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# Prevalence and genetic diversity of *Campylobacter* spp. in the production chain of broiler chickens in Lebanon and its association with the intestinal protozoan *Blastocystis* sp.

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**ABSTRACT** *Campylobacter jejuni* is recognized as the most common foodborne pathogen associated with human gastroenteritis worldwide. Broilers are frequently infected by the bacteria and are considered the main source of exposure to humans. However, despite its public health impact, no recent data are currently available in Lebanon about *Campylobacter* spp. in poultry and human population. Therefore, this study aimed to determine the prevalence and genetic diversity of *Campylobacter* spp. in 227 ceca and on 227 carcasses of broiler chickens collected in Lebanese slaughterhouses. Overall, the prevalence of *Campylobacter* was shown to reach 67.0% in ceca and 17.2% on carcasses of Lebanese poultry. The only 2 *Campylobacter* species identified were *C. jejuni* and *C. coli*, with a slightly higher prevalence of *C. coli* in ceca and of *C. jejuni* on carcasses. A high level of genetic diversity was reported among the 51 *C. jejuni* isolates selected, since 25 distinct profiles were identified according to the comparative genomic fingerprinting typing method based on a subset of 40 genes using the 90% similarity threshold. Predominant

clusters observed in Lebanese poultry isolates were also frequently found among French human clinical cases, highlighting that broiler chickens represent a potential reservoir for human campylobacteriosis. In addition, a significantly higher prevalence of *Campylobacter* spp. was found in slaughterhouse workers than in a cohort of hospitalized patients with no contact with poultry, confirming that contaminated broiler chickens in slaughterhouse appeared to be a non-negligible source of *Campylobacter* spp. transmission. Interestingly, a significant association between *Campylobacter* spp. and *Blastocystis* sp. has been observed. This correlation suggested that the presence of *Campylobacter* spp. would be favored when *Blastocystis* sp. is present and, similarly, the absence of one would favor the absence of the other. This is the first large-scale investigation focusing on the impact of *Campylobacter* spp. in broiler chickens in Lebanon and confirmed the need to implement prevention and control measures in the poultry production to reduce the burden of campylobacteriosis in the human population.

**Key words:** *Campylobacter*, *Blastocystis*, zoonosis, genetic diversity, poultry

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## INTRODUCTION

*Campylobacter* species are the most commonly reported gastrointestinal bacterial pathogens in humans worldwide (FAO/WHO, 2009) that cause an enteric foodborne zoonotic disease called campylobacteriosis. In the European Union (EU), this disease ranks first in terms of prevalence, exceeding the total number of human zoonoses caused respectively by *Salmonella*,

*Yersinia*, and *Escherichia coli* (EFSA and ECDC, 2017). Campylobacteriosis induces a self-limiting gastroenteritis that generally resolves within 2 to 3 wk, but may lead to severe post-infectious sequelae such as Guillain-Barré syndrome and reactive arthritis (Mortensen et al., 2009; Jackson et al., 2014). *Campylobacter* spp. are regarded as common inhabitants of the intestinal tract of many domestic and wild birds, as well as warm-blooded animals such as cattle and pigs, in which these bacteria seem to establish a commensal relationship. Humans become infected either directly by animal contact or indirectly through handling and ingestion of contaminated food products derived from these animals such as undercooked chicken

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and pork, improperly cooked beef, and raw milk or untreated drinking water (Lévesque et al., 2008; Mossong et al., 2016). Several avian species are considered the main reservoirs of *Campylobacter* spp., in particular broiler chickens intended for human consumption. Indeed, these bacteria can grow at the higher body temperature of birds, i.e., 42°C. In addition, infected poultry are mostly asymptomatic and shed the bacteria in high numbers, thereby facilitating transmission of *Campylobacter* spp. (Sarp et al., 2016; Garcia-Sanchez et al., 2017; Pergola et al., 2017).

Human campylobacteriosis is mainly caused by the 2 species, *Campylobacter jejuni* and *C. coli*, which are responsible for around 90% of all cases diagnosed in the EU. More than 200,000 confirmed cases of campylobacteriosis were reported in 2016, with chickens being the main reservoir of transmission according to the European Food Safety Authority (EFSA and ECDC, 2017). According to a baseline study conducted in Europe in 2008, the prevalence of *Campylobacter* spp. was globally shown to reach 71.2% and 75.8% in live broiler chickens and broiler carcasses, respectively (EFSA, 2010). Moreover, the prevalence within member states ranged from 2.0 to 100% for cecal contents and from 4.9 to 100% for carcasses. In Middle Eastern countries, a large-scale study reported that 57 and 100% of poultry slaughterhouses were contaminated by *Campylobacter* spp. in Bahrain and Saudi Arabia, respectively, with *C. jejuni* being the major species identified in both surveys (Senok and Botta, 2009). Moreover, 48% of chickens supplied to supermarkets by Saudi (53%) and Qatari (45.9%) producers were found to be contaminated with *Campylobacter* spp. (Abu-Madi et al., 2016). In Lebanon, very little data are currently available regarding the burden of *Campylobacter* spp. in human and broiler chicken populations. The few smaller-scale studies conducted in this country showed low (0.7 and 2.5%) (Talhouk et al., 1998; Bechara et al., 2016) to moderate (11.1%) (Dabboussi et al., 2012) prevalence of *Campylobacter* spp. among diarrheic patients, with a relative predominance of *C. jejuni*. Moreover, 100% of fecal and cloacal swabs collected from a poultry farm and 50% of breast swabs obtained from the corresponding slaughterhouse were shown to be contaminated by either *C. jejuni* or *C. coli* or both species (Hajj Semaan et al., 2014). In addition, 22.7% of ceca collected from meat chicken breeder farms and 9.7% of raw chicken carcasses obtained from shops in Lebanon were also positive for the bacteria, with predominance of *C. coli* (Talhouk et al., 1998).

In addition to *Campylobacter* spp. and other pathogenic bacterial species, cosmopolitan enteric protozoans such as *Blastocystis* sp. also frequently colonize humans (Clark et al., 2013; Stensvold and Clark, 2016) and a wide range of animals, including birds (Cian et al., 2017). In Lebanon, the infection rate exceeds 50% in various human cohorts (Osman et al., 2016; Greige et al., 2018) and 30% of poultry samples collected from slaughterhouses (Greige et al., 2018). The

zoonotic transmission of *Blastocystis* sp. was confirmed through repeated and direct contact between chickens and their handlers (Greige et al., 2018). Although the pathogenicity of this parasite is still uncertain, mainly because of its significant asymptomatic carriage (Andersen and Stensvold, 2016), infection with *Blastocystis* sp. in humans is associated with various digestive disorders and urticaria (Tan et al., 2010; Lepczynska et al., 2015). Interestingly, a direct association between the presence of this parasite and variations in the composition of bacterial communities in the gut was recently demonstrated (Andersen et al., 2015; Audebert et al., 2016; Forsell et al., 2017; Nieves-Ramirez et al., 2018), suggesting that *Blastocystis* sp. plays a major role, at least within the human microbiome.

