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Members of the family Campylobacteraceae: Campylobacter jejuni, Campylobacter coli.

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2017

Pitkänen , T & Hänninen , M-L 2017 , Members of the family Campylobacteraceae: Campylobacter jejuni, Campylobacter coli. in A Pruden , N Asbolt & J Miller (eds) , Global Water Pathogens Project. <http://www.waterpathogens.org> : Part 3 Specific excreted pathogens: environmental and epidemiology aspects <http://www.waterpathogens.org/book/campylobacter> . Michigan State University, E. Lansing, MI, UNESCO . < <http://www.waterpathogens.org/book/campylobacter> >

<http://hdl.handle.net/10138/232581>

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GLOBAL WATER PATHOGEN PROJECT

**PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND
EPIDEMIOLOGY ASPECTS**

MEMBERS OF THE FAMILY CAMPYLOBACTERACEAE: CAMPYLOBACTER JEJUNI, CAMPYLOBACTER COLI

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Citation:

Pitkanen, T. and Hanninen, M-L. 2017. Members of the family Campylobacteraceae: *Campylobacter jejuni*, *Campylobacter coli*. In: J.B. Rose and B. Jiménez-Cisneros, (eds) Global Water Pathogens Project. <http://www.waterpathogens.org> (A. Pruden, N. Ashbolt and J. Miller (eds) Part 3 Bacteria) <http://www.waterpathogens.org/book/campylobacter> Michigan State University, E. Lansing, MI, UNESCO.

Acknowledgements: K.R.L. Young, Project Design editor; Website Design (<http://www.agroknow.com>)

Published: January 15, 2015, 10:26 am, **Updated:** August 1, 2017, 10:30 am

Summary

Campylobacter infection is one the most widely spread bacterial gastroenteric disease worldwide. The global estimate of the burden of campylobacteriosis for 2010 was 7.5 million disability-adjusted life years (DALYs). The knowledge of the incidence, prevalence, clinical output and epidemiology of *Campylobacter* as a common cause of human acute gastroenteritis has been published since 1977, but the coverage of prevalence and incidence data from different parts of the world is still highly variable. In the industrialized countries, the number of reported cases has increased during the last 10 to 15 years. However, national statistics underestimate the actual population incidence because only a fraction of the *Campylobacter* cases present to health services, and many of these are not investigated further. In addition, co-infections with other enteric pathogens are common. In high-income regions, the most common symptoms of a *Campylobacter* infection include an acute, self-limiting gastroenteritis, with an incubation period of 2 to 5 days. In developing regions, watery diarrhea is the most common presentation of the illness, commonly seen during the first years of life. In the latter regions, due to the endemic nature and acquired immunity after the repeated exposure to *Campylobacter*, adults are only infrequently affected.

Currently, 29 species and 12 subspecies are classified in the genus *Campylobacter*. The most important *Campylobacter* species in human gastroenteritis is *Campylobacter jejuni*, which accounts for 90 to 95% of all campylobacteriosis cases reported. The majority of the remaining cases are caused by *Campylobacter coli*, but the importance of *C. coli* as an enteric pathogen varies between regions. The role of some other species (*Campylobacter lari*, *Campylobacter upsaliensis*, *Campylobacter concisus*) is less well characterized. Based on the data of experimental infections and indirectly from waterborne outbreaks, it is known that exposure to only a few hundred cells may result in illness. Due to uncertainties in estimating the dose-response relation at the typically low doses from water exposures, risk estimations for *C. jejuni* exposures are highly uncertain, and in the absence of dose-response data for *C. coli* or other *Campylobacter* species, not directly possible.

A wide variety of animals, especially poultry, wild birds, cattle and sheep carry high numbers of *C. jejuni* and *C. coli* as commensals in their intestines, all being potential reservoirs. Fecal contamination of food, recreational water and drinking water contributes to human infections and the fecal material of infected persons (symptomatic and asymptomatic) spread the organisms back to environment through sewage plants and toilets. Reported *Campylobacter* numbers in non-disinfected wastewater effluents vary between 100 and 100,000 CFU/ L. *Campylobacter* are frequently isolated from surface waters from different parts of the world despite the fact that the isolation of *Campylobacter* from water requires specialized culture-based techniques and may fail due to insufficient method sensitivity or difficulties in separation of *Campylobacter* from the non-target bacteria. The potential false negative

results due to the occurrence of viable-but-not-culturable *Campylobacter* and other difficult to culture situations has facilitated the development of nucleic acid-based molecular *Campylobacter* detection methods.

Mechanisms that enable *Campylobacter* to survive in the environment are poorly understood. Different survival characteristics and abilities of *Campylobacter* species under environmental conditions affect our understanding of *Campylobacter* ecology and diversity. For example, it has been reported that *C. lari* can survive better in aquatic environments than *C. jejuni* and *C. coli*. The survival of *Campylobacter* is favoured by low temperature, the absence of sunlight and by low numbers of indigenous microbiota. In general, it can be assumed that the same water purification techniques that are capable of removing *Escherichia coli* from water, remove or inactivate *Campylobacter* cells as efficiently. The exact *Campylobacter* removal rates, however, remain unknown due to the semi-quantitative nature of the *Campylobacter* detection methods.

Campylobacter spp.

1.0 Epidemiology of the Disease and Pathogen(s)

1.1 Global Burden of Disease

Campylobacteriosis is one the most common bacterial gastroenteric diseases worldwide (Blaser, 1997; Murray et al., 2012; Scallan et al., 2015). Most infections are sporadic and outbreaks are rather uncommon. Since, Skirrow (1977) published data showing *C. jejuni* was a much more common cause of human acute gastroenteritis than previously estimated, data on the incidence, prevalence and epidemiology has vastly improved, yet data is still highly variable. Most reliable data based on notification requirement of transmissible infectious diseases to public health authorities relates to developed countries where surveillance systems have been active for a long time (e.g. Member States of European Union, United States of America and Canada). On the other hand less detailed and unsystematic data production systems are available from developing regions that still provide useful data.

The global estimate of the burden of campylobacteriosis for 2010 was 7.5 million disability-adjusted life years (DALYs). This was a clear improvement from 1990, when the burden of campylobacteriosis was estimated to be 16.6 million DALYs. However, *Campylobacter* enteritis accounted for 8.5% of the total burden of diarrheal disease, ranking fourth after rotavirus, cryptosporidiosis and *Escherichia coli* diarrhea (combined enterotoxigenic and enteropathogenic *E. coli* infections) (Murray et al., 2012). Thus, *Campylobacter* remains one of the most frequently occurring bacterial causes of gastrointestinal diseases worldwide. Several countries have performed country-level analyses of DALYs. In the USA in 2006, 845,000 people were estimated to have acquired domestic *Campylobacter* infection and the estimated DALY was 22,500 (10,400 to 38,600; 90% Credible Interval, population size approx. 325

million). In this number the sequelae (IBD, Guillan-Barre/Miller Fisher syndromes, and reactive arthritis) increased the years of life disabled (YLD) by more than 70% of the total DALY (Scallan et al., 2015). Further, in the Netherlands (population approx. 17 million; estimated number of cases 108,000 (33,000-271,000) *Campylobacter* DALY was estimated for 2011 to be 3,633 (Mangen et al., 2015). In Australia (population 22 million, *Campylobacter* cases 774,003) estimated DALY for 2010 was 18 222 showing very high impact on public health (Gibney et al., 2014).

1.1.1 Global Distribution

An overview on global distribution of reported campylobacteriosis cases is presented in Table 1. The epidemiology and demography of *Campylobacter* infections differs between developing and industrialized countries. In developing countries, symptomatic disease is most

commonly seen only during the first two years of life, and symptomatic illness in adults is rare due to the endemic nature of *Campylobacter* in these regions. The clinical picture also differs from that seen in industrialized countries, as watery diarrhea is the most common presentation. The peak in the very young and lack of clinical illness in adults is believed to be due to acquired immunity after repeated exposure to *Campylobacter*, and supporting this a high percentage of asymptomatic seropositive individuals have been reported in several studies (Blaser, 1997; Kirkpatrick and Tribble, 2011; Platts-Mills et al., 2015). In addition, co-infection with other enteric pathogens is relatively common. In developing countries monitoring data is poorly available. In a recent, prospective, multi-center, case-control study of acute moderate severe diarrhea in children (Enterics Multi-Center Study, GEMS), using culture based techniques *Campylobacter* spp. were found to be in the top five causes in Bangladesh, Pakistan and India among 2 to 5 year olds (Kotloff et al., 2013; Platts-Mills et al., 2015).

Table 1. Incidence of diagnosed Campylobacteriosis disease

Area	Study Period	Patient Age	Incidence (Annual)/ Unless Otherwise Noted	Reference
Australia	2010	All ages	0.11% ^a (112.3/100,000)	NNDSS, 2012
Bolivia	NR	NR	Prevalence: 10.5% in diarrhea and 9.6% in asymptomatic persons	Fernández, 2011
Canada	2006	All ages	0.45% ^b (447.2/100,000) (estimated)	Thomas et al., 2013
Chile	NR	NR	Prevalence: 9.2 to 14.1% of gastroenteritis	Fernández, 2011
Czech Republic	2014	All age groups	0.20% (197.4/100,000)	EFSA, 2015
Denmark	2014	All age groups	0.07% ^c (67/100,000)	EFSA, 2015
Finland	2014	All age groups	0.09% ^c (89.7/100,000)	EFSA, 2015
France	2014	All age groups	0.05% (45.2/100,000)	EFSA, 2015
Guatemala	2008 to 2012	Children (< 5 y)	0.19 to 1.29% (185.5 to 1288.8/100,000)	Benoit et al., 2014
		Other age groups	0.09 to 0.22% (92.5 to 215.8/100,000)	
Japan (Miuagi Prefecture)	2005	All age groups	1.51% (1,512/100,000)	Kubota et al., 2011
Kenya	1997 to 2003	NR	7%	Brooks et al., 2006
New Zealand	2003	All ages	0.40% ^d (396/100,000)	Lane and Briggs, 2014
	2012		0.16% (159/100,000)	
Peru	NR	NR	Prevalence: 15 to 23% of gastroenteritis	Fernández, 2011
Poland	2014	NR	0.0017% (1.7/100,000)	EFSA, 2015

Area	Study Period	Patient Age	Incidence (Annual)/ Unless Otherwise Noted	Reference
Romania	2014	NR	0.0011% (1.1/100,000)	EFSA, 2015
Sweden	2014	All age groups	0.09% ^c (85.9/100,000)	EFSA, 2015
Tanzania	2012 to 2013	Children (< 5 y)	Prevalence: 9.7%	Deogratias et al., 2014
Thailand	1992	Children (< 5 y)	Prevalence: 15% diarrheal patients	Taylor et al., 1993
The Netherlands	2014	All age groups	0.05% ^e (47.5/100,000)	EFSA, 2015
United Kingdom	2014	All age groups	0.10% ^f (104/100,000)	EFSA, 2015
		All ages	0.01% ^c (13.3/100,000)	
		< 5	0.02% (22/100,000)	
USA	2014	5 to 9	0.01% (9/100,000)	CDC, 2014
		60 to 69	0.02% (15/100,000)	

NR: Not Reported

^aNo clear seasonality; ^bSeasonal peak in summer; ^cPeak in June to August; ^dSeasonal peak: January; ^ePeak in August; ^fPeak in May to June.

Most countries in Europe and North America have existing annual reporting and notification systems for incidence/prevalence of campylobacteriosis indicating that reliable data on prevalence of the infections among populations exists as well as trends of infections during longer periods of time are available. These trend data from Europe and North America have shown that the number of reported cases has increased during the last 10 to 15 years (EFSA, 2015; MMWR, 2014; Thomas et al., 2013). The data collection also includes information on the prevalence of *C. jejuni* and *C. coli* in their domestic animal reservoirs and certain foods improving assessment of potential sources of human infections. Some countries also have data on foreign travel-associated cases separated from those acquired from domestic sources (EFSA, 2015; Nichols et al., 2012; Kendall et al., 2012). This travel-associated data is important for targeting potential interpretation measures to decrease health burden of the illness. The incidence/prevalence data available from low-income countries is often lacking or fragmented but data collected from different sources indicate that the epidemiology of campylobacteriosis is different than in developed regions (Kotloff et al., 2013; Mason et al., 2013).

The European Union (EU) notification rate of campylobacteriosis was 64.8 per 100,000 population in 2013, but varied considerably between the member states (MS); from <2 to 173.7 per 100,000 population in Latvia and Czech Republic, respectively (Table 1), indicating the surveillance is not similarly developed within the EU countries. However, the true incidence of *Campylobacter*

infection is higher than reported. The national statistics grossly underestimate the actual population incidence because only a fraction of the campylobacteriosis cases are presented to health services, and many of these are not investigated further. For instance in the UK, Tam et al. (2012) reported that every campylobacteriosis case reported to the national surveillance represented 9.3 community campylobacteriosis cases between 2008 and 2009. Overall, the true incidence is expected to range between 4.4 and 9.3 per 1,000 person-years population in high-income countries. The underreporting level varies between countries depending on functionality of the surveillance system.

1.1.2 Symptomatology

The most important *Campylobacter* species in human gastroenteritis is *Campylobacter jejuni*, which accounts for 90 to 95% of all campylobacteriosis cases identified. The majority of the remaining cases are caused by *Campylobacter coli*, but the importance of *C. coli* as an enteric pathogen varies between regions, degree of urbanization and age of the patient suggesting that the epidemiology of *C. coli* infection differs among countries (Blaser, 1997). However, the predominance of *C. jejuni* and *C. coli* may be biased by the selective cultivation media employed (Lastovica and Allos, 2008). The role of some other species (*Campylobacter fetus*, *Campylobacter lari*, *Campylobacter upsaliensis*, *Campylobacter concisus*) is less

well characterized, especially their role in infections in developing countries.

In high-income regions, the most common symptoms of a *Campylobacter* infection include an acute, self-limiting gastroenteritis, and the incubation period is typically 2 to 5 days (Blaser, 1997; Skirrow and Blaser, 2000), but up to 8 days has been reported. Half of the patients experience a febrile period with fever, malaise and abdominal pain preceding the diarrhea. Fresh blood in the stool is common, while vomiting occurs in approximately 15% of the cases. The pathological lesions seen in humans suffering from *Campylobacter* infection include acute inflammatory enteritis extending to colon and rectum, with terminal ileitis and colitis (Blaser, 1997). Short lasting immunity to homologous strains has been reported, and immunity has been reported under natural conditions, for instance after repeated occupational exposure. Normally the diarrhea lasts for 2 to 3 days, while discomfort can persist for weeks. The most severe cases, however, need hospitalization (Feodoroff et al., 2010). The incidence peaks in young children and again during early adulthood. In developing countries, *C. jejuni* infections are common in the very young children (< 5 years), causing watery diarrhea (Lengerh et al., 2013), while adults are only infrequently affected due to acquired immunity (Kotloff et al., 2013; Allos, 2001). Only a few hundred cells are thought to cause infections (Robinson, 1981), but as no dose-response for symptoms was detected (Black et al., 1988) interpretation of the infectious dose from the data of experimental infections is complicated because, interpretation of the conditional fraction that go onto disease was problematic. Medema et al., (1996) using the experimental data of Black et al., (1988) fitted it to a Beta-Poisson dose-response model but considered the low dose data too unreliable to be of value (further discussion of appropriate dose-response models and parameter estimates can be found in chapter Risk Assessment for Sanitation). While data on the infectious dose of *C. coli* are not available from human volunteer studies, data from waterborne outbreaks suggests the infection dose is low (Guzman-Herrador et al., 2015).

The most severe, but rare complication following a *C. jejuni* infection is Guillain-Barré syndrome (GBS) (incidence in developed regions range from 0.4 to 4.0 cases per 100,000), a postinfectious autoimmune disorder of the peripheral nervous system. Up to 20% of the patients having GBS require mechanical ventilation. However, mortality rates of GBS have been reduced to 2 to 3% in the developed regions, although it remains higher in less developed area (Allos, 1997; Tam et al., 2007). Reactive arthritis is another complication following 1% to 5% of campylobacteriosis cases, and mainly affects the bigger joints, but even the smaller joints can be involved (Hannu et al., 2002; Townes, 2010). Other rare complications include extra-intestinal infections and bacteremia, occurring mainly in immune-compromised patients. Furthermore, several studies have reported an association between *C. jejuni* infection and post-infectious irritable bowel syndrome (IBS)

as well as inflammatory bowel disease (IBD) (Marshall et al., 2006; Kirkpatrick and Tribble, 2011). These post-infectious complications increase the DALY of the disease. In most cases campylobacteriosis is self-limiting and does not require any antimicrobial treatment. However, macrolides or quinolones are used in severe, complicated or systemic infections or in immune-compromised patients (Allos, 2001). Increased worldwide resistance to fluoroquinolones has, however, made the treatment management more complex.

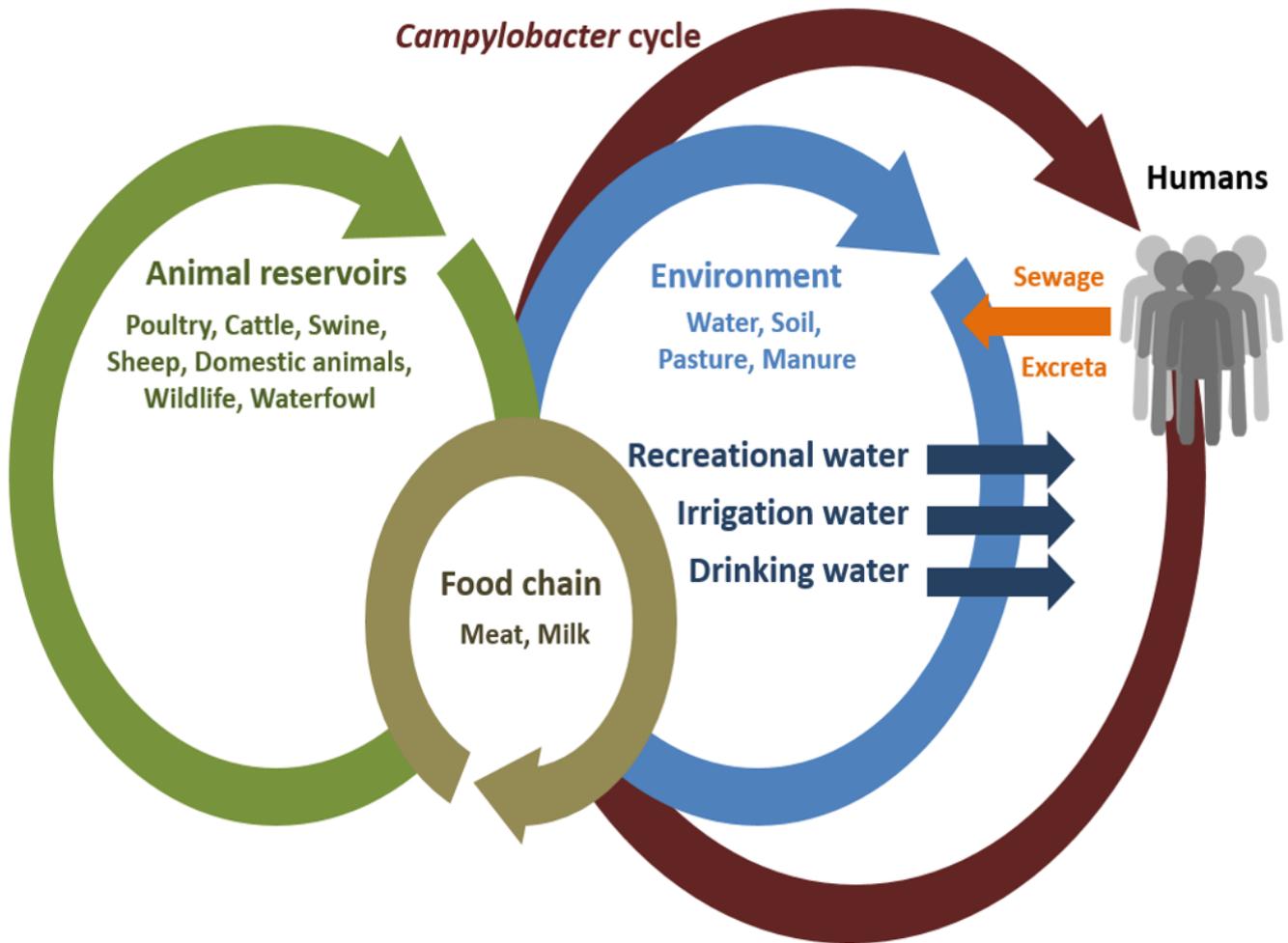
1.2 Taxonomic Classification of the Agent(s)

The family *Campylobacteraceae* includes the genera *Arcobacter*, *Sulfurospirillum*, *Dehalospirillum* and *Campylobacter* and are Gram-negative, generally microaerophilic organisms with a low G+C content and a relatively small genome. Currently, 29 species and 12 subspecies are classified in the genus *Campylobacter*, of which seven species and one subspecies were described after 2009 (<http://www.bacterio.net/campylobacter.html>, accessed 02.04.2015). A majority of the species are animal commensals colonising the reproductive organs, lower parts of intestinal tract or oral cavity. Several species are opportunistic or primary zoonotic pathogens. Two most clinically important species in human infections are *C. jejuni* (two subspecies, *C. jejuni* subsp. *jejuni* and *C. jejuni* subsp. *doylei*) and *C. coli*. The pathogenic role of *C. jejuni* subsp. *doylei* is unclear, but it has been isolated from infants with bacteremia. The two subspecies differ biochemically and can be separated by polymerase chain reaction (PCR) of nitrate reductase locus (Miller et al., 2007).

Campylobacter spp. are spiral curved rods with a polar flagellum occurring at one end of the cell that enables the display of rapid corkscrew-like darting and spinning motions (Butzler, 2004; Debruyne et al., 2008). *C. jejuni* is microaerophilic and a slender, spirally curved, non-spore forming, Gram-negative rod, which measures 0.2 to 0.8 µm wide and 0.5 to 5 µm long. In older cultures or in unfavorable environments, coccoid forms of *C. jejuni* cells are seen. Thermotolerant *Campylobacter* spp. are able to grow at 42°C but not below 30°C, and this group includes the species *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* (ISO 17995, 2005). *C. jejuni* cannot utilize carbohydrates for energy (some strains utilize fucose), but rather uses amino acids and tricarboxylic acid cycle intermediates as primary energy source (Debruyne et al., 2008). *C. lari* strains, often isolated from wild birds and surface water, have been differentiated from *C. jejuni* and *C. coli* due to their resistance to nalidixic acid (Debruyne et al., 2008). Traditional phylogenetic analysis to describe the taxonomy of *Campylobacter* spp. is based on the similarity of 16S rRNA gene (Debruyne et al., 2008).

1.3 Transmission

1.3.1 Routes of transmission



Most human *Campylobacter* infections are thought to be food- or waterborne (Allos, 2001; Guzman-Herrador et al., 2015; Mughini Gras et al., 2012; Pitkänen, 2013). *C. jejuni* and *C. coli* are zoonotic pathogens which have a broad variety of different animal hosts (Wagenaar et al., 2015). This extremely broad host spectrum from domestic and pet animals through mammalian wildlife to wild birds gives for this organism an almost unique position among zoonotic pathogens and makes detection of all potential sources and transmission routes very complicated. Fecal contamination of the food chain and environment transmits *Campylobacter* to human foods, recreational water and drinking water. Further, fecal material of infected persons (symptomatic and asymptomatic) spread the organisms back to environment through human excreta and sewage systems. Fecal input is high and increases the possibility that susceptible humans will become infected by fecal-oral route through contaminated food and water both in highly developed as well as in less developed regions. Direct contact with animals is also a likely route of transmission. Fly borne transmission has also been postulated. Person-to-person transmission is infrequent and has only been described in young children (Butzler, 2004). A diagram emphasizing the cyclic nature of *C. jejuni* and *C. coli* transmission from animal reservoirs and human excreta through food and water environment is presented in Figure 1.

Figure 1. Cyclic nature of *Campylobacter* spp. transmission from animal reservoirs to humans through fecal contamination of food and water. Adapted with permission from Christian Penny, LIST, Luxembourg

1.3.2 Reservoirs

A wide variety of animal species carry *C. jejuni* and *C. coli* as commensals in their intestines, being potential reservoirs and sources of human infections (Ogden et al., 2009). During the last 10 to 15 years when modern population genetic tools, such as multilocus sequencing typing (MLST) has been more systematically applied to characterize *Campylobacter* populations colonizing different animal species and human patients, new information on the role of different animal species as reservoir/source has accumulated (Kärenlampi et al., 2007). These studies often show that certain animal species are

more often colonized with MLST types overlapping with those of human patients (generalist types) indicating that they could be a reservoir/source (de Haan et al., 2010; Sheppard et al., 2009; Lévesque et al., 2013). Further source attribution models combining MLST data and mathematical modeling indicate which animal reservoirs play the most important role in human infections (Mughini-Gras et al., 2012; de Haan et al., 2010). Several animal species also have MLST types which are strictly associated with the host. These types do not commonly occur in other animal species or human patients (host-adapted types) and they can be regarded as less virulent even though more data will be required for final conclusions (Cody et al.,

2015; Llarena et al., 2015a). The most relevant reservoirs/sources of human infections are presented next.

1.3.2.1 Livestock - cattle and sheep

In addition to avian sources, dairy and beef cattle are typically colonized by *C. jejuni* and *C. hyointestinalis*. Colonization is age dependent with young animals are more often carrying *C. jejuni* than older animals. Both slaughterhouse surveys as well farm studies show variations in prevalence (between 12.5% and 89.4%) depending on study (Wesley et al., 2000; Nielsen, 2002; Gilpin et al., 2008; Kwan et al., 2008; Hakkinen et al., 2007). Within-herd prevalence, similarly, will vary depending on study site from 0% to 100% in dairy cattle (Gilpin et al., 2008; Pradhan et al., 2009; Hakkinen et al., 2007), and from 5.4% to 83% in beef cattle (Inglis et al., 2004; Berry et al., 2006; Oporto et al., 2007). The bacterial counts in bovine fecal samples are usually low ($< 10^3$ per gram) and therefore often detected only by enrichment (range in different studies: not detected up to 10^6 per gram) but temporary fluctuations in counts are common. In different source attribution models bovine as source has presented approximately 20 to 40%. Raw milk-associated outbreaks are common indicating that *C. jejuni* strains colonizing cattle are capable of infecting humans. Further, reports from case-control studies have also identified the consumption of unpasteurised milk as an important risk factor for campylobacteriosis among humans (Studahl and Andersson, 2000; Kapperud et al., 2003; Neimann et al., 2003; Michaud et al., 2004) as well as handling raw beef (Schönberg-Norio et al., 2004). Molecular epidemiological studies have shown overlapping genotypes between cattle and humans further revealing that cattle can be a source of human infection. Certain MLST types such as ST-61 have adapted to cattle, but are also able to cause symptomatic infections in humans. Also sheep can be colonized by *C. jejuni* and *C. coli* genotypes overlapping with those isolated from human patients, as reported in Scotland (Sheppard et al., 2009). In principle several other animal species carrying *Campylobacter* spp. can be source of human infections but these are usually sporadic cases.

1.3.2.2 Poultry

Epidemiology studies performed during the last 30 years clearly indicate that poultry, especially, chickens are one of the most important reservoirs/sources of human campylobacteriosis worldwide (Skirrow, 1977; Llarena et al., 2015; Wagenaar et al., 2015). According to recent evaluation by EU Biohazard Panel (EFSA, 2011), 20 to 30% of human *Campylobacter* infections in the EU member states can be explained by handling and consumption of contaminated chicken meat (direct exposure), but chicken production as whole plays major role (explains 30 to 50% of cases) because chicken production acts as amplification and generation site of *Campylobacter* (reservoir). The bacteria can spread from farms, slaughterhouses and through other routes to the environment, causing human exposure through environmental vehicles (Figure 1). The reason why poultry is an important source of *C. jejuni* and also *C. coli* is that their prevalence in commercial flocks is

up to 70% in many industrialized countries, yet there are significant spatial and temporal differences in prevalence (EFSA, 2015; Kovats et al., 2005). In industrialized countries chickens are produced in units containing 10,000 to 50,000 birds per a house and a farm can have several houses where birds are reared for five to seven weeks (differences in the breeding times exist). Commonly if a flock is *Campylobacter* positive, all birds carry the bacterium in their intestines, at concentrations up to 10^8 per gram of fecal material at time of slaughter. Industrialized production cycles have usually strict biosecurity control systems protecting the production from contamination. However, the capability to have high biosecurity level during all production steps is challenging. Biosecurity also includes the safe handling of the animal waste including manure and slaughterhouse waste, which are treated in scheduled ways to prevent pathogens (*Campylobacter* spp.) spreading to the outside environment (EFSA, 2011).

The UN report's the world's stock of chickens was 19 billion in 2012, meaning almost three chickens per a human being (<http://www.thepoultrysite.com/focus/global-poultry-trends/2400/global-poultry-trends-region-select-track-poultry-trends-across-the-world>, accessed 07.02.2016). The production is centralized: 50% of the poultry production takes place in three countries, USA, Brazil and China. In the EU, approximately 6 million chickens are slaughtered each year. The production is local but the market is global meaning that the ecology of *Campylobacter* is mostly local concerning the transmission of the strains but also spreading globally through exported meat contaminated by *Campylobacter*. On the other hand free range and organic production systems are increasing and may cause even higher contamination risks in production. The situation in rural developing areas can be different because on those regions poultry production often happens at small scale at family-own farms where humans, poultry and other animals are living in close contact (backyard rearing) making the spreading of *Campylobacter* strains between different hosts and the environment including water a common phenomenon.

1.3.2.3 Wild birds

Wild birds are a huge diverse group of different bird species with differing ecologies and life styles that also represent an important reservoir and source of *Campylobacter* species causing human infections (Cody et al., 2015; Llarena et al., 2015a; Muchini-Gras et al., 2012; Kwan et al., 2014; Griekspoor et al., 2013). They can cause a direct transmission through their use as foods or transmit indirectly by contaminating foods, bathing water or drinking water reservoir by fecal material and they can work as reservoirs to transmit *Campylobacter* spp. to domestic and other wild animals (Wagenaar et al., 2015). There are both bird species which have their life cycles locally and species which migrate long distances and are then able to transmit *Campylobacter* spp. between distantly located areas. Studies performed on different areas in the world from all continents to Antarctica reveal that

Campylobacter spp. are widely spread among wild birds (Griekspoor et al., 2013). Several *Campylobacter* species colonize birds, most important are *C. jejuni*, *C. coli* and *C. lari*, a bird-adapted species. Most studies are targeted on wild birds as reservoir transferring *Campylobacter* spp. to domestic animals or humans. Bird species seem to differ how commonly they carry *Campylobacter* spp.; some have high prevalence and some low or nonexistent colonization. Bird species known to be commonly colonized are ducks (family Anatidae), shorebirds (several families), corvids, gulls, pigeons, doves, thrushes and starlings (Griekspoor et al., 2013). Bird species which are living in close connection to agriculture and other human activities (urban areas, close to landfills etc.) seem to be often colonized. Epidemiological studies show evidence that fecal contamination of non-disinfected drinking water by wild bird feces has been associated to some waterborne outbreaks (Pitkänen et al., 2008). However, comparison of wild bird and human patient isolates seldom indicate overlapping sero- or genotypes thus making final conclusion on the role of wild birds as contamination sources unclear. Modern molecular genotyping methods are applied and used to compare similarity of genotypes of wild birds and human patients or domestic animals. MLST typing in combination with mathematical source attribution models have been applied and they generally show that some types overlap between humans, animals and birds (ST-45, ST-48, ST-21 and ST-257) but birds also have types which seem to be unique for the species (e.g. ST 1034 in barnacle geese (Llarena et al., 2015a). Interestingly the bird-associated MLST types occur in certain bird species all over the world further supporting the idea on host-associated types. A recent UK study using MLST typing data and a mathematical model estimated that wild bird-attributed isolates could account annually for 2 to 3% of human cases in Oxfordshire, UK (Cody et al., 2015). Also isolated outbreaks point to human-infectious *C. jejuni* in some flocks of waterfowl (e.g. Sandhill Cranes [Lu et al., 2013]), versus largely absent in others (e.g. California Gull [Lu et al., 2011]).

1.3.2.4 Water and soil

Waterborne *Campylobacter* spp. are likely to originate from recent fecal contamination of water by waterfowl and farm animal manure runoff, in addition to discharges of poorly or non-disinfected sewage effluent or septic seepage (Koenraad et al., 1994; Obiri-Danso and Jones, 1999; Abulreesh et al., 2006; Kuusi et al., 2005; Jokinen et al., 2010; Hellein et al., 2011). River and lake water have several potential contamination sources such as cattle and sheep on pasture and manure spreading on fields within watersheds, direct fecal contamination by wild birds and animals, human untreated waste in some regions and treated municipal wastewater (Figure 1). The relative importance of different sources and extent of contamination is highly dependent upon the hydrogeology of the watershed. While farming and urban areas are important sources, remote regions may still have *Campylobacter* contamination due to wild animals. Also, bacterial discharge from farmland to waterways is highly dependent on rain/snow events that mobilize fecal deposits (Muirhead

et al., 2006; Ferguson et al., 2010). Humans may also acquire campylobacteriosis from natural water by bathing, recreation/canoeing, or drinking directly from within the watershed.

1.3.3 Incubation period

Campylobacter infection is an acute self-limited gastrointestinal illness with an incubation period that is longer than most other intestinal infections: commonly two to five days but up seven days have been reported. The mean incubation period is 3.2 days ranging approximately from 1 to 10 days (Blaser, 1997; Feodoroff et al., 2010; Nichols et al., 2012).

1.3.4 Period of communicability

Patients shed high numbers of *C. jejuni* in voided material during their symptomatic infection phase, which commonly last three to five days. Duration of shedding lasts from a few hours to three weeks (average 15 days) (Nichols et al., 2012; Feodoroff et al., 2010; Butzler, 2004). Limited data is available on the concentration in human feces, but in three patients' concentrations varied from 6×10^6 to 10^9 CFU/g (Blaser and Wang, 1980). Nonetheless, human-to-human transmission of *Campylobacter* is uncommon (Blaser, 1997).

Campylobacter enteritis most frequently affects children aged < 4 years and the infection rate is higher in boys and adult males than in girls and adult females (Allos, 2001; Kotloff et al., 2013). The infection frequency displays a strong seasonal variation in temperate climates with infections being more common in summer than in winter (Nylen et al., 2002; Schönberg-Norio et al., 2004; Kovats et al., 2005). The reason for the summer peak of human infections remains unknown but it may be affected by the higher prevalence of sporadic foodborne infections together with higher probability of waterborne outbreaks in conjunction with summer activities, such as outdoor food handling practices and the use of non-community water supplies (Olson et al., 2008). The seasonal variation is less distinct in tropical and subtropical areas (Kovats et al., 2005) and fly transmission has been hypothesized as an explanatory factor (Nichols, 2005).

1.3.5 Population susceptibility

Antibodies to *Campylobacter* antigens are present for several months conferring a short-term immunity to the homologous strain (Black et al., 1992). In developed regions where infections are uncommon, population level immunity is usually low and active immunity after symptomatic infection disappears rapidly and does not provide protective immunity against non-homologous strains (Allos, 2001; Blaser, 1997). Occupational exposure (farmers, slaughterhouse workers etc.) may acquire immunity and may occasionally be asymptomatic shedders of campylobacters (Ellström et al., 2014). Epidemiological features of infections in low-income countries suggest that due to several infection episodes in childhood leading to acquired immunity, older persons rarely experience

symptomatic infection but may shed the pathogen.

1.4 Population and Individual Control Measures

1.4.1 Vaccines

Several attempts have been performed to develop a vaccine either for prevention of human infections or more commonly vaccines to prevent colonization of chickens by *C. jejuni* and so reduce its spread to humans. Currently, there is no vaccine approved by any global regulatory authority to prevent *Campylobacter*-associated illness. Biological feasibility of human vaccine development is supported by data from epidemiologic and human challenge studies. Several candidates are under development (http://www.who.int/immunization/research/meetings_workshops/Campylobacter_VaccineRD_Sept2014.pdf).

1.4.2 Hygiene measures

Hygienic food preparation needs high quality water in food production and related processing areas (for cleaning food handling surfaces and utensils, hands etc.). Further, availability for proper hand washing facilities, toilets and clean water are of primary importance for safe food production. The prevention of fecal contamination of drinking water and maintenance of a residual chlorine concentration in the distributed drinking water are critical control measures to reduce the numbers of waterborne *Campylobacter* infections in regions with centralized water supplies and distribution. Private wells should be protected from fecal contamination by constructing them in such a manner that any fecal contamination source is not close to well and surface water cannot contaminate the well, e.g. after heavy rain/flooding. Protection of raw water sources from fecal contamination is one of the first hygienic measures, promoting the production of drinking water in a way requiring less costly water treatment (http://www.who.int/water_sanitation_health/en/). Many cases of *C. jejuni* infections are associated with drinking water cross-connection with wastewater infrastructure (Pitkänen, 2013). The water safety plan (WSP) approach introduced by WHO is a risk management framework that supports the production of high quality drinking water worldwide with multiple benefits (Gunnarsdóttir et al., 2012).

2.0 Environmental Occurrence and Persistence

2.1 Detection Methods

Campylobacter detection methods have been developed since *Campylobacter jejuni* was first isolated from the feces of patients with gastrointestinal disease in the 1970s (Dekeyser et al., 1972) and include culture-based and direct molecular methods. *Campylobacter* species are fastidious bacteria that are oxygen sensitive and are grown in a microaerobic atmosphere containing 3 to 10% (v/v) oxygen and 5 to 10% (v/v) carbon dioxide (Kelly, 2001). Some species also require hydrogen for their growth. Colonies directly grown on solid media are usually incubated at

41.5°C with fecal samples that usually contain high numbers of viable *Campylobacter* cells (Jacobs-Reitsma et al., 2008; Ugarte-Ruiz et al., 2012). Thermotolerant *Campylobacter* can also be cultivated from feces or mixed environmental media with antimicrobial-containing selective media (blood-based or charcoal blood-free) under microaerophilic conditions at an elevated temperature (42°C). However, initial liquid enrichment is generally used before plating on solid selective media to promote the recovery of low numbers of cultivable *Campylobacter* cells from environmental samples (Hänninen et al., 2003; Jacobs-Reitsma et al., 2008; Kim et al., 2016). The selective agents most often used include cefoperazone, amphotericin B, trimethoprim and vancomycin, and the media also contain sterile sheep or horse blood, or charcoal to neutralize the toxic effects of oxygen and light (Jacobs-Reitsma et al., 2008; Kim et al., 2016).

Since cultivation of *Campylobacter* spp. requires specific conditions including microaerobic atmosphere, the isolation of *Campylobacter* spp. from water is rarely successful or attempted, and was only reported in eight out of the 29 outbreaks reviewed by Pitkänen (2013). Thus far, studies have shown that it is difficult to isolate *Campylobacter* spp. from water related to waterborne *Campylobacter* outbreaks mainly due to insufficient and delayed water sampling and analysis (Bopp et al., 2003; Hänninen et al., 2003; Jakopanec et al., 2008). Generally, failure to isolate *Campylobacter* spp. is due to deficiencies in the detection methods applied to water samples (Rollins and Colwell, 1986; Jacobs-Reitsma et al., 2008), i.e. insufficient sample volumes and selective culture (Miller and Mandrell, 2005). The current standard method for the detection of thermotolerant *Campylobacter* species from water is based on membrane filtration as an initial concentration step (ISO 17995, 2005), followed by liquid enrichment before plating onto a solid selective medium, so as to recover low numbers of *Campylobacter* cells usually existing in environmental samples (Hänninen et al., 2003; Jacobs-Reitsma et al., 2008; Ugarte-Ruiz et al., 2015). Enrichment using Bolton broth is the primary method used for isolation of *Campylobacter* spp. in drinking water (ISO 17995, 2005), and the use of increased sample volume is recommended even though it may require the use of larger diameter filters (Hijnen et al., 2000) than the conventional 47 mm in cases where the water under examination is turbid. Direct plating methods employed for stool samples are unsuitable for quantitative recovery of low numbers of *Campylobacter* spp. present in environmental samples (Rollins and Colwell, 1986; Hänninen et al., 2003), and in water with high microbial biomass, non-targeted bacterial groups may out-compete the growth of *Campylobacter* spp. during the enrichment step (Abulreesh et al., 2005). For sewage influent and effluent samples, the selectivity of the enrichment may be increased by use of Preston broth at an elevated temperature of 41.5°C instead of 37°C (Koenraad et al., 1995) and a 21 h enrichment time as compared to the standard time of 44 h (Pitkänen, 2013), or addition of a wider range of antimicrobial agents (Kim et al., 2016). Alternatively a filter method, where motile *Campylobacter* spp. move through the pores in membrane filter on unselective medium prior to incubation at the growth temperature, have been used for separation of

Campylobacter spp. from the non-target bacteria (Diergaardt et al., 2004; Rechenburg and Kistemann, 2009).

Methods for species identification and strain typing are not matrix-dependent, i.e. any method available for *Campylobacter* typing may be utilized in further characterization of *Campylobacter* isolates. The traditional subtyping methods include phenotyping, serotyping, biotyping, and phage typing (Butzler, 2004). The minimum standard for identifying *Campylobacter* spp. includes colony morphology, Gram's stain, motility, and an oxidase test. To separate *C. jejuni* from *C. coli*, a hippurate-hydrolysis test has traditionally been used, although there are also hippurate-negative *C. jejuni* (Totten et al., 1987).

More recently, a wide range of nucleic acid-based methods have been used to confirm presumptive *Campylobacter* isolates from media. Due to the biochemical inertness of *Campylobacter* spp., molecular methods such as PCR and sequencing of the 16S rRNA gene are now generally used to differentiate *Campylobacter*-species. However, the sequence variability in the 16S rRNA gene in *C. jejuni* and *C. coli* does not differentiate these species, necessitating the use of other gene targets of PCR and sequencing of the amplicon, for example, the *hipO*-gene (Linton et al., 1996) or *groEL*- and *rpoB*-gene (Kärenlampi et al., 2004; Korczak et al., 2006). Indeed, PCR methods targeted to *hipO* gene may provide a more accurate differentiation between *C. jejuni* and *C. coli* than the phenotypic hippurate test (Abu-Halaweh et al., 2005; Abulreesh et al., 2006; Jensen et al., 2005). In addition, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) (Mandrell et al., 2005) and a number of genotyping methods, such as pulsed-field gel electrophoresis (PFGE), restriction fragment analysis of *Campylobacter* flagellin genes (*fla*-RFLP), amplified fragment length polymorphism (AFLP), MLST and direct sequencing (Butzler, 2004; On et al., 2008; Wassenaar and Newell, 2000; Clark et al., 2003; Kovanen et al., 2014) can be used to aid in the identification of *Campylobacter* species.

Recently whole genome MLST (wgMLST) has been increasingly applied for studies of outbreak-associated *C. jejuni* isolates (Kovanen et al., 2016). A recent study using wgMLST of *C. jejuni* isolates from a waterborne outbreak revealed that sometimes pulsed-field gel electrophoresis (PFGE) may overestimate the clonal relationship between some of the apparent outbreak-related *C. jejuni* strains (Revez et al., 2014). It is possible that in previous years, when PFGE was solely utilized for genotyping of outbreak isolates, some unrelated, sporadic human cases were mistakenly classified as part of the waterborne outbreaks. The use of molecular tools such as wgMLST is increasing in outbreak investigations (Moffatt et al., 2016).

The reliance on conventional cultivation alone for *Campylobacter* spp. detection and subsequent subtyping is not practical, since viable but not culturable (VBNC) *Campylobacter* cells occur (Tholozan et al., 1999; Abulreesh et al., 2006; Bronowski et al., 2014; Oh et al., 2015). The coupling of PCR detection after enrichment to the culture-based method increases the work load but

substantially shortens the time requirement for detection and confirmation of *Campylobacter* spp. (i.e. from two or more days down to five hours). The use of PCR after enrichment may reduce the presence of false negative analysis results in cases where the *Campylobacter* colonies are overgrown by the growth of non-target bacteria on a solid agar medium. Ideally quantitative real-time PCR for *Campylobacter* detection should be applied directly to water samples (Nogva et al., 2000; Yang et al., 2003; Nam et al., 2005; VanDyke et al., 2010; Clark et al., 2011; Park et al., 2011). These studies have utilized *Campylobacter* PCR assays targeting the 16S rRNA gene (Bang et al., 2002; Moreno et al., 2003; Josefsen et al., 2004; Hellein et al., 2011), the 23S rRNA gene (Engvall et al., 2002), 16S-23S rDNA internal transcribed (ITS) region (Khan and Edge, 2007) and the flagellin genes *flaA* and *flaB* (Waage et al., 1999; Moore et al., 2001). Other molecular assays reported have targeted genes such as *glyA* (Jensen et al., 2005), *gafF* and *ceuE* (Nayak et al., 2005; Hellein et al., 2011), ORF-C sequence (Sails et al., 2003) and *cpn60* gene (Banihashemi et al., 2012).

However, the problem of detecting both viable and non-viable cells has so far restricted applications of PCR techniques. Therefore, techniques differentiating living from dead cells have been studied and may well provide novel tools for rapid and accurate detection of viable microbes (Cenciarini-Borde et al., 2009; Fittipaldi et al., 2012; Pitkänen et al., 2013). For example, it has been suggested that RT-qPCR assay based *Campylobacter* spp. specific rRNA signals are associated to active bacterial populations in environmental waters since they were in better agreement with the culture-based results of thermotolerant *Campylobacter* spp. than that of rDNA-based assays (Pitkänen et al., 2013). Novel molecular platforms such as isothermal amplification techniques and high-throughput sequencing approaches may provide new possibilities for *Campylobacter* spp. detection. Isothermal amplification coupled with microarrays provide opportunity to simultaneously study a large number of microbial targets, including genes of *C. jejuni* in less than 20 minutes (Tourlousse et al., 2012).

2.2 Data on Occurrence

2.2.1 Excreta in environment

Campylobacter spp. occurrence data in human fecal waste other than sewage is limited. Further, there are gaps in the incidence data (see Table 1) especially from developing regions where waterless or no sanitation systems occur. Nonetheless, *Campylobacter* spp. are one of the most frequently occurring pathogens causing human gastrointestinal diseases worldwide, hence, always present in community fecal waste.

Most warm-blooded animals are asymptomatic carriers of *Campylobacter* spp. The presence of *Campylobacter* spp. in mammal and bird feces: cat, dog, cow, seagull, chicken, duck, geese and pigs is commonly reported (Hellein et al., 2011). For example, in Canada, the presence of *Campylobacter* spp. was shown in a total of 37 animal fecal samples (35.6%) out of 104 samples collected from goat,

cow, chicken, goose, duck and pig feces (Jokinen et al., 2010). However, *Campylobacter* spp. was absent from rabbit and rat feces and present in less than 15% of horse, dog, llama, alpaca and sheep fecal samples (n=113; Jokinen et al., 2010). In Queensland, Australia, 24 out of 40 possum fecal samples (60%) contained 2.0×10^5 to 2.0×10^7 *Campylobacter* spp. cells/g (Ahmed et al., 2012). In the same study, 24% of bird fecal samples had 6.6×10^4 to 6.6×10^6 *Campylobacter* spp. cells/g (n=38). Noting that the presence of animal *Campylobacter* spp. does not mean they are infectious in humans, strain typing is necessary (e.g. Mughini Gras et al., 2013).

2.2.2 Sewage

Sewage is a major source of *Campylobacter* spp. in the environment. Septic seepage into well waters and cross-connections between sewerage and drinking water systems have caused several waterborne outbreaks (Pitkänen, 2013). Non-disinfected wastewater effluents discharge *Campylobacter* spp. to environmental waters (Lauria-Filgueiras and Hofer, 1998; Hokajärvi et al., 2013; Rechenburg and Kistemann, 2009). Table 2 summarizes the reported *Campylobacter* spp. numbers in raw sewage and wastewater treatment plant effluents from developed regions.

Table 2. Occurrence of *Campylobacter* in sewage, effluents and sludges

Area	Period of Study	Pathogen Type	Sample Volume/ Method	Percent Positive (# of Samples)	Concentrations Average (Ranges) MPN ^a , CFU ^b or Cells ^c /L	Reference
Matrix raw sewage						
Brazil (Rio de Janeiro)	1990 to 1991	<i>Campylobacter</i> spp.	120 ml centrifuged at 3000 rpm for 30 min or Moore's swab adaptations	52.3% (68/130)	NR ^d	Lauria-Filgueiras and Hofer, 1998
		<i>C. jejuni</i>	Culture	23.8% (31/130)		
		<i>C. coli</i>	Culture	13.1% (17/130)		
Canada (British Columbia)	2004 to 2006	<i>Campylobacter</i> spp.	500 mL Culture	36.4% (4/11)	NR (<i>C. jejuni</i> confirmed by PCR from all positive samples)	Jokinen et al., 2010
Germany (North-Rhine Westphalia)	NR	<i>Campylobacter</i> spp.	100 mL, 10 ml and 1 ml or less using dilutions Culture	% NR (39)	1.1E+06 CFU (median) (1.0E+02 to 1.0E+07 CFU)	Rechenburg and Kistemann, 2009
Germany (Weisse Elster)	NR	<i>Campylobacter</i> spp.	NR Culture	% NR (13)	1.1E+04 CFU	Stelzer and Jacob, 1991
Italy (Bologna)	1990 to 1991	<i>Campylobacter</i> spp.	1 mL, 0.1 mL and 0.01 mL Culture	% NR (22)	1.6E+04 MPN	Stampi et al., 1992
Italy (Piedmont region)	2014 to 2015	<i>Campylobacter</i> spp.	100 mL Gene detection (PCR after enrichment)	83% (10/12)	NR	Bonetta et al., 2016
		<i>C. jejuni</i>		50% (6/12)	NR	
		<i>C. coli</i>		25% (3/12)	NR	
Spain (Catalonia)	2004 to 2006	<i>Campylobacter</i> spp.	0.0001 to 10 mL, centrifugation at 7700g for 20 min Culture	85.7% (24/28)	2.5E+03 MPN (geomean) (< 4.0E+01 to 1.5E+05 MPN)	Rodriguez and Araujo, 2010
USA (Pensacola, Florida)	2008 to 2009	<i>Campylobacter</i> spp.	10 mL qPCR for cell counts, PCR for speciation	26.3% (15/57)	4.1E+06 Cells (mean of +ve) (< 1.0E+02 to 6.2E+07 Cells)	Hellein et al., 2011
Matrix sewage effluent^e						

Area	Period of Study	Pathogen Type	Sample Volume/ Method	Percent Positive (# of Samples)	Concentrations Average (Ranges) MPN ^a , CFU ^b or Cells ^c /L	Reference
Brazil (Rio de Janeiro)	1990 to 1991	Campylobacter spp.	120 ml centrifuged at 3000 rpm for 30 min or Moore's swab adaptations	25.4% (33/130)	NR	Lauria-Filgueiras and Hofer, 1998
		<i>C. jejuni</i>		6.2% (8/130)		
		<i>C. coli</i>		4.6% (6/130)		
Finland	2006 to 2007	Campylobacter spp.	0.1, 1 and 10 mL Culture	52.9% (18/34)	5.0E+02 CFU (median)	Hokajärvi et al., 2013
Germany (North-Rhine Westphalia)	NR	Campylobacter spp.	100 mL, 10 ml and 1 ml or less using dilutions Culture	% NR (37)	1.0E+03 CFU (median) (< 1.0E+01 to 1.0E+05 CFU)	Rechenburg and Kistemann, 2009
Germany (Weisse Elster)	NR	Campylobacter spp.	NR Culture	% NR (11)	1.3E+03 CFU	Stelzer and Jacob, 1991
Germany (Weisse Elster)	NR	Campylobacter spp.	NR Culture	42.8% (3/7)	NR (Not found in the digested sludge (age >90 days))	Stelzer and Jacob, 1991
Italy (Bologna)	1990 to 1991	Campylobacter spp.	50 and 100 mL Culture	% NR (22)	3.0E+02 MPN	Stampi et al., 1992
		Campylobacter spp.		% NR (22)	Not detected Post chlorination	
Italy (Piedmont region)	2014 to 2015	Campylobacter spp.	1000 mL Gene detection (PCR after enrichment)	42% (5/12)	NR	Bonetta et al., 2016
		<i>C. jejuni</i>		8% (1/12)		
		<i>C. coli</i>		25% (3/12)		
Matrix sewage sludge				9% (6/64)	NR	Sahlström et al., 2004
Sweden	NR	<i>C. coli</i>	Vol NR Culture	(2% (1/69) in treated sludge)		
Sweden	NR	<i>C. jejuni</i>	Vol NR Culture	20% (13/64) (4% (2/69) in treated sludge)	NR	Sahlström et al., 2004
The Netherlands (Gelderland)	1991 to 1993	Campylobacter spp.	Vol NR Culture	88% (27/30)	2.5E+03 MPN (3.2E+02 to 6.3 E+05 MPN)	Koenraad et al., 1994

^aMPN: Most probable number; ^bCFU: Colony forming units; ^cQuantification was based on a standard curve constructed relating the number of bacterial cells to the cycle threshold (CT) of qPCR for known concentrations of bacteria determined by culture (CFU); ^dNR: Not reported; ^eActivated sludge

2.2.3 Sludge

Table 2 summarizes the published reports of *Campylobacter* spp. numbers in sludge. For example, at sewage treatment plants in Sweden, 20.3% (13/64) and 9.4% (6/64) of raw sludge samples contained viable *C. jejuni* and *C. coli*, respectively, whereas only 2.9% (2/69)

and 1.4% (1/69) of treated sludge samples were *C. jejuni* and *C. coli* positive (Sahlström et al., 2004).

2.2.4 Animal manure

Because *C. jejuni* and *C. coli* commonly colonize a large numbers of animal hosts, their presence in fresh fecal

material is common. For example, 100% presence of *Campylobacter* spp. in poultry wastewater (n=13; geometric mean 6.0×10^6 MPN/L) and in pig slurries (n=6; geometric mean 4.9×10^6 MPN/L) in Mediterranean area has been reported (Rodríguez and Araujo, 2010). Origin of manure, the manure treatment process, climate and other environmental factors play a role in the survival and numbers of *Campylobacter* spp. in manure and after spreading to land (Nicholson et al., 2005).

2.2.5 Surface waters

Campylobacter spp. are frequently isolated from fresh surface waters (Stelzer and Jacob, 1991; Koenraad et al., 1997; Jones 2001; Savill et al., 2001; Diergaardt et al., 2004; Hörman et al., 2004; Vereen et al., 2007; Hellein et al., 2011; Jokinen, et al., 2012; Hokajärvi et al., 2013).

Table 3 summarizes the published studies reporting the detection of *Campylobacter* spp. and available *Campylobacter* spp. counts in surface waters. The high variability in the reported detection rates and counts of *Campylobacter* spp. in surface water is in part due to the different methods used for detecting *Campylobacter* spp. from water. For example, the *Campylobacter* counts have been analyzed by direct plating (Vereen et al., 2007), using *Campylobacter* spp. specific quantitative PCR methods (Hellein et al., 2011; Van Dyke et al., 2010) and by semi-quantitative enrichment method (Hokajärvi et al., 2013; Diergaardt et al., 2004) - making comparison between studies rather pointless given the different forms (dead or culturable) reported. *Campylobacter* spp. counts also naturally vary, due to processes that mobilize them off surfaces and their dilution within surface waters (Sterk et al., 2016).

Table 3. Occurrence of *Campylobacter* spp. in surface waters^a

Area	Period of study	Sample Volume/ Method MATRIX	Percent Positive (# of samples)	Concentrations Average (Range) MPN ^b , CFU ^c or Cells ^d /L	Comments	Reference
Canada	2006 to 2007	1000 mL centrifuged 14000g for 20 min Culture	49.0% (377/769)	4.0E+02 MPN (median)	C. jejuni, C. coli and C. lari occurred in 45%, 34% and 19% of all <i>Campylobacter</i> positive samples, respectively 93.4% and 7.9% of positive samples contained C. jejuni and C. coli, respectively	Khan et al., 2014
Canada (British Columbia)	2004 to 2006	500 mL Culture	43.0% (80/186)	NR ^e		Jokinen et al., 2010
Canada (Southern Alberta)	2005 to 2007	500 mL Culture RIVER	26.6% (91/342)	NR		Jokinen et al., 2012
Canada (Waterloo)	2005 to 2008	100 mL qPCR RIVER	69.8% (275/394)	5.5E+01 Cells (median) (< 1.0E+02 to 1.3E+03 Cells)		Van Dyke et al., 2010
		1000 mL Culture RIVER	12.8% (44/344)	NR	Culture results only qualitative	
Finland	2006 to 2007	10, 100 and 1000 mL Culture RECREATIONAL WATER	58.0% (29/50)	5.0E+01 CFU (median) (< 1.0E+00 to 1.0E+02 CFU)	C. jejuni, C. coli, C. lari and unidentified <i>Campylobacter</i> spp. isolates found	Hokajärvi et al., 2013

Area	Period of study	Sample Volume/ Method MATRIX	Percent Positive (# of samples)	Concentrations Average (Range) MPN ^b , CFU ^c or Cells ^d /L	Comments	Reference
Germany (North-Rhine Westphalia)	NA	100 mL, 10 ml and 1 ml Culture RIVER	86% (87/101)	1.0E+03 CFU (median) (< 1.0E+01 to 1.0E+06 CFU)		Rechenburg and Kistemann, 2009
Germany (Weisse Elster)	NA	NR Culture RIVER	82.1%	From <1.0E+02 to >2.4E+03 CFU	Species identified: <i>C.</i> <i>jejuni</i> (92.2%), <i>C. coli</i> (7.8%),	Stelzer and Jacob, 1991
Greece	1990 to 1991	100 mL Culture (without enrichment)	16.3% (14/86)	NR	For <i>C.</i> <i>jejuni</i> only	Arvanitidou et al., 1995
New Zealand	NA	500 mL, 100 mL and 10 mL Culture RIVER	60% (18/30)	1.8E+00 MPN (median) (<1.2E+00 to >1.1E+02 MPN)		Savill et al., 2001
Northern Ireland	NA	400 mL PCR RIVER	0% (0/17)	BDL	All samples below the detection limit	Moore et al., 2001
Norway (Oslo)	1986 to 1987	3 × 100 mL Culture RIVER	43.8% (42/96)	NR		Brennhovd et al., 1992
Norway (Telemark County)	1999	100 ML Culture RIVER	53.3% (32/60)	NR	Isolates identified as <i>C.</i> <i>coli</i> (44%), <i>C.</i> <i>jejuni</i> (34.6%), <i>C. lari</i> (14.7%) and not- classified (6.7%). Species identified: <i>C.</i> <i>jejuni</i> ssp. <i>jejuni</i> 1 (18.1%), <i>C.</i> <i>jejuni</i> ssp. <i>doylei</i> (7.2%), <i>C. coli</i> (4.8%), <i>C. lari</i> (3.6%) and <i>C.</i> <i>sputorum</i> ssp. <i>fecalis</i> (3.6%)	Rosef et al., 2001
Poland (Western Pomerania)	1998 to 1999	10 mL, 1 mL and 0.1 mL Culture RIVER	19.7% (29/147)	<1.0E+04 CFU		Dackowska-Kozon and Brzostek- Nowakowska, 2001
Spain (Catalonia)	2004 to 2006	1 to 1000 mL, centrifugation at 7700g for 20 min Culture RIVER	81.8% (45/55)	1.3E+01 MPN (geomean) (< 4.0E-01 to 1.1E+04 MPN)		Rodriguez and Araujo, 2010
Taiwan	2007 to 2008	1000 mL Culture RIVER	2.7% (2/75)	NR	Isolates identified as <i>C.</i> <i>jejuni</i>	Hu and Kuo, 2011
The Netherlands (Gelderland)	1991 to 1993	NR Culture RIVER	69.4% (25/36)	1.7E+02 MPN (3.2 E+01 to 1.6E+04 MPN)		Koenraad et al., 1994
UK (Lancashire)	1982 to 1983	500 mL Culture RIVER	43% (134/312)	<1.0E+02 to 2.3E+03 CFU	Identified species: <i>C.</i> <i>jejuni</i> , <i>C. coli</i> , <i>C. lari</i>	Bolton et al., 1987

Area	Period of study	Sample Volume/ Method MATRIX	Percent Positive (# of samples)	Concentrations Average (Range) MPN ^b , CFU ^c or Cells ^d /L	Comments	Reference
USA (Georgia)	2003 to 2004	0.1 mL Culture (without enrichment)	59.5% (44/74)	4.6E+04 CFU (< 1.0E+04 to 6.0E+05 CFU)		Vereen et al., 2007
USA (Pensacola, Florida)	2008 to 2009	500 mL qPCR	31.3% (10/32)	3.3E+05 Cells/L (mean of +ve samples only) (< 1.0E+02 to 4.6E+06 Cells/L)	Results per sites (not per samples)	Hellein et al., 2011

^aUndescribed surface water unless matrix specified; ^bMPN: Most probable number; ^cCFU: Colony forming units; ^dQuantification was based on a standard curve constructed relating the number of bacterial cells to the cycle threshold (CT) of qPCR for known cell numbers of target bacteria determined by epifluorescent microscopy or culture; ^eNR: Not reported.

Noting the bias of culture methods to detect *C. jejuni* and *C. coli*, *C. jejuni* has been the species identified most frequently from surface waters (Thomas et al., 1999a), yet *C. coli* and *C. lari* are also commonly reported (Thomas et al., 1999a; Denis et al., 2011; Hokajärvi et al., 2013), associate with the presence of agricultural runoff or large flocks of waterfowl (Bolton et al., 1987; Obiri-Danso and Jones, 1999). In a study in Ontario, Canada, 73% of isolates from the samples of waterfowl feces (n=15 out of a total of

52 samples) were identified as *C. jejuni*, 13% were *C. coli* and 27% *C. lari* (Van Dyke et al., 2010). By using gene detection after enrichment, it has been noted that the variety of *Campylobacter* species in watersheds is even broader, including *C. consicus*, *C. gracilis*, *C. hyointestinalis*, *C. helveticus* among others in addition to *C. jejuni*, *C. coli* and *C. lari* detected using culturing (Table 4) (Tambalo et al., 2016).

Table 4. Occurrence and distribution in water of *Campylobacter* species in Canadian (Saskatchewan) and French studies

Pathogen Type	Percent Positive (# of Samples)
Canada study; 200 ml sample volume with culture and PCR after enrichment (2009 to 2013) (Source: Tambalo et al., 2016)	
<i>Campylobacter</i> spp.	38.7% (101/261)
<i>C. coli</i>	11% (10/93)
<i>C. consicus</i>	11% (10/93)
<i>C. curvus</i>	1% (1/93)
<i>C. fetus</i>	0% (0/93)
<i>C. gracilis</i>	8% (7/93)
<i>C. helveticus</i>	5% (5/93)
<i>C. hyointestinalis</i>	6% (6/93)
<i>C. jejuni</i>	32% (30/93)
<i>C. lari</i>	87% (81/93)

Pathogen Type	Percent Positive (# of Samples)
<i>C. mucosalis</i>	0% (0/93)
<i>C. rectus</i>	0% (0/93)
<i>C. spurotum</i>	3% (3/93)
<i>C. upsaliensis</i>	0% (0/93)
French study (Brittany); 500 ml sample volume of river water using culture (2006) (Source: Denis et al., 2011)	
<i>Campylobacter</i> spp.	50% (30/60)
<i>C. jejuni</i>	33.3% (20/60)
<i>C. coli</i>	16.7% (10/60)
<i>C. lari</i>	5.0% (3/60)

2.2.6 Groundwaters

Campylobacter spp. are absent in groundwater not influenced by surface water, but many groundwaters may be contaminated during events, such as after flooding, hence the general need for disinfection. The majority of the *Campylobacter* outbreaks have been linked to relatively small groundwater works without adequate disinfection (Pitkänen, 2013; Guzman-Herrador et al., 2015). In some regions the occurrence of *Campylobacter* spp. in groundwater may be continuous as reported from wells in Canterbury, New Zealand, where *Campylobacter* spp. was detected in 12% (16/135) of the wells associated with intensive dairying combined with border strip irrigation, of which 11 samples out of 135 contained *C. jejuni* (8.1%) and the *Campylobacter* counts varied from <0.6 to 3.1 MPN/ L (Close et al., 2008). In another study from shallow groundwater aquifers in New Zealand, low counts of *Campylobacter* spp. were detected in 75% of samples (n=18; Savill et al., 2001).

2.2.7 Drinking waters

Drinking water is a common vehicle reported for *Campylobacter* outbreaks (Frost, 2001) that may affect thousands of individuals at the same time (Miller and Mandrell, 2005), but generally outbreaks result from smaller supplies and affect fewer people (Pitkänen, 2013; Guzman-Herrador et al., 2015). Waterborne *Campylobacter* outbreaks probably remain under-reported in many countries, due to the lack of specific notification system or testing. The majority of reported outbreaks have occurred in Nordic countries (Hänninen et al., 2003; Pitkänen, 2013; Guzman-Herrador et al., 2015).

The contribution of drinking water to the burden of sporadic *Campylobacter* infections may be significantly underestimated. It is particularly difficult to recognize the association with water when private wells and untreated

surface water is used as drinking water (Kapperud et al., 2003; Schönberg-Norio et al., 2004; Olson et al., 2008). For example, in Norway (Brennhovd et al., 1992) and Finland (Pitkänen et al., 2015), no *Campylobacter* spp. were recovered from water samples, but this is not always the case. Positive recoveries have been reported for Northern Ireland, 2.2% (2/91) drinking water from domestic household taps were PCR positive for *Campylobacter* spp. (Moore et al., 2001), and in New Zealand, 29.2% (7/24) of drinking water samples from centralized water supply were reported to be *Campylobacter* spp. positive (Savill et al., 2001). Also, 21% (5/24) of household taps fed from rainwater tanks in Southeast Queensland, Australia contained 5.0 to 1.0×10^2 *Campylobacter* cells/L when assayed as 16S rRNA gene copies (Ahmed et al., 2012). In all of these detections, however, the infection risk to the public is only of potential concern as the *Campylobacter* counts detected were presumably low, not necessarily viable cells nor genotypes of human-infective species, like *C. lari* (Savill et al., 2001).

2.2.8 Seawaters

C. jejuni has also been detected in 2% of the sea water samples examined in Greece (n=200) and represented the only *Campylobacter* species identified in the study (Arvanitidou et al., 1995). In Catalonia, Spain, *Campylobacter* spp. were absent from seawater samples (n=8) but *Campylobacter* spp. numbers below 5.0×10^2 MPN/L were detected in 78% of marsh water samples (n=9) from the same area (Rodriguez and Araujo, 2010). In Northern Ireland, *Campylobacter* spp. was absent from seawater samples (n=6) but were detected using a PCR method in 4.3% of the estuary lough water samples from protected bays (n=23; Moore et al., 2001). The presence of *Campylobacter* spp. is clearly sampling site dependent, as was seen in a Polish study where *Campylobacter* spp. were confirmed in 19.7% of the river water sampling sites nearby

the contamination sources, but only 5.6% (3/54) of the lagoon and none of the 20 marine bay water samples (Daczowska-Kozon and Brzostek-Nowakowska, 2001). The formation of VBNC cells in the saline environments may contribute to the low prevalence of *Campylobacter* spp. in seawater, when culture-based detection is used (Jackson et al., 2009; Gin and Goh, 2013).

2.2.9 Soil and sediments

Manure spreading operations are known to spread *Campylobacter* spp. to agricultural fields, and grazing livestock deposit *Campylobacter* spp. in their manure on pastures (Brown et al., 2004). The extent of wildlife feces in spreading *Campylobacter* spp. into terrestrial forest soils is less known.

Intertidal sediments are known reservoirs of *Campylobacter* spp. as reported from EU bathing sites in North West England where urease positive thermophilic campylobacters (UPTC) and *C. lari* were detected (Obiri-Danso et al., 2001).

2.2.10 Irrigation water and on crops

Campylobacter spp. are commonly present in natural water systems (rivers, lakes, ponds) and their presence needs to be considered when natural water is used for irrigation (for occurrence in environmental water see Section 2.2.5) (Banting et al., 2016; Gu et al., 2013).

Agricultural application of raw sludge or anaerobically digested biosolids to arable land has long been recognized as a *Campylobacter* source to horticultural produce (Koenraad, 1997; Park and Sanders, 1992).

2.2.11 Fish and shellfish

There are no reports about *Campylobacter* spp. in fish, but shellfish harvested from fecally contaminated waters frequently contain *Campylobacter* spp. (Wilson and Moore, 1996). However, in the *Campylobacter*-positive shellfish samples, most were other species than the common human pathogens (*C. jejuni* or *C. coli*), such as *C. lari* variants (Debruyne et al., 2009).

2.2.12 Air

Airborne transmission of campylobacteriosis is not considered significant for *Campylobacter* spp., which are oxygen intolerant bacteria causing infection through the fecal-oral route. However, flies are common carriers of *Campylobacter* spp. especially in conditions where sanitation and excreta disposal is underdeveloped (Khalil et al., 1994).

2.3 Persistence

Campylobacter spp. isolated from the environment are likely to have originated from fresh fecal material. *Campylobacter* spp. are not expected to regrow in the environment since they are considered unable to multiply outside the intestines of warm-blooded host animals and birds (Jones, 2001). Nonetheless, laboratory studies indicate the potential for *C. jejuni* to grow within soil/water amoebae (reviewed within Vaerewijck et al., 2014). *C. jejuni* survives at 4°C in moist, sun-sheltered environments (Bolton et al., 1987; Obiri-Danso and Jones, 1999; Kärenlampi and Hänninen, 2004), but is readily killed by temperatures reached in pasteurization, cooking, and frying (Habib et al., 2010). Under UK field conditions *Campylobacter* spp. survives in livestock manure slurries for 32 days, but only 2 to 4 days in solid manure heaps in temperatures greater than 55°C (Nicholson et al., 2005). Maximum survival periods following the land spreading of manure and slurry varied from four days to the more than 32 days with some indication of longer survival in clay loam grassland than in sandy arable soil (Nicholson et al., 2005). An *in vitro* study also showed that the survival of *C. coli* in swine manure was for at least 25 days at + 4°C but decreased to a few days at +22°C (Bui et al., 2011).

Table 5, Appendix A and Appendix B summarize the available *Campylobacter* spp. persistence data in different water matrices. Different survival characteristics of the *Campylobacter* spp. under environmental conditions may influence the diversity of different campylobacters (Korhonen and Martikainen 1991; Thomas et al., 1999b). For example, *C. lari* can survive better in aquatic environments than *C. jejuni* and *C. coli* (Obiri-Danso et al., 2001), and culturable *C. jejuni* survive longer than *C. coli* in lake water (Korhonen and Martikainen, 1991). Furthermore, *C. coli* and *C. upsaliensis* have demonstrated comparable survival characteristics (Thomas et al., 1999b).

Table 5. Persistence in manure, river water and seawater

Area	Bacterial Species	Matrix	Initial Number (CFU/ml)	Model Equation	T90 Days	Temperature (°C)	Other Experimental Conditions	Reference
New Zealand (Lincoln)	<i>C. jejuni</i> (mixture of strains)	River water	1.1E+06	$k_D^a(h^{-1}) = 0.028$	3.4	14	Dark, DO 8 to 9 ppm	
	<i>C. jejuni</i> (mixture of strains)	River water	1.1E+06	$k_S^b(m^2 MJ^{-1}) = 2.74,$ $ns^c=20.4$	0.07	14	Sunlight, S90 ^d =1.65 MJ m ⁻² , winter, DO 8 to 9 ppm	
	<i>C. jejuni</i> (mixture of strains)	River water	1.1E+06	$k_S^b(m^2 MJ^{-1}) = 1.93,$ $ns=no$ shoulder	0.03	14	Sunlight, S90=1.68 MJ m ⁻² , summer, DO 8 to 9 ppm	Sinton et al., 2007
	<i>C. jejuni</i> (mixture of strains)	Seawater	1.1E+06	$k_D^a(h^{-1}) = 0.066$	1.5	14	Dark, DO 8 to 9 ppm	
	<i>C. jejuni</i> (mixture of strains)	Seawater	1.1E+06	$k_S^b(m^2 MJ^{-1}) = 2.46,$ $ns=2.87$	0.05	14	Sunlight, S90=1.28 MJ m ⁻² , winter, DO 8 to 9 ppm	
	<i>C. jejuni</i> (mixture of strains)	Seawater	1.1E+06	$k_S^b(m^2 MJ^{-1}) = 3.99,$ $ns=13.3$	0.03	14	Sunlight, S90=1.38 MJ m ⁻² , summer, DO 8 to 9 ppm	
United Kingdom (Northern Ireland)	<i>C. jejuni</i> (B/86/4451 in pasteurized beef cattle slurry)	Manure slurry	1.0E+04	NA ^e	Not reached	4 17	pH 6.9 to 7.5	Kearney et al., 1993
United Kingdom (Northern Ireland)	<i>C. jejuni</i> (B/86/4451 in pasteurized beef cattle slurry)	Manure slurry	1.0E+05	NA ^f	Not reached ^g	35	Anaerobic digestion at 35, pH increase from 7.26 to 7.83 during 71 days.	Kearney et al., 1993

^a k_D : Dark inactivation coefficient; ^b k_S : Sunlight inactivation coefficient; ^c ns : Shoulder constant; ^dS90: Insolation needed to achieve a 90% reduction in CFUs; ^eNA: Not available, unchanged after 112 days at both temperatures; ^fNA: Not available, no reduction detected; ^gEstimate over 510 days (batch)/over 793 days (semi-continuous)

Campylobacter spp. do not carry many of the classic stress response mechanisms, but still employ a number of strategies enabling it to survive in the environment (Bronowski et al., 2014). However, the *Campylobacter* survival mechanisms in the environment are poorly understood and more data to predict the survival characteristics of *Campylobacter* spp. in water is needed (Gonzalez and Hänninen, 2012). The mechanisms may differ from those for most of the other enteric bacteria (Murphy et al., 2006). As with other enterics, *C. jejuni* enter into VBNC state under unfavorable conditions, however, what is different is that the size of cells is often reduced; with the shape of cells transformed from helical rods to cocci (Rollins and Colwell 1986; Jones 2001; Oliver 2005). In a recent study *C. jejuni* maintained the ability to adhere to intestinal cells after three weeks incubation in freshwater at 4°C indicating that the VBNC forms may retain their infectivity (Patrone et al., 2013). In artificial sea water at 4°C, *C. jejuni* strains survived in the VBNC form through a period lasting from 138 to 152 days (Baffone et

al., 2006). In drinking water at 4°C, the origin of the strain and the culture conditions were an important factor determining campylobacter survival (Cools et al., 2003).

The key factors affecting the survival of *Campylobacter* spp. in aquatic environments include temperature, light, biotic interactions and concentrations of oxygen and nutrients (Thomas et al., 1999b). The survival of campylobacters is favoured by low temperature and the absence of sunlight as shown in the inactivation rate studied conducted in Catalonia, Spain (Rodríguez and Araujo 2012). Not only were campylobacters more susceptible to high light intensities than other enteric bacteria, but they appear to be unable to repair damage to DNA (Jones 2001). It has been reported that low numbers of indigenous microbiota favor the culturable detection of stressed *Campylobacter* cells (Korhonen and Martikainen, 1991). However, the viability of *Campylobacter* spp. in water systems is favoured by biofilms (Svensson et al., 2008) and by free-living amoebae harboring bacteria intracellularly (Snelling et al., 2005). The presence of protozoan grazers such as *Acanthamoeba* spp. has been

reported to increase the survival of *C. jejuni* in vitro studies in water (Axelsson-Olsson et al., 2005).

3.0 Reductions by Sanitation Management

Overall, very little comparative data was available to identify surrogates for campylobacter inactivation by different sanitation management systems. Nonetheless, based on what is understood and summarized above (and in the chapter Using indicators to assess microbial treatment and disinfection efficacy), *E. coli*, enterococci and other fecal indicators may be reasonable surrogates. What is understood for campylobacters by sanitation systems is summarized below. In general, the absence of fecal indicator bacteria can be considered as a reliable proof of absence of *Campylobacter* spp. after the sanitation treatments. In oxygen-rich processes, *Campylobacter* spp. are presumably less persistent than fecal indicator bacteria. On the contrary, in anaerobic processes, the survival of *Campylobacter* spp. might be even better than fecal indicator bacteria (see section 3.1.5) and must be taken into consideration when evaluating the treatment efficiency.

3.1 Excreta and Wastewater Treatment

3.1.1 Onsite Sanitation

Campylobacter spp. might be present in effluents from on-site sand filters as shown by Kauppinen et al. (2014). The exact removal rates, however, remain unknown due to the semi-quantitative nature of the *Campylobacter* detection method and the inconsistent presence of *Campylobacter* spp. in the influent water.

3.1.1.1 Dry sanitation with inactivation by storage

Only little is known about *Campylobacter* spp. reductions by storage of human fecal material, but it is assumed survival would be similar as seen for animal manures. It is likely that *Campylobacter* survival in fecal material is dependent on available water (a_w), as reported in studies related to foodstuffs (Fernandez et al., 1985). While other food processing pathogens have a number of mechanisms to withstand stresses (Burgess et al., 2016), *Campylobacter* spp. are considered uniquely sensitive to a number of environmental conditions and are generally less able to tolerate environmental stress than other foodborne pathogens (Park, 2002).

In experiments with swine manure in Denmark, *C. coli* remained viable for up to 24 days at 4°C, based on both culture and RT-qPCR results (Bui et al., 2011). Survival in swine feces was dependent on storage temperature, surviving one week at 15°C but only a few hours at 42°C. Hence, Bui et al. (2011) suggests simply treated feces to temperature up to 42°C or even hotter for few hours before application on the agricultural soil to inactivate campylobacters; such as through solarization treatment (Simmons et al., 2013).

3.1.1.2 Pit latrines, vault toilets, dry toilets

Potential pathogen content of urine is low compared to feces (Höglund et al., 2002). For example, in a study of pathogens in source-diverted urine in South Africa, no *Campylobacter* was not detected in any of the urine samples (Bischel et al., 2015). Urine diversion enhances the dehydration of separately stored feces and thus, the a_w decrease in the fecal material may result in a higher reduction in *Campylobacter* spp. numbers in dry toilets using urine diversion compared to combined systems.

3.1.1.3 Composting

Temperature and oxygen-content are considered major factors influencing *Campylobacter* spp. inactivation during sludge and manure composting, yet only limited data on reductions are available. For example, in France, aerated composting containing thermophilic phase (50 to 65°C) reduced the *C. jejuni* gene copy numbers from approximate 1.0×10^5 copies/g level to non-detect (Wery et al., 2008). In a pen manure (20 to 50°C) in Australia, the numbers of *C. jejuni* gene copies declined below the detection limit within one week (Klein et al., 2011), but a windrow compost configuration was not able to inactivate culturable *C. coli* from the sewage sludge in Sweden (Sahlström et al., 2004), possible due to uneven heating and poor mixing during the composting process.

3.1.1.4 Other residuals (solids) management: with the intention of reuse as fertilizer for agriculture/food, etc.

The low infectious dose of *C. jejuni* and its demonstrated ability to survive in the top 5 cm of soils for at least 25 days at 10°C must be noted prior to any reuse of solids that contain fecal matter (Ross & Donnison, 2006). As a residual management strategies, the stockpiling of livestock bedding waste from animal pens to a secondary store, and storing them under conditions conducive for increased temperature is considered a simple and cost-effective treatment for rapidly lowering levels of zoonotic agents, including *Campylobacter* spp. in solid farm wastes (Hutchