in a house and turkeys reared in the other house, suggesting that flies may be responsible for cross-contamination between different flocks, as already hypothesized for broiler chickens (Hald *et al.*, 2004). On several occasions, also STs (i.e. STs 104 and 2850 for *C. jejuni* and STs 832 and 6689 for *C. coli*) of strains isolated from *Alphitobius diaperinus* matched with those isolated from fattening turkeys. Unlike flies, these insects are unlikely to be responsible for cross-contamination between flocks reared in the same farm or being the source of introduction of *Campylobacter* spp. in the farm because they usually live within a poultry house, hiding and persisting over time in floor and wall cracks and joints (Kaufman *et al.*, 2002). Lesser mealworms may rather act as ongoing reservoirs of contamination within a poultry flock and could be involved in carry-over between subsequent flocks (Bates *et al.*, 2004). Overall, we consider that flies and lesser mealworms are likely to play a significant role in the transmission of *Campylobacter* in turkey farms, at least as vectors.

It is interesting to note that some environmental samples (i.e. dust from the anteroom door and fans, air from inside the house, water from the in-house collection tank and beetles) and those taken during downtime tested always negative for *Campylobacter* spp. Besides the fact that some samples (e.g. air and dust) may be unfavourable to *Campylobacter* growth and survival due to a lack of moisture (Newell *et al.*, 2011), a concentration of bacterial cells at undetectable levels or in a non-cultivable form and the culture-based procedure employed in this study may have failed to

detect *Campylobacter* (Krüger *et al.*, 2014). It is widely accepted that *Campylobacter* is able to survive under environmentally stressful conditions as viable but non-culturable forms, which are not detected by culture-based methods (Chaisowwong *et al.*, 2012). However, it has been demonstrated that viable but non-culturable forms may persist in the farm environment, even after cleaning and disinfection procedures, and colonize successive flocks (Magajna & Schraft, 2015; Battersby *et al.*, 2016). Unfortunately, in the present study, no analyses could be carried out to detect these forms, but it seems highly advisable to do so in future studies. Nevertheless, in accordance with El-Adawy *et al.* (2012), we can consider downtime cleaning and disinfection procedures in our turkey farms to have been effective in reducing *Campylobacter* spp. in the farm environment. Although knowledge gained in this study does not differ from previous findings in broilers, we were able to demonstrate that some sources are critical in turkeys as in broilers.

In this study, MLST analysis showed high genetic diversity among *C. jejuni* and *C. coli* isolates from fattening turkeys and the farm environment. Also, studies in which MLST was used to study the population structure of *Campylobacter* spp. colonizing turkeys, demonstrated a very high diversity (D'Lima *et al.*, 2007; Gu *et al.*, 2009; El-Adawy *et al.*, 2013; Kashoma *et al.*, 2014; Manfreda *et al.*, 2016). A relatively higher level of genetic relatedness was observed among *C. coli* isolates when compared to those of *C. jejuni*, which is in contrast to Kashoma *et al.* (2014), who reported a higher genetic (ST) variation in *C. coli* than in *C. jejuni* from turkeys. In our study, MLST showed that multiple genotypes were present in turkey flocks (and in some environmental sources, such as drinkers, in-house overshoes, flies, and lesser mealworms) at the same time, and a succession of different genotypes was observed over time. Additionally, some predominant genotypes (e.g. *C. coli* ST 832 found in most of the samplings and production cycles, and in both houses and farms) were detected. All these data suggest that multiple and/or common routes of exposure may have been involved in *Campylobacter* introduction and dissemination in turkey flocks. On the other hand, genetic relatedness was documented among *C. jejuni* and *C. coli* from fattening turkeys and the farm environment. Indeed, overlapping STs between strains isolated from flocks of different origin reared in the same farm (e.g. *C. jejuni* ST 2850 and *C. coli* ST 829, as well as *C. jejuni* STs 51 and 104, and *C. coli* ST 1170 identified concurrently in fattening turkeys reared in a house and in anteroom overshoes and flies in the adjoining house, respectively) suggests a cross-contamination between flocks or a common source of transmission (e.g. the farmer and/or the flies moving from one house to the other). Moreover, STs (i.e. STs 2116 and 2274 for *C. jejuni* and STs 825, 829, 832, and 1170 for *C. coli*)

matching between subsequent production cycles observed in both farms may be attributed to a carry-over between production cycles or to a possible existence of other sources (e.g. utensils and other fomites, feed, dust, and personnel) harbouring *Campylobacter* in the farm environment, since no *Campylobacter* spp. were isolated during downtime. Genetic relatedness was also observed among *Campylobacter* strains from turkeys and environmental sources isolated from the two different farms (i.e. STs 2863, 2274, 2850, and 2116 for *C. jejuni* and STs 829 and 832 for *C. coli*). Genotyping by MLST has been rarely used for studying *Campylobacter* spp. populations in commercial turkeys. In the present study, several STs were identified in either *C. jejuni* or *C. coli* isolates from breeders, fattening turkeys, and the environmental sources. Out of the 13 and 12 STs identified

in *C. jejuni* and in *C. coli* isolates belonging to six and one CCs, respectively, only one *C. jejuni* ST (i.e. 51) and three *C. coli* STs (i.e. 825, 829, and 1170), but all the *C. jejuni* and *C. coli* CCs have been previously reported in turkey isolates from the USA, Germany, Denmark, Scotland, and Italy (Colles *et al.*, 2003; Miller *et al.*, 2006; D'Lima *et al.*, 2007; Sheppard *et al.*, 2009; Lang *et al.*, 2010; Sheppard *et al.*, 2010; Zautner *et al.*, 2011; El-Adawy *et al.*, 2013; Kashoma *et al.*, 2014; Manfreda *et al.*, 2016). Furthermore, the *C. coli* ST 829 (CC 828) has been identified in retail ground turkey meat in the USA (Thakur *et al.*, 2009). Only a single study (Manfreda *et al.*, 2016) is currently available on MLST diversity of *C. jejuni* strains isolated from Italian turkey farms in 1998. Although most of the STs (except for ST 51 of a single isolate) identified by Manfreda *et al.* (2016) did not match with those

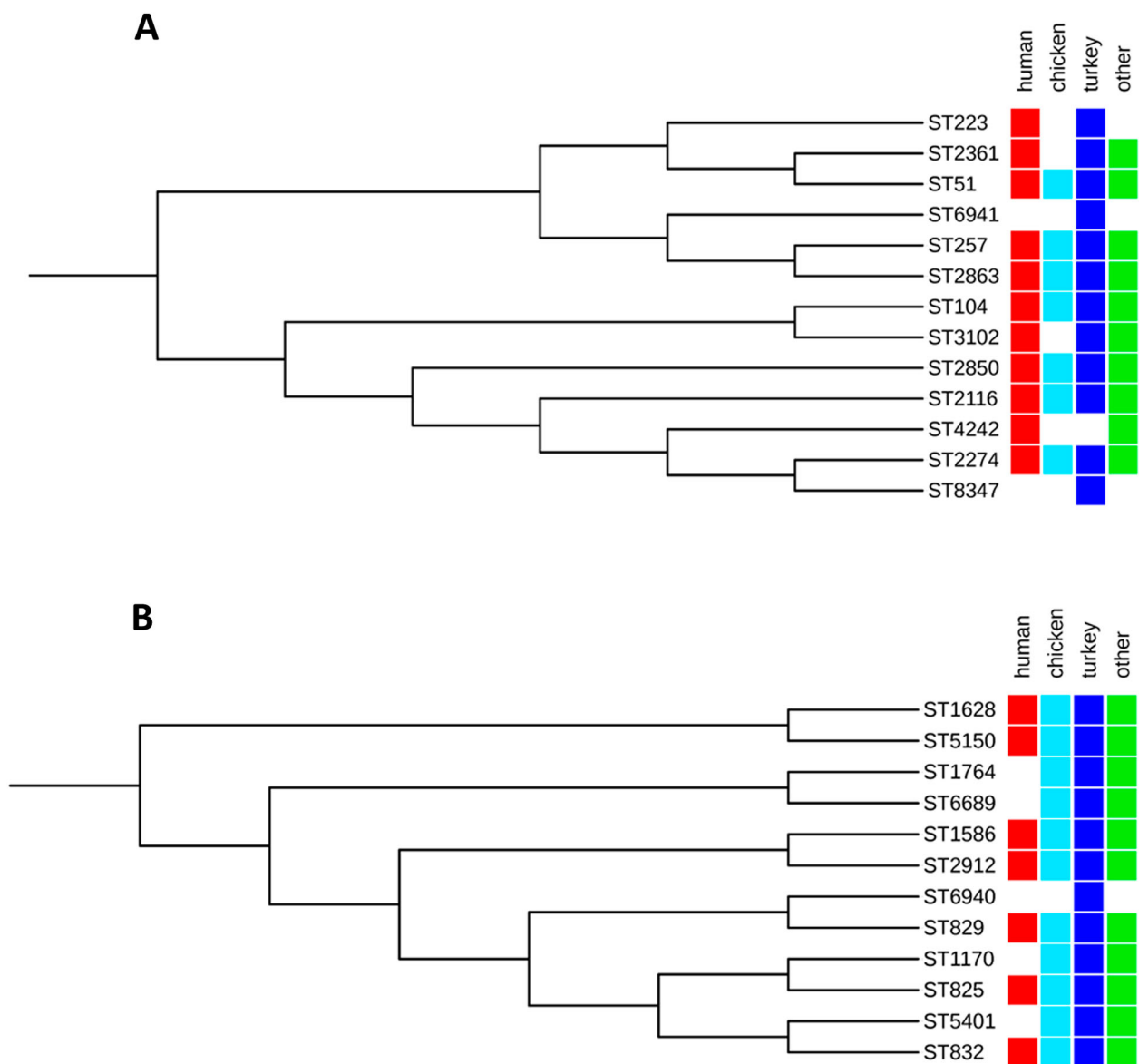


Figure 3. Occurrence of *C. jejuni* (A) and *C. coli* (B) STs from this study and from poultry (i.e. chickens and turkeys) and human samples downloaded from the *Campylobacter* MLST database (<http://pubmlst.org/campylobacter/>). Other = other animals (e.g. cattle, sheep, goat, pig, dog, wild birds), environmental water, farm environment, and soil.

identified in the present study, common CCs (i.e. 21, 257, 443, and 446) were found. Surprisingly, the only matching genotype was from turkeys reared in the same region, i.e. Veneto (North-Eastern Italy), where our study was carried out, suggesting the presence of locally predominant strains circulating in the turkey production sector. Finally, 11/13 *C. jejuni* (i.e. 51, 104, 223, 257, 2116, 2274, 2361, 2850, 2863, 3102, and 4242) and 7/12 *C. coli* (i.e. 825, 829, 832, 1586, 1628, 2912, and 5150) STs identified in this study matched with those isolated from human campylobacteriosis cases (Figure 3), suggesting that turkeys may represent a potential source of human *Campylobacter* infection.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

- Adkin, A., Hartnett, E., Jordan, L., Newell, D. & Davison, H. (2006). Use of a systematic review to assist the development of *Campylobacter* control strategies in broilers. *Journal of Applied Microbiology*, 100, 306–315.
- Agunos, A., Waddell, L., Léger, D., Taboada, E. & Kimman, T. (2014). A systematic review characterizing on-farm sources of *Campylobacter* spp. for broiler chickens. *PLoS One*, 9, e104905.
- Ahmed, M.F., El-Adawy, H., Hotzel, H., Tomaso, H., Neubauer, H., Kemper, N., Hartung, J. & Hafez, H.M. (2016). Prevalence, genotyping and risk factors of thermophilic *Campylobacter* spreading in organic Turkey farms in Germany. *Gut Pathogens*, 8, 3547.
- Alter, T., Weber, R.M., Hamedy, A. & Glünder, G. (2011). Carry-over of thermophilic *Campylobacter* spp. between sequential and adjacent poultry flocks. *Veterinary Microbiology*, 147, 90–95.
- Arsenault, J., Letellier, A., Quessy, S., Normand, V. & Boulianne, M. (2007). Prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. *Preventive Veterinary Medicine*, 81, 250–264.
- Bates, C., Hiatt, K.L. & Stern N.J. (2004). Relationship of *Campylobacter* isolated from poultry and from darkling beetles in New Zealand. *Avian Diseases*, 48, 138–147.
- Battersby, T., Whyte, P. & Bolton, D.J. (2016). The pattern of *Campylobacter* contamination on broiler farms; external and internal sources. *Journal of Applied Microbiology*, 120, 1108–1118.
- Bull, S.A., Allen, V.M., Domingue, G., Jørgensen, F., Frost, J.A., Ure, R., Whyte, R., Tinker, D., Corry, J.E., Gillard-King, J. & Humphrey, T.J. (2006). Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. *Applied and Environmental Microbiology*, 72, 645–652.
- Callicott, K.A., Frithriksdottir, V., Reiersen, J., Lowman, R., Bisaillon, J.R., Gunnarsson, E., Berndtson, E., Hiatt, K.L., Needleman, D.S. & Stern, N.J. (2006). Lack of evidence for vertical transmission of *Campylobacter* spp. in chickens. *Applied and Environmental Microbiology*, 72, 5794–5798.
- Cawthraw, S.A., Wassenaar, T.M., Ayling, R. & Newell, D.G. (1996). Increased colonization potential of *Campylobacter jejuni* strain 81116 after passage through chickens and its implication on the rate of transmission within flocks. *Epidemiology and Infection*, 117, 213–215.
- Chaisowong, W., Kusumoto, A., Hashimoto, M., Harada, T., Maklon, K. & Kawamoto, K. (2012). Physiological characterization of *Campylobacter jejuni* under cold stresses conditions: its potential for public threat. *Journal of Veterinary Medical Science*, 74, 43–50.
- Colles, F.M., Dingle, K.E., Cody, A.J. & Maiden, M.C. (2008). Comparison of *Campylobacter* populations in wild geese with those in starlings and free-range poultry on the same farm. *Applied and Environmental Microbiology*, 74, 3583–3590.
- Colles, F.M., Jones, K., Harding, R.M. & Maiden, M.C. (2003). Genetic diversity of *Campylobacter jejuni* isolates from farm animals and the farm environment. *Applied and Environmental Microbiology*, 69, 7409–7413.
- Cox, N.A., Richardson, L.J., Maurer, J.J., Berrang, M.E., Fedorka-Cray, P.J., Buhr, R.J., Byrd, J.A., Lee, M.D., Hofacre, C.L., O’Kane, P.M., Lammerding, A.M., Clark, A.G., Thayer, S.G. & Doyle, M.P. (2012). Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. *Journal of Food Protection*, 75, 1896–1902.
- Dingle, K.E., Colles, F.M., Falush, D. & Maiden M.C. (2005). Sequence typing and comparison of population biology of *Campylobacter coli* and *Campylobacter jejuni*. *Journal of Clinical Microbiology*, 43, 340–347.
- Dingle, K.E., Colles, F.M., Wareing, D.R., Ure, R., Fox, A.J., Bolton, F.E., Bootsma, H.J., Willems, R.J., Urwin, R. & Maiden M.C. (2001). Multilocus sequence typing system for *Campylobacter jejuni*. *Journal of Clinical Microbiology*, 39, 14–23.
- D’lima, C.B., Miller, W.G., Mandrell, R.E., Wright, S.L., Siletzky, R.M., Carver, D.K. & Kathariou, S. (2007). Clonal population structure and specific genotypes of multidrug-resistant *Campylobacter coli* from Turkeys. *Applied and Environmental Microbiology*, 73, 2156–2164.
- El-Adawy, H., Hotzel, H., Tomaso, H., Neubauer, H. & Hafez, H.M. (2012). Elucidation of colonization time and prevalence of thermophilic *Campylobacter* species during turkey rearing using multiplex polymerase chain reaction. *Poultry Science*, 91, 454–459.
- El-Adawy, H., Hotzel, H., Tomaso, H., Neubauer, H., Taboada, E.N., Ehrlich, R. & Hafez, H.M. (2013). Detection of genetic diversity in *Campylobacter jejuni*

- isolated from a commercial Turkey flock using *flaA* typing, MLST analysis and microarray assay. *PLoS One*, 8, e51582.
- Ellis-Iversen, J., Ridley, A., Morris, V., Sowa, A., Harris, J., Atterbury, R., Sparks, N. & Allen, V. (2012). Persistent environmental reservoirs on farms as risk factors for *Campylobacter* in commercial poultry. *Epidemiology and Infection*, 140, 916–924.
- European Food Safety Authority. (2011). Scientific opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal*, 9, 2105.
- European Food Safety Authority & European Centre for Disease Prevention and Control. (2017). The European Union summary report on trends and sources of Zoonoses, Zoonotic agents and food-borne outbreaks in 2016. *EFSA Journal*, 15, 5077.
- Giacomelli, M., Andrighetto, C., Lombardi, A., Martini, M. & Piccirillo A. (2012). A longitudinal study on thermophilic *Campylobacter* spp. in commercial turkey flocks in Northern Italy: occurrence and genetic diversity. *Avian Diseases*, 56, 693–700.
- Gu, W., Siletzky, R.M., Wright, S., Islam, M. & Kathariou, S. (2009). Antimicrobial susceptibility profiles and strain type diversity of *Campylobacter jejuni* isolates from turkeys in eastern North Carolina. *Applied and Environmental Microbiology*, 75, 474–482.
- Hald, B., Skovgård, H., Bang, D.D., Pedersen, K., Dybdahl, J., Jespersen, J.B. & Madsen, M. (2004). Flies and *Campylobacter* infection of broiler flocks. *Emerging Infectious Diseases*, 10, 1490–1492.
- Hald, B., Skovgård, H., Pedersen, K. & Bunkenborg, H. (2008). Influxed insects as vectors for *Campylobacter jejuni* and *Campylobacter coli* in Danish broiler houses. *Poultry Science*, 87, 1428–1434.
- Hansson, I., Vågsholm, I., Svensson, L. & Olsson Engvall, E. (2007). Correlations between *Campylobacter* spp. prevalence in the environment and broiler flocks. *Journal of Applied Microbiology*, 103, 640–649.
- Hermans, D., Van Deun, K., Messens, W., Martel, A., Van Immerseel, F., Haesebrouck, F., Rasschaert, G., Heyndrickx, M. & Pasmans, F. (2011). *Campylobacter* control in poultry by current intervention measures ineffective: urgent need for intensified fundamental research. *Veterinary Microbiology*, 152, 219–228.
- Humphrey, T., O'Brien, S. & Madsen, M. (2007). *Campylobacter* as zoonotic pathogens: a food production perspective. *International Journal of Food Microbiology*, 117, 237–257.
- Huneau-Salaün, A., Denis, M., Balaine, L. & Salvat, G. (2007). Risk factors for *Campylobacter* spp. colonization in French free-range broiler-chicken flocks at the end of the indoor rearing period. *Preventive Veterinary Medicine*, 80, 34–48.
- Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. & Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, 28, 687–720.
- Kashoma, I.P., Kumar, A., Sanad, Y.M., Gebreyes, W., Kazwala, R.R., Garabed, R. & Rajashekara, G. (2014). Phenotypic and genotypic diversity of thermophilic *Campylobacter* spp. in commercial Turkey flocks: a longitudinal study. *Foodborne Pathogens and Disease*, 11, 850–860.
- Kaufman, P.E., Burgess, M., Rutz, D.A. & Glenister C. (2002). Population dynamics of manure inhabiting arthropods under an integrated pest management (IPM) program in New York poultry facilities-3 case studies. *The Journal of Applied Poultry Research*, 11, 90–103.
- Keener, K.M., Bashor, M.P., Curtis, P.A., Sheldon, B.W. & Kathariou, S. (2004). Comprehensive review of *Campylobacter* and poultry processing. *Comprehensive Reviews in Food Science and Food Safety*, 3, 105–116.
- Kiess, A.S., Kenney, P.B. & Nayak, R.R. (2007). *Campylobacter* detection in commercial turkeys. *British Poultry Science*, 48, 567–572.
- Krüger, N.J., Buhler, C., Iwobi, A.N., Huber, I., Ellerbroek, L., Appel, B. & Stingl, K. (2014). “Limits of control” – crucial parameters for a reliable quantification of viable *Campylobacter* by real-time PCR. *PLoS One*, 9, e88108.
- Lang, P., Lefebure, T., Wang, W., Pavinski Bitar, P., Meinersmann, R.J., Kaya, K. & Stanhope, M.J. (2010). Expanded multilocus sequence typing and comparative genomic hybridization of *Campylobacter coli* isolates from multiple hosts. *Applied and Environmental Microbiology*, 76, 1913–1925.
- Lee, B.C., Reimers, N., Barnes, H.J., D’Lima, C., Carver, D. & Kathariou, S. (2005). Strain persistence and fluctuation of multiple-antibiotic resistant *Campylobacter coli* colonizing turkeys over successive production cycles. *Foodborne Pathogens and Disease*, 2, 103–110.
- Lee, M.D. & Newell, D.G. (2006). *Campylobacter* in poultry: filling an ecological niche. *Avian Diseases*, 50, 1–9.
- Letunic, I. & Bork, P. (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research*, 44, W242–W245.
- Luangtongkum, T., Morishita, T.Y., Ison, A.J., Huang, S., McDermott, P.F. & Zhang, Q. (2006). Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. *Applied and Environmental Microbiology*, 72, 3600–3607.
- Lyngstad, T.M., Jonsson, M.E., Hofshagen, M. & Heier B.T. (2008). Risk factors associated with the presence of *Campylobacter* species in Norwegian broiler flocks. *Poultry Science*, 87, 1987–1994.
- Magajna, B.A. & Schraft, H. (2015). *Campylobacter jejuni* biofilm cells become viable but non-culturable (VBNC) in low nutrient conditions at 4°C more quickly than their planktonic counterparts. *Food Control*, 50, 45–50.
- Manfreda, G., Parisi, A., De Cesare, A., Mion, D., Piva, S. & Zanoni, R.G. (2016). Typing of *Campylobacter jejuni* isolated from Turkey by genotypic methods, antimicrobial susceptibility, and virulence gene patterns: a retrospective study. *Foodborne Pathogens and Disease*, 13, 93–100.
- Meerburg, B. G. & Kijlstra, A. (2007). Role of rodents in transmission of *Salmonella* and *Campylobacter*. *Journal of the Science of Food and Agriculture*, 87, 2774–2781.
- Miller, W.G., Englen, M.D., Kathariou, S., Wesley, I.V., Wang, G., Pittenger-Alley, L., Siletz, R.M., Muraoka, W., Fedorka-Cray, P.J. & Mandrell, R.E. (2006). Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. *Microbiology*, 152, 245–255.
- Mughini-Gras, L., Penny, C., Ragimbeau, C., Schets, F.M., Blaak, H., Duim, B., Wagenaar, J.A., de Boer, A., Cauchie, H.M., Mossong, J. & van Pelt, W. (2016). Quantifying potential sources of surface water contamination with *Campylobacter jejuni* and *Campylobacter coli*. *Water Research*, 101, 36–45.
- Nascimento, M., Sousa, A., Ramirez, M., Francisco, A.P., Carriço, J.A. & Vaz, C. (2017). Phyloviz 2.0: providing scalable data integration and visualization for multiple

- phylogenetic inference methods. *Bioinformatics*, 33, 128–129.
- Newell, D.G., Elvers, K.T., Dopfer, D., Hansson, I., Jones, P., James, S., Gittins, J., Stern, N.J., Davies, R., Connerton, I., Pearson, D., Salvat, G. & Allen, V.M. (2011). Biosecurity-based interventions and strategies to reduce *Campylobacter* spp. on poultry farms. *Applied and Environmental Microbiology*, 77, 8605–8614.
- Ogden, I.D., MacRae, M., Johnston, M., Strachan, N.J., Cody, A.J., Dingle, K.E. & Newell, D.G. (2007). Use of multilocus sequence typing to investigate the association between the presence of *Campylobacter* spp. in broiler drinking water and *Campylobacter* colonization in broilers. *Applied and Environmental Microbiology*, 73, 5125–5129.
- Parkhill, J., Wren, B.W., Mungall, K., Ketley, J.M., Churcher, C., Basham, D., Chillingworth, T., Davies, R.M., Feltwell, T., Holroyd, S., Jagels, K., Karlyshev, A.V., Moule, S., Pallen, M.J., Penn, C.W., Quail, M.A., Rajandream, M.A., Rutherford, K.M., van Vliet, A.H., Whitehead, S. & Barrell, B.G. (2000). The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature*, 403, 665–668.
- Pérez-Boto, D., García-Pena, F.J., Abad-Moreno, J.C., Hurtado-Pizarro, M.D., Pérez-Cobo, I. & Echeita, M.A. (2010). Drinking water as the source of *Campylobacter coli* infection in grandparent heavy breeders. *Avian Pathology*, 39, 483–487.
- Perko-Mäkelä, P., Isohanni, P., Katzav, M., Lund, M., Hänninen, M.L. & Lyhs, U. (2009). A longitudinal study of *Campylobacter* distribution in a Turkey production chain. *Acta Veterinaria Scandinavica*, 51, 18.
- Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- Sahin, O., Kassem, I.I., Shen, Z., Lin, J., Rajashekara, G. & Zhang, Q. (2015). *Campylobacter* in poultry: ecology and potential interventions. *Avian Diseases*, 59, 185–200.
- Sheppard, S.K., Dallas, J.F., MacRae, M., McCarthy, N.D., Sproston, E.L., Gormley, F.J., Strachan, N.J., Ogden, I.D., Maiden, M.C. & Forbes, K.J. (2009). *Campylobacter* genotypes from food animals, environmental sources and clinical disease in Scotland 2005/6. *International Journal of Food Microbiology*, 134, 96–103.
- Sheppard, S.K., Dallas, J.F., Wilson, D.J., Strachan, N.J., McCarthy, N.D., Jolley, K.A., Colles, F.M., Rotariu, O., Ogden, I.D., Forbes, K.J., Maiden, M.C. & Hartskeerl, R.A. (2010). Evolution of an agriculture-associated disease causing *Campylobacter coli* clade: evidence from national surveillance data in Scotland. *PLoS One*, 5, e15708.
- Skarp, C.P., Hänninen, M.L. & Rautelin, H.I. (2016). *Campylobacteriosis*: the role of poultry meat. *Clinical Microbiology and Infection*, 22, 103–109.
- Smith, K., Reimers, N., Barnes, H.J., Lee, B.C., Siletzky, R. & Kathariou, S. (2004). *Campylobacter* colonization of sibling Turkey flocks reared under different management conditions. *Journal of Food Protection*, 67, 1463–1468.
- Thakur, S., White, D.G., McDermott, P.F., Zhao, S., Kroft, B., Gebreyes, W., Abbott, J., Cullen, P., English, L., Carter, P. & Harbottle, H. (2009). Genotyping of *Campylobacter coli* isolated from humans and retail meats using multilocus sequence typing and pulsed-field gel electrophoresis. *Journal of Applied Microbiology*, 106, 1722–1733.
- Wallace, J.S., Stanley, K.N. & Jones, K. (1998). The colonization of turkeys by thermophilic campylobacters. *Journal of Applied Microbiology*, 85, 224–230.
- Wright, S.L., Carver, D.K., Siletzky, R.M., Romine, S., Morrow, W.E. & Kathariou, S. (2008). Longitudinal study of prevalence of *Campylobacter jejuni* and *Campylobacter coli* from turkeys and swine grown in close proximity. *Journal of Food Protection*, 71, 1791–1796.
- Zautner, A.E., Herrmann, S., Corso, J., Tareen, A.M., Alter, T. & Gross, U. (2011). Epidemiological association of different *Campylobacter jejuni* groups with metabolism-associated genetic markers. *Applied and Environmental Microbiology*, 77, 2359–2365.
- Zweifel, C., Scheu, K.D., Keel, M., Renggli, F. & Stephan, R. (2008). Occurrence and genotypes of *Campylobacter* in broiler flocks, other farm animals, and the environment during several rearing periods on selected poultry farms. *International Journal of Food Microbiology*, 125, 182–187.