This study has been performed within a joint-cooperation program (Partenariat Hubert Curien, programme Cedre) between France and Lebanon. In France, the National Reference Laboratory for *Campylobacter* has access to a large and representative collection of isolates, representative of the diversity of poultry isolates. This collection is a valuable reference to this first investigation conducted in Lebanon in order to map the isolates collected according to their genotypes. Therefore, the aims of this study were to estimate the prevalence of *Campylobacter* spp. in the ceca and on carcasses of farmed broiler chickens collected in Lebanese slaughterhouses, to identify the main species isolated from positive samples and analyze the genetic diversity of *C. jejuni* isolates. The genetic profiles obtained herein were compared to those of the French poultry database. On another hand, because of the lack of surveillance program for campylobacteriosis in the Lebanese population, the genetic profiles of Lebanese poultry isolates were compared to those of French human clinical isolates (Thépault et al., 2018a) in order to predict the possible exposure of humans to the types of isolates prevalent in Lebanon. Finally, the possible association between *Campylobacter* spp. and *Blastocystis* sp. in broiler chickens was evaluated, since this protozoan was already reported with high prevalence in the same collection of poultry ceca samples screened herein (Greige et al., 2018).

## MATERIALS AND METHODS

### Ethics Statement

The study was approved by the research ethics committee “Comité Interne d’Ethique de la Recherche Médicale (CIER) du Groupement des Hôpitaux de l’Institut Catholique de Lille (GHICL)” (France) with reference number 2016–04–02. Oral and written informed consents were obtained from all subjects included in this study after a clear explanation of the research objectives prior to enrolment. The subject data was collected anonymously (with encryption of the identity of individuals). This study was conducted in accordance with the Code of Ethics of the World

Medical Association (Declaration of Helsinki). All chicken samples were collected after slaughter under the supervision of slaughterhouses staff. No approval from the Institutional Animal Care and Use Committee or ethics committee was necessary, as no experiments involving live chickens were performed.

### Study Sites

The study was conducted throughout 2016 at 3 of the biggest poultry slaughterhouses in Lebanon. The first is located in the governorate of North Lebanon (7 million birds per year), while the other 2 slaughterhouses are in the governorate of Mount Lebanon (4,750,000 and 3,750,000 birds per year). All chickens included in this study belonged to the subspecies *Gallus gallus domesticus* and were aged from 29 to 47 D at the time of slaughter. The animals were raised on a total of 74 farms located near the corresponding slaughterhouses. The clinical epidemiological survey was conducted at Hamidi Medical Centre in Tripoli.

### Poultry Sample Collection

A total of 227 cecal and 227 neck skin samples were collected from the 3 slaughterhouses supplied by the 74 farms, each of which corresponded to 1 batch. For each farm, 5 ceca from 5 randomly selected chickens from the same batch were collected separately by the slaughterhouse staff in the evisceration area. Each cecum was recovered in a sterile bag, respecting the conditions of asepsis and hygiene. At the laboratory, the lower end of each of the 5 ceca collected was cut using a pair of sterile tweezers and its contents were recovered into a sterile cup and pooled for further processing of what was considered a single sample. In parallel, 5 neck skins of eviscerated and chilled carcasses (chilled water bath for 1 h 40 min) were collected from 5 other randomly selected chickens of the same batch under aseptic conditions after chemical treatment with either chlorine (25 ppm in wash water after evisceration for 40 min and 50 ppm in the cooling water used for chilling) or peracetic acid (PAA: 25 ppm) depending on the slaughterhouse, then recovered in a sterile bag. For each of the farms, 1 to 4 samples of ceca and neck skins were collected throughout the year (1 pooled sample per season). All samples were stored in isothermal containers with ice and transported as quickly as possible to the LMSE of the AZM Centre in Tripoli.

### Human Sample Collection

With the aim of evaluating the zoonotic potential of *Campylobacter* spp. isolates identified in poultry, human stool samples were obtained during the same period from 50 individuals in contact with broiler chickens (workers in the slaughtering area, drivers for the delivery service, and a veterinarian) and thus classi-

fied as “exposed” to the bacteria. In parallel, a second cohort included 50 volunteering patients classified as “non-exposed” because they reported no contact with poultry and were followed up for different pathologies at Hamidi Medical Centre in Tripoli.

### Sample Microbiological Analysis

The detection of *Campylobacter* spp. in cecal and neck skin samples was performed 1 D after specimen collection following an adapted version of the ISO 10272–1 standard (ISO, 2006). Briefly, *Campylobacter* species were isolated from cecal samples by direct plating of pooled intestinal content onto the solid modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) selective medium (Scharlau Chemicals, Barcelona, Spain). The agar plates were incubated under microaerophilic conditions at 42°C for 48 h. All neck skin samples from the same batch were swabbed using the same sterile cotton-tipped swab stick and then plated directly onto mCCDA agar plate. In parallel, 5 neck skin samples from the same batch were also minced with sterile scissors, pooled, and added to 225 mL of peptone water, homogenized for 1 min using a stomacher, then 5 mL was transferred to 45 mL of Bolton broth (Scharlau Chemicals, Barcelona, Spain) in a sterile container for pre-enrichment at 37°C for 4 to 6 h, followed by enrichment at 42°C for 48 h (Hue et al., 2011). After incubation, the broth was streaked on an mCCDA agar plates which were then incubated under microaerophilic conditions at 42°C for 48 h. All agar plates were subsequently examined for the presence of typical colonies of *Campylobacter* spp. For each positive sample, 1 colony per plate was selected, subcultured on blood agar (Bio-Rad, Marnes-la-Coquette, France), and incubated for 48 h at 42°C. Following incubation, 1 colony per plate was used to perform standard Gram staining and microscopic examination. Colonies consisting of curved or spiral motile Gram-negative bacilli presumed to be *Campylobacter* spp. were finally subcultured on blood agar.

### Identification of *Campylobacter* spp. by Multiplex-Polymerase Chain Reaction

Genomic DNA was isolated from 1-D colony subcultured bacteria grown on blood agar using the boiling method. Briefly, cell lysates were prepared by suspending 2 or 3 bacterial colonies from the same positive sample in 300  $\mu$ L of sterile distilled water in a microcentrifuge tube placed in boiling water for 5 min before being centrifuged at 13,000 rpm for 10 min. Then, 100  $\mu$ L of the supernatant was transferred to a new sterile tube and stored at –20°C for further analysis. Genomic DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Gometz le Châtel, France). Multiplex PCR (m-PCR) amplification of 20 to 100 ng genomic DNA with the 2 primer

pairs MDmapA1/MDmapA2 and COL3/MDCOL2 was performed for the simultaneous identification of *C. jejuni* and *C. coli*, respectively. The sequence primers and m-PCR conditions were previously described by Denis et al. (1999). The first primer pair targets the *mapA* gene of *C. jejuni* encoding an outer membrane lipoprotein, while the second allows the amplification of the *ceuE* gene of *C. coli* encoding a protein involved in siderophore transport. Positive (DNA obtained from *C. coli* ATCC 33559 and *C. jejuni* ATCC BAA-1062) and negative (DNA matrix replaced by water) m-PCR controls were performed.

### Comparative Genomic Fingerprinting Typing of *C. jejuni* Isolates

Of the 79 *C. jejuni* isolates identified in this study, 51 (65%) were randomly selected for subtyping using the comparative genomic fingerprinting typing method based on a subset of 40 genes (CGF40) as developed by Taboada et al. (2012). The selected isolates were almost equally distributed between cecum (26) and neck skin (25, obtained after detection by swabbing and direct plating (22) and enrichment (3)) samples. To generate a CGF40 fingerprint for each selected *C. jejuni* isolate, eight 5-plex PCRs were performed using 40 primer sets following PCR conditions detailed previously (Thépault et al., 2018a). The CGF40 fingerprints were then visualized using a standard gel electrophoresis containing 2% of agarose stained with GelRed (Interchim, Montluçon, France) according to the manufacturer's recommended procedures. The reference *C. jejuni* strain NCTC11168 was used as positive control in all assays, since it exhibits the 40 targeted markers in its genome. The PCR results were converted to binary values, with 0 representing the absence of the marker gene and 1 indicating its presence in the bacterial genome, resulting in a 40-digit binary profile for each isolate. The isolate profiles were stored in BioNumerics software (v7.6, Applied Maths, Sint Martens-Latem, Belgium). A dendrogram was thus built using the simple matching distance and unweighted-pair group method using average linkages (UPGMA) of clustering in BioNumerics, with 90% fingerprinting similarity to analyze the population structure of isolates (Taboada et al., 2012). CGF40–90% clusters including at least 4% of the isolates of the broiler chickens population were defined as predominant in the population. For comparison purposes, a selection of CGF40–90% profiles from 471 isolates of *C. jejuni* (Thépault et al., 2018a) collected in France during the EU baseline investigation and representative of the national broiler production (Hue et al., 2011) were

used, as well as a collection of 514 *C. jejuni* clinical isolates originating from human cases of campylobacteriosis reported in France (Thépault et al., 2018a,b).

### Statistical Analysis

The seasonal variation of the prevalence of *Campylobacter* spp., the comparison of human cohorts, and the association between *Campylobacter* spp. and *Blas-tocystis* sp. were assessed using the chi-square test. The general significance level was set at a *P* value of below 0.05.

## RESULTS

### Prevalence and Identification of *Campylobacter* spp. in Poultry Samples

Of the 227 samples of chicken ceca screened herein from 74 farms, 152 were found to be positive for *Campylobacter* spp., which corresponded to a prevalence of 67.0% (Table 1). Of the positive specimens, 67 (44.1%) corresponded to single contaminations by *C. coli* and 60 (39.5%) by *C. jejuni*, while the remaining 25 (16.4%) were identified as co-contaminations by both *Campylobacter* species. Although bacterial contamination was detected during the whole year, significant seasonal variation in prevalence of *Campylobacter* spp. was observed among ceca samples, since the bacteria were more prevalent in autumn (31.6%) and summer (28.9%) than in winter (23.7%) and spring (15.8%) (chi-square test, *P* = 0.012). Of the 227 neck skin samples of broiler carcasses collected after evisceration and treatment with either chlorine or PAA, 17.2% (39/227) were positive for *Campylobacter* spp. Of the positive specimens, 19 (48.7%) corresponded to single contamination by *C. jejuni*, 17 (43.6%) by *C. coli*, and 3 (7.7%) to co-contamination by both *Campylobacter* species (Table 1). By merging all these data regarding single contaminations, a total of 79 *C. jejuni* and 84 *C. coli* isolates were identified among all the animal samples screened in this study. Moreover, a farm or a batch was considered positive for *Campylobacter* spp. if on one of the sampling occasions, at least one broiler sample was positive either for cecum or neck skin specimens. Therefore, about 92% (68/74) of the farms analyzed herein were contaminated with *Campylobacter* spp., with slight predominance of single contaminations by *C. coli* (51.5 vs. 48.5% for single contaminations by *C. jejuni*) (Table 1).

**Table 1.** Prevalence and distribution of *Campylobacter* species in Lebanese broiler chicken samples.

Broiler samples	Positive/total	% positive	<i>C. jejuni</i>	<i>C. coli</i>	Mixed infections
Cecum	152/227	67.0%	60 (39.5%)	67 (44.1%)	25 (16.4%)
Neck skin	39/227	17.2%	19 (48.7%)	17 (43.6%)	3 (7.7%)

### Prevalence of *Campylobacter* spp. in Human Cohorts

Single stool specimens were collected from a total of 100 individuals divided into 2 groups. The first group of 50 individuals was considered to be exposed to potential contamination by *Campylobacter* spp. by including only slaughterhouse staff members reporting direct and repeated contact with broiler chickens. Six of these 50 individuals (12.0%) were shown to be colonized with *Campylobacter* spp. Single colonization with *C. coli* were identified in 4 out of the positive individuals, while the 2 remaining positive staff members exhibited mixed colonization with *C. coli* and *C. jejuni*. Interestingly, no cases of *Campylobacter* spp. colonization were found in the control group consisting of 50 individuals hospitalized at Hamidi Medical Centre in Tripoli for various pathologies and reporting no contact with poultry. The difference in *Campylobacter* spp. colonization between the exposed and control groups was statistically significant (chi-square test,  $P = 0.012$ ), confirming that exposure to poultry represents a source of human contamination. Unfortunately, typing of human isolates was not performed due to the absence of single infection with *C. jejuni*.

### CGF40 Typing of Lebanese *C. jejuni* Broiler Isolates and Comparison with French Poultry and Human Isolates

CGF40 fingerprints were generated for 51 selected *C. jejuni* isolates among the 79 identified in this study and obtained from cecum and neck skin samples of Lebanese broiler chickens. These 51 isolates were clustered into 25 profiles revealed by the CGF40–90% analysis. The level of genetic diversity observed through the CGF40–90% was considered to have an acceptable balance allowing both high discriminatory power and identification of clades with genetically similar isolates (Thépault et al., 2018a). According to this level, 5 *C. jejuni* isolates belonged to cluster 13 (11.7%), 5 to clusters 1 and 30 (9.8% each), 4 to cluster 38 (7.7%), 3 to clusters 3 and 11 (5.8% each), and 2 to clusters 6, 9, 18, 19, 21, and 22 (3.9% each) (Figure 1). Each of the remaining thirteen *C. jejuni* isolates presented a unique profile (2% each). Six of all these clusters (13, 1, 30, 38, 3, and 11) included at least 4% of the *C. jejuni* isolates and were thus defined as predominant in the Lebanese broiler population. Together, these 6 clusters accounted for 51.0% (26/51) of the isolates.

These profiles were first compared to those previously obtained from 471 poultry isolates in France, leading to the identification of 70 clusters considering a 90% similarity threshold. Cluster 13 was predominant and represented 11.8% of the Lebanese *C. jejuni* isolates, but only 1.5% of the French isolates (Figure 2). Two other clusters (30 and 38) represented 9.8 and 7.8% of the Lebanese poultry isolates, respectively, and were

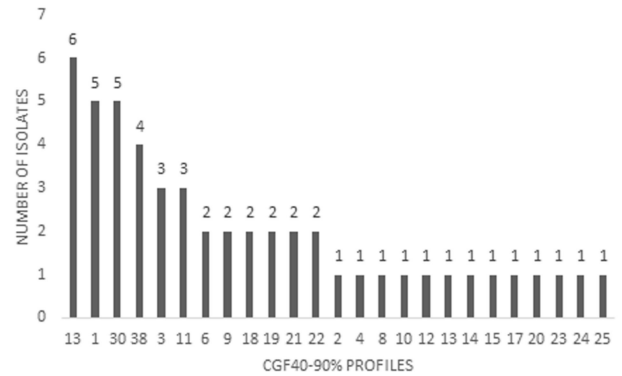


Figure 1. Distribution of CGF40–90% profiles within the population of 51 *C. jejuni* isolates from broiler chickens in Lebanon.

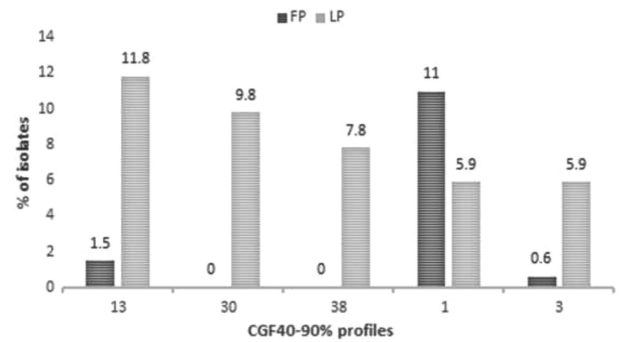
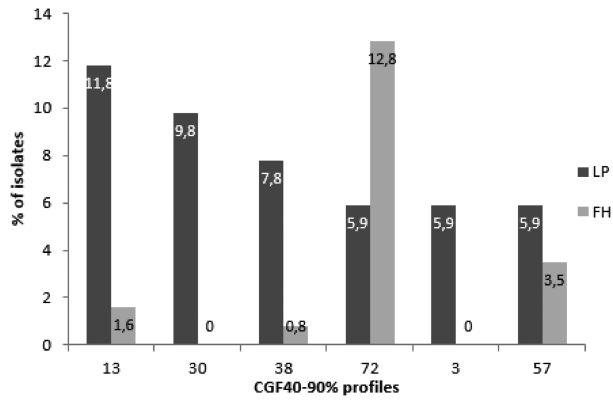


Figure 2. Distribution of the main CGF40–90% profiles within the 2 groups of *C. jejuni* isolates from Lebanese (LP) and French (FP) broiler chickens.

not found in the French broiler collection. Cluster 1, which represented 5.9% of the Lebanese *C. jejuni* isolates, was predominant among the French broiler isolates (11%). To complete these data, cluster 3 included 5.9 and 0.6% of the Lebanese and French poultry isolates, respectively.

Because of the absence of single infection with *C. jejuni* among slaughterhouse staff members and, more generally, the lack of surveillance programme for campylobacteriosis in the Lebanese population, the profiles obtained from the 51 *C. jejuni* isolates of Lebanese broiler chickens were compared to those identified from a total of 514 *C. jejuni* clinical isolates originating from human cases of campylobacteriosis in France. CGF40–90% analysis revealed 110 distinct profiles among a total of 565 *C. jejuni* isolates, divided into 6 predominant clusters (13, 30, 38, 72, 3, and 57) accounting for 47% (24/51) of the Lebanese broiler isolates (Figure 3). Of the Lebanese poultry isolates, cluster 13 was the predominant profile (11.8%) followed by the clusters 30 (9.8%), 38 (7.8%), 72, 3, and 57 (5.9% each). Interestingly, the profile 72 that was found to be predominant among French human isolates (12.8%) was also identified in 5.9% of Lebanese poultry isolates. Similarly, profiles 13, 38, and 57 were also shown to be shared by various proportions of both Lebanese poultry and French human isolates. In contrast, clusters 30 and 3 grouping a significant number of Lebanese poultry



**Figure 3.** Distribution of the main CGF40–90% profiles within the 2 groups of *C. jejuni* isolates from Lebanese broiler chickens (LP) and French humans (FH).

isolates were not identified among the French human isolates.

### Assessment of the Association Between *Campylobacter* spp. and *Blastocystis* sp. Infection in Lebanese Broiler Chickens

Previously, 223 of the 227 cecum samples from broiler chickens screened herein for the presence of *Campylobacter* spp. were analyzed to determine the infection rate of the parasitic protozoan *Blastocystis* sp. (Greige et al., 2018). The remaining 4 cecum specimens collected in this study were rapidly tested for the presence of *Blastocystis* sp. by real-time quantitative PCR (Poirier et al., 2011) and were found to be negative. The potential association between the 2 microorganisms was thus assessed through the results of the analysis of these 227 samples. Of these, 55 were co-infected by *Campylobacter* spp. and *Blastocystis* sp., 59 were negative for both microorganisms, 16 were positive only for *Blastocystis* sp., and 97 were positive only for *Campylobacter* spp. (Table 2). A statistically significant difference (chi-square test,  $P = 0.023$ ) was obtained between the observed number of cases of infection by each of these 2 microorganisms and the theoretically expected number in the absence of association of *Blastocystis* sp. and *Campylobacter* spp. According to our data, the presence of *Campylobacter* spp. would be associated with the presence of *Blastocystis* sp. and, similarly, the absence of *Campylobacter* spp. would be associated with the absence of *Blastocystis* sp.

## DISCUSSION

This study focused on broiler chickens because they represent the largest poultry market sector in Lebanon and are considered a major source of human campylobacteriosis (Silva et al., 2011; EFSA and ECDC, 2016; Thépault et al., 2017, 2018b). One of the main objectives of this survey was to assess the rate of contamination of Lebanese broiler chickens by *Campylobacter* spp. at the farm and slaughterhouse levels. Strikingly, these bacteria were detected in 94% of sampled poultry farms and with higher prevalence in autumn and summer than in winter and spring. This high prevalence of *Campylobacter* spp. in broiler flocks has already been described, for instance in France as in other European countries (EFSA 2010; Hue et al., 2010; Allain et al., 2014). Moreover, the seasonal variation in prevalence of the bacteria observed here was probably due to various factors, including temperature and environmental reservoirs of contamination, such as flies, insects, rodents, and birds, in the summer and early autumn period (Huneau-Salaün et al., 2007; Ellis-Iversen et al., 2009; Jorgensen et al., 2011; EFSA and ECDC, 2016). Within the farms, the prevalence of *C. coli* was slightly higher than that of *C. jejuni*, while conversely *C. jejuni* was slightly predominant in slaughterhouses. Another smaller-scale survey conducted in Lebanon reported predominance of *C. coli* in cecum and on carcass samples (Talhok et al., 1998), while, on the contrary, a second study aiming to assess the infection rate of domestic broiler chickens by *Campylobacter* spp. on farms and throughout the slaughtering process in the same geographical region reported predominance of *C. jejuni* (Hajj Semaan et al., 2014). While *C. jejuni* is generally predominant in poultry worldwide (EFSA 2010, Hue et al., 2011; Allain et al., 2014; García-Sánchez et al., 2017), the predominance of *C. coli* in Lebanese poultry farms was, however, in agreement with various surveys conducted in European countries including Bulgaria, Hungary, Italy, Luxembourg, Malta, Portugal, Ireland, Spain, and Greece (EFSA 2010; Marinou et al., 2012; Nobile et al., 2013; Sahin et al., 2015; Mezher et al., 2016; Pergola et al., 2017). Since all except Luxembourg and Ireland represent southern European countries, most of which surround the Mediterranean Sea, the predominance of *C. coli* on poultry farms could be associated with the geographical area. Regarding Lebanese broiler carcasses contaminated with *Campylobacter* spp., our results indicating lower prevalence on

**Table 2.** Comparison of the observed and expected number of cases of infection by *Blastocystis* sp. and *Campylobacter* spp. in cecum samples from Lebanese broiler chickens.

No. of cases of infection	Negative for <i>Blastocystis</i> sp. (observed/expected)	Positive for <i>Blastocystis</i> sp. (observed/expected)	Total
Negative for <i>Campylobacter</i> spp. (observed/expected)	59 (51.5)	16 (23.5)	75
Positive for <i>Campylobacter</i> spp. (observed/expected)	97 (104.5)	55 (47.5)	152
Total	156	71	227

carcass than in cecum samples were roughly similar to those observed in Cyprus and Sweden (EFSA, 2010). Moreover, lower prevalence of *Campylobacter* spp. was observed on skin samples in Lebanon in comparison to those reported in studies conducted in German, Croatian, and French slaughterhouses (EFSA, 2010; Hue et al., 2010; Hue et al., 2011; EFSA and ECDC, 2015; Alpigiani et al., 2017). This could be explained by the absence of decontamination in these European countries associated with regulations prohibiting the use of chemical decontaminants on broiler carcasses. However, the decontamination process using chlorine or PAA performed in Lebanese slaughterhouses was clearly not sufficiently effective, since 17% of carcasses remained positive for the bacteria at the end of the slaughter chain in this study. In a study conducted in commercial processing plants in the USA where decontamination is allowed, it was shown that the use of chlorine in the chill tank was more efficient than chlorinated carcass wash. However, the presence of *Campylobacter* on carcasses was still possible even after decontamination (Berrang et al., 2007). Similarly, Nagel et al., 2013 showed that the use of peracetic acid in a post-chill immersion tank is more effective than chlorine as an application for reducing *Campylobacter* on carcasses (Nagel et al., 2013). In another study aiming at evaluating the effect of chlorine in immersion chillers on *Campylobacter* reduction on broiler carcasses in New Zealand, it was found that chlorine at a concentration of 50 ppm is optimal to reduce the level of *Campylobacter* counts on carcasses. However, this intervention did not inhibit completely the presence of *Campylobacter* and is not advocated to replace hygienic practices (Van Der Logt et al., 2015).

A comprehensive molecular characterization of *C. jejuni* isolated from Lebanese poultry on farms and during the slaughtering process was performed. Briefly, the majority of Lebanese broiler isolates were distributed into 6 profiles, most of which were present in the French broiler production, indicating a weak association between the genetic profile and the geographical origin of the bacteria as previously suggested by Thépault et al. (2017). Interestingly, most of these profiles were also identified in French human cases of campylobacteriosis, highlighting the potential zoonotic risk of transmission to the Lebanese population. Thus, these results confirmed the high genetic diversity of *C. jejuni* and the importance of poultry as a source of contamination for humans (Strachan et al., 2012; Thépault et al., 2017, 2018a,b). Although only 51 *C. jejuni* isolates were subtyped, profile 30 was identified as potentially specific to Lebanese broiler production, since it was reported neither in French avian nor in French human isolates. In addition to foodborne exposure, work-related exposure to *Campylobacter* spp. may also represent a major source of infection (Castillo Neyra et al., 2012). Interestingly, 12% of Lebanese slaughterhouse workers were contaminated by the bacteria, while all non-directly exposed individuals were negative for *Campylobacter* spp. Our findings provided evidence that humans working

with poultry may be at increased risk of exposure to *Campylobacter* spp., as previously suggested (Vegosen et al., 2015). Two studies conducted in Sweden and the USA reinforced this statement, since 25% of Swedish poultry abattoir workers (de Perio et al., 2013; Ellström et al., 2014) and 83% of workers for less than a month in American slaughterhouses were positive for *Campylobacter* spp. (de Perio et al., 2013).

Finally, a relatively large proportion (24.2%) of cecum samples from Lebanese poultry were co-infected by the eukaryotic single-celled *Blastocystis* sp. and *Campylobacter* spp. Interestingly, this protozoan was previously identified as a co-pathogen with *Campylobacter* spp. in a patient hospitalized with gastroenteritis (Jansen et al., 2008). Moreover, in the context of 2 described outbreaks of the apicomplexan protozoa *Cryptosporidium* occurring in the United Kingdom, a few individuals were also shown to be infected concurrently with *Campylobacter* spp. (Casemore et al., 1986; Duke et al., 1996). Recent studies conducted in humans have described bacterial microbiota changes associated with *Blastocystis* sp. colonization, with mainly an increase in bacterial alpha diversity (Scanlan et al., 2014; Audebert et al., 2016; Nieves-Ramirez et al., 2018). It was postulated that *Blastocystis* sp. may exert a predatory effect on intestinal bacteria of highly abundant taxa (Chabé et al., 2017; Nieves-Ramirez et al., 2018). Consequently, this predation may decrease competition for nutrients and space, thus leading to an increase in bacterial richness. Although no data are currently available regarding the impact of *Blastocystis* sp. on the bacterial intestinal microbiota of chickens, the latter observation could therefore explain, at least partly, the infection rate of *Campylobacter* spp. in *Blastocystis* sp.-colonized animals by favoring the colonization of the host by the bacteria. In addition, *Blastocystis* sp. is also known to have immunomodulatory properties (Ajjanpur and Tan, 2016). In particular, the parasite produces cysteine proteases which are able to degrade human secretory immunoglobulin A (Puthia et al., 2005). Since IgA plays a crucial role in regulation of host–bacteria interactions in the gut, the supposed immunomodulatory effect of the parasite in infected chickens could also facilitate colonization by other enteric pathogens such as *Campylobacter* spp. Future studies focusing on *Blastocystis*-colonized broiler chickens are necessary to test all these hypotheses.

In conclusion, this study highlighted the high prevalence and genetic diversity of *Campylobacter* spp. in Lebanese poultry production and strongly suggested that broiler chickens represented a significant reservoir for human campylobacteriosis. In particular, their in-contact workers in poultry slaughterhouses exhibited a higher risk of infection by the bacteria through zoonotic transmission. Consequently, a surveillance programme is needed in Lebanon, together with effective measures in terms of hygiene practices, with the aim of controlling the burden of *Campylobacter* spp. in both animal and human populations.



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## REFERENCES

- Abu-Madi, M., J. M. Behnke, A. Sharma, R. Bearden, and N. Al-Banna. 2016. Prevalence of virulence/stress genes in *Campylobacter jejuni* from chicken meat sold in Qatari retail outlets. *PLoS One* 11:e0156938.
- Ajjampur, S. S. R., and K. S. W. Tan. 2016. Pathogenic mechanisms in *Blastocystis* spp.—interpreting results from *in vitro* and *in vivo* studies. *Parasitol. Int.* 65:772–779.
- Allain, V., M. Chemaly, M. J. Laisney, S. Rouxel, S. Quesne, and S. Le Bouquin. 2014. Prevalence of and risk factors for *Campylobacter* colonisation in broiler flocks at the end of the rearing period in France. *Br. Poult. Sci.* 55:452–459.
- Alpigiani, I., J. C. Abrahantes, V. Michel, A. Huneau-Salaün, M. Chemaly, L. J. Keeling, A. Gervelmeyer, C. Bacci, F. Brindani, S. Bonardi, and F. Berthe. 2017. Associations between animal welfare indicators and *Campylobacter* spp. in broiler chickens under commercial settings: a case study. *Prev. Vet. Med.* 147:186–193.
- Andersen, L. O., I. Bonde, H. B. Nielsen, and C. R. Stensvold. 2015. A retrospective metagenomics approach to studying *Blastocystis*. *FEMS Microbiol. Ecol.* 91:1–9.
- Andersen, L. O., and C. R. Stensvold. 2016. *Blastocystis* in health and disease: are we moving from a clinical to a public health perspective? *J. Clin. Microbiol.* 54:524–528.
- Audebert, C., G. Even, and A. Cian. The *Blastocystis* Investigation Group Loywick, A., S. Merlin, E. Viscogliosi, and M. Chabé. 2016. Colonization with the enteric protozoa *Blastocystis* is associated with increased diversity of human gut bacterial microbiota. *Sci. Rep.* 6:25255.
- Bechara, R., M. Hosny, I. AL-Kassaa, F. Dabboussi, H. Mallat, and M. Hamze. 2016. Detection of pathogenic bacteria in diarrheal stool collected from children in north lebanon by using conventional stool culture and microarray technique (clart® enterobac). *Leb. Sci. J.* 17:233–239.
- Berrang, M. E., J. S. Bailey, S. F. Altekruse, B. Patel, W. K. Shaw, JR., R. J. Meinersmann, and P. J. Fedorka-Cray. 2007. Prevalence and numbers of *Campylobacter* on broiler carcasses collected at Rehag and Postchill in 20 U.S. processing plants. *J. Food Prot.* 70:1556–1560.
- Casemore, D. P., E. G. Jessop, D. Douce, and F. B. Jackson. 1986. *Cryptosporidium* plus *Campylobacter*: an outbreak in a semi-rural population. *J. Hyg.* 96:95–105.
- Castillo Neyra, R., L. Vegosen, M. F. Davis, L. Price, and E. K. Silbergeld. 2012. Antimicrobial-resistant bacteria: an unrecognized work-related risk in food animal production. *Saf. Health Work* 3:85–91.
- Chabé, M., A. Lokmer, and L. Ségurel. 2017. Gut protozoa: friends or foes of the human gut microbiota? *Trends Parasitol.* 33:925–934.
- Cian, A., D. El Safadi, M. Osman, R. Moriniere, N. Gantois, S. Benamrouz-Vanneste, P. Delgado-Viscogliosi, K. Guyot, L. L. Li, S. Monchy, C. Noël, P. Poirier, C. Nourrisson, I. Wawrzyniak, F. Delbac, S. Bosc, M. Chabe, T. Petit, G. Certad, and E. Viscogliosi. 2017. Molecular epidemiology of *Blastocystis* sp. in various animal groups from two French zoos and evaluation of potential zoonotic risk. *PLoS One* 12:e0169659.
- Clark, C. G., M. van der Giezen, M. A. Alfellani, and C. R. Stensvold. 2013. Recent developments in *Blastocystis* research. *Adv. Parasitol.* 82:1–32.
- Dabboussi, F., S. Alam, H. Mallat, S. Hlais, and M. Hamze. 2012. Preliminary study on the prevalence of *Campylobacter* in childhood diarrhoea in North Lebanon. *East Mediterr. Health J.* 18:1225–1228.
- de Perio, M. A., R. T. Niemeier, S. J. Levine, K. Gruszynski, and J. D. Gibbins. 2013. *Campylobacter* infection in poultry-processing workers, Virginia, USA, 2008–2011. *Emerg. Infect. Dis.* 19:286–288.
- Denis, M., C. Soumet, K. Rivoal, G. Ermel, D. Blivet, G. Salvat, and P. Colin. 1999. Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Lett. Appl. Microbiol.* 29:406–410.
- Duke, L. A., A. S. Breathnach, D. R. Jenkins, B. A. Harkis, and A. W. Codd. 1996. A mixed outbreak of *Cryptosporidium* and *Campylobacter* infection associated with a private water supply. *Epidemiol. Infect.* 116:303–308.
- EFSA. 2010. Analysis of the baseline survey on the prevalence of *Campylobacter* in broiler batches and of *Campylobacter* and *Salmonella* on broiler carcasses in the EU, 2008. *EFSA J.* 8:1503.
- EFSA, and ECDC. 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. *EFSA J.* 13:3991.
- EFSA, and ECDC. 2016. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA J.* 14:4634.
- EFSA, and ECDC. 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. *EFSA J.* 15:5077.
- Ellis-Iversen, J., F. Jorgensen, S. Bull, L. Powell, A. J. Cook, and T. J. Humphrey. 2009. Risk factors for *Campylobacter* colonisation during rearing of broiler flocks in Great Britain. *Prev. Vet. Med.* 89:178–184.
- Ellström, P., I. Hansson, C. Söderström, E. O. Engvall, and H. Rautelin. 2014. A prospective follow-up study on transmission of *Campylobacter* from poultry to abattoir workers. *Foodborne Pathog. Dis.* 11:684–688.
- FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). 2009. Risk assessment of *Campylobacter* spp. in broiler chickens: Interpretative Summary. *Microbiological Risk Assessment Series* No 11. Geneva. 35 pp.
- Forsell, J., J. Bengtsson-Palme, M. Angelin, A. Johansson, B. Evengård, and M. Granlund. 2017. The relation between *Blastocystis* and the intestinal microbiota in Swedish travellers. *BMC Microbiol.* 17:231.
- García-Sánchez, L., B. Melero, I. Jaime, M. L. Hänninen, M. Rossi, and J. Rovira. 2017. *Campylobacter jejuni* survival in a poultry processing plant environment. *Food Microbiol.* 65:185–192.
- Greige, S., D. El Safadi, N. Bécu, N. Gantois, B. Pereira, M. Chabé, S. Benamrouz-Vanneste, G. Certad, R. El Hage, M. Chemaly, M. Hamze, and E. Viscogliosi. 2018. Prevalence and subtype distribution of *Blastocystis* sp. isolates from poultry in Lebanon and evidence of zoonotic potential. *Parasit. Vectors* 11:389.
- Hajj Semaan, E., H. Dib, R. Mrad, C. Chami, and R. Jalkh. 2014. Dynamic of *Campylobacter* species contamination along a poultry slaughtering chain. *Ital. J. Food Saf.* 3:2246.
- Hue, O., V. Allain, M. J. Laisney, S. Le Bouquin, F. Lalande, I. Petetin, S. Rouxel, S. Quesne, P. Y. Gloaguen, M. Picherot, J. Santolini, S. Bougeard, S. Gilles, and M. Chemaly. 2011. *Campylobacter* contamination of broiler caeca and carcasses at the slaughterhouse and correlation with *Salmonella* contamination. *Food Microbiol.* 28:862–868.
- Hue, O., S. Le Bouquin, M. J. Laisney, V. Allain, F. Lalande, I. Petetin, S. Rouxel, S. Quesne, P. Y. Gloaguen, M. Picherot, J. Santolini, S. Gilles, S. Bougeard, and M. Chemaly. 2010. Prevalence of and risk factors for *Campylobacter* spp. contamination of broiler chicken carcasses at the slaughterhouse. *Food Microbiol.* 27:992–999.

- Humeau-Salaün, A., M. Denis, L. Balaine, and G. Salvat. 2007. Risk factors for *Campylobacter* spp. colonization in French free-range broiler-chicken flocks at the end of the indoor rearing period. *Prev. Vet. Med.* 80:34–48.
- ISO - International Organization for Standardization. 2006. ISO 10272 microbiology of food and animal feeding stuff - horizontal method for detection and enumeration of *Campylobacter* spp. - Part 1: enrichment method; Part 2: enumeration method. Geneva, Switzerland.
- Jackson, B. R., J. A. Zegarra, H. López-Gatell, J. Sejvar, F. Arzate, S. Waterman, A. S. Núñez, B. López, J. Weiss, R. Q. Cruz, D. Y. Murrieta, R. Luna-Gierke, K. Heiman, A. R. Vieira, C. Fitzgerald, P. Kwan, M. Zarate-Bermudez, D. Talkington, V. R. Hill, and B. Mahon. 2014. Binational outbreak of Guillain-Barré syndrome associated with *Campylobacter jejuni* infection, Mexico and USA, 2011. *Epidemiol. Infect.* 142:1089–1099.
- Jansen, A., K. Stark, J. Kunkel, E. Schreier, R. Ignatius, O. Liesenfeld, D. Werber, U. B. Göbel, M. Zeitz, and T. Schneider. 2008. Aetiology of community-acquired, acute gastroenteritis in hospitalised adults: a prospective cohort study. *BMC Infect. Dis.* 8:143.
- Jorgensen, F., J. Ellis-Iversen, S. Rushton, S. A. Bull, S. A. Harris, S. J. Bryan, A. Gonzalez, and T. J. Humphrey. 2011. Influence of season and geography on *Campylobacter jejuni* and *C. coli* subtypes in housed broiler flocks reared in Great Britain. *Appl. Environ. Microbiol.* 77:3741–3748.
- Lepczynska, M., W. C. Chen, and E. Dzika. 2015. Mysterious chronic urticarial caused by *Blastocystis* spp.? *Int. J. Dermatol.* 55:259–266.
- Lévesque, S., E. Frost, R. D. Arbeit, and S. Michaud. 2008. Multilocus sequence typing of *Campylobacter jejuni* isolates from humans, chickens, raw milk, and environmental water in Quebec, Canada. *J. Clin. Microbiol.* 46:3404–3411.
- Marinou, I., S. Bersimis, A. Ioannidis, C. Nicolaou, A. Mitrousi-Ziouva, N. J. Legakis, and S. Chatzipanagiotou. 2012. Identification and antimicrobial resistance of *Campylobacter* species isolated from animal sources. *Front. Microbiol.* 3:58.
- Mezher, Z., S. Saccares, R. Marciandò, P. De Santis, E. M. F. Rodas, V. De Angelis, and R. Condoleo. 2016. Occurrence of *Campylobacter* spp. in poultry meat at retail and processing plants' levels in central Italy. *Ital. J. Food Saf.* 5:5495.
- Mortensen, N. P., M. L. Kuijff, C. W. Ang, P. Schiellerup, K. A. Krogfelt, B. C. Jacobs, A. van Belkum, H. P. Endtz, and M. P. Bergman. 2009. Sialylation of *Campylobacter jejuni* lipooligosaccharides is associated with severe gastro-enteritis and reactive arthritis. *Microbes Infect.* 11:988–994.
- Mossong, J., L. Mughini-Gras, C. Penny, A. Devaux, C. Olinger, S. Losch, H. M. Cauchie, W. van Pelt, and C. Ragimbeau. 2016. Human campylobacteriosis in Luxembourg, 2010–2013: a case-control study combined with multilocus sequence typing for source attribution and risk factor analysis. *Sci. Rep.* 6: 20939.
- Nagel, G. M., L. J. Bauermeister, C. L. Bratcher, M. Singh, and S. R. McKee. 2013. *Salmonella* and *Campylobacter* reduction and quality characteristics of poultry carcasses treated with various antimicrobials in a post-chill immersion tank. *Int. J. Food Microbiol.* 165:281–286.
- Nieves-Ramírez, M. E., O. Partida-Rodríguez, I. Laforest-Lapointe, L. A. Reynolds, E. M. Brown, A. Valdez-Salazar, P. Morán-Silva, L. RojasVelázquez, E. Morien, L. W. Parfrey, M. Jin, J. Walter, J. Torres, M. C. Arrieta, C. Ximénez-García, and B. B. Finlay. 2018. Asymptomatic intestinal colonization with protist *Blastocystis* is strongly associated with distinct microbiome ecological patterns. *mSystems* 3:e00007–18.
- Nobile, C. G. A., R. Costantino, A. Bianco, C. Pileggi, and M. Pavia. 2013. Prevalence and pattern of antibiotic resistance of *Campylobacter* spp. in poultry meat in Southern Italy. *Food Control* 32:715–718.
- Osman, M., D. El Safadi, A. Cian, S. Benamrouz, C. Nourrisson, P. Poirier, B. Pereira, R. Razakandrainibe, A. Pinon, C. Lambert, I. Wawrzyniak, F. Dabboussi, F. Delbac, L. Favennec, M. Hamze, E. Viscogliosi, and G. Certad. 2016. Prevalence and risk factors for intestinal protozoan infections with *Cryptosporidium*, *Giardia*, *Blastocystis* and *Dientamoeba* among schoolchildren in Tripoli, Lebanon. *PLoS Negl. Trop. Dis.* 10:e0004496.
- Pergola, S., M. P. Franciosini, F. Comitini, M. Ciani, S. De Luca, S. Bellucci, L. Menchetti, and P. Casagrande Proietti. 2017. Genetic diversity and antimicrobial resistance profiles of *Campylobacter coli* and *Campylobacter jejuni* isolated from broiler chicken in farms and at time of slaughter in central Italy. *J. Appl. Microbiol.* 122:1348–1356.
- Poirier, P., I. Wawrzyniak, A. Albert, H. El Alaoui, F. Delbac, and V. Livrelli. 2011. Development and evaluation of a real-time PCR assay for detection and quantification of *Blastocystis* parasites in human stool samples: prospective study of patients with hematological malignancies. *J. Clin. Microbiol.* 49:975–983.
- Puthia, M. K., A. Vaithilingam, J. Lu, and K. S. W. Tan. 2005. Degradation of human secretory immunoglobulin A by *Blastocystis*. *Parasitol. Res.* 97:386–389.
- Sahin, O., I. I. Kassem, Z. Shen, J. Lin, G. Rajashekara, and Q. Zhang. 2015. *Campylobacter* in poultry: ecology and potential interventions. *Avian Dis.* 59:185–200.
- Scanlan, P. D., C. R. Stensvold, M. Rajilić-Stojanović, H. G. H. J. Heilig, W. M. De Vos, P. W. O'Toole, and P. D. Cotter. 2014. The microbial eukaryote *Blastocystis* is a prevalent and diverse member of the healthy human gut microbiota. *FEMS Microbiol. Ecol.* 90:326–330.
- Senok, A. C., and G. A. Botta. 2009. *Campylobacter* enteritis in the Arabian Gulf. *J. Infect. Dev. Ctries* 3:74–82.
- Silva, J., D. Leite, M. Fernandes, C. Mena, P. A. Gibbs, and P. Teixeira. 2011. *Campylobacter* spp. as a foodborne pathogen: a review. *Front. Microbiol.* 2:200.
- Skarp, C. P. A., M. L. Hänninen, and H. I. K. Rautelin. 2016. Campylobacteriosis: the role of poultry meat. *Clin. Microbiol. Infect.* 22:103–109.
- Stensvold, C. R., and C. G. Clark. 2016. Current status of *Blastocystis*: a personal view. *Parasitol. Int.* 65:763–771.
- Strachan, N. J. C., M. MacRae, A. Thomson, O. Rotariu, I. D. O'Gden, and K. J. Forbes. 2012. Source attribution, prevalence and enumeration of *Campylobacter* spp. from retail liver. *Int. J. Food Microbiol.* 153:234–236.
- Taboada, E. N., S. L. Ross, S. K. Mutschall, J. M. MacKinnon, M. J. Roberts, C. J. Buchanan, P. Kruczkiewicz, C. C. Jokinen, J. E. Thomas, J. H. E. Nash, V. P. J. Gannon, B. Marshall, F. Pollari, and C. G. Clark. 2012. Development and validation of a comparative genomic fingerprinting method for high-resolution genotyping of *Campylobacter jejuni*. *J. Clin. Microbiol.* 50:788–797.
- Talhok, R., R. El-Dana, G. Araj, E. Barbour, and F. Hashwa. 1998. Prevalence, antimicrobial susceptibility and molecular characterization of *Campylobacter* isolates recovered from humans and poultry in Lebanon. *J. Med. Leban.* 46:310–316.
- Tan, K., H. Mirza, J. Teo, B. Wu, and P. Macary. 2010. Current views on the clinical relevance of *Blastocystis* spp. *Curr. Infect. Dis. Rep.* 12:28–35.
- Thépault, A., M. Guyard-Nicodème, V. Rose, S. Quesne, M. Queguiner, E. Houard, F. Mégraud, K. Rivoal, and M. Chemaly. 2018a. A representative overview of the genetic diversity and lipooligosaccharide sialylation in *Campylobacter jejuni* along the broiler production chain in France and its comparison with human isolates. *Int. J. Food Microbiol.* 274:20–30.
- Thépault, A., G. Méric, K. Rivoal, B. Pascoe, L. Mageiros, F. Touzain, V. Rose, V. Béven, M. Chemaly, and S. K. Sheppard. 2017. Genome-wide identification of host-segregating epidemiological markers for source attribution in *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 83:e03085–16.
- Thépault, A., V. Rose, S. Quesne, T. Poezevara, V. Béven, E. Hirschfeld, F. Touzain, P. Lucas, G. Méric, L. Mageiros, S. K. Sheppard, M. Chemaly, and K. Rivoal. 2018b. Ruminant and chicken: important sources of campylobacteriosis in France despite a variation of source attribution in 2009 and 2015. *Sci. Rep.* 8:9305.
- Van der Logt, P., R. Biggs, P. Greenhead, M. Callander, S. Wagener, J. Lee, and S. Hathaway. 2015. Chlorination in immersion chillers. 18th International workshop on *Campylobacter*, *Helicobacter* and related organisms – CHRO. New Zealand.
- Vegosen, L., P. N. Breyse, J. Agnew, G. C. Gray, I. Nachamkin, K. Sheikh, F. Kamel, and E. Silbergeld. 2015. Occupational exposure to swine, poultry, and cattle and antibody biomarkers of *Campylobacter jejuni* exposure and autoimmune peripheral neuropathy. *PLoS One* 10:e0143587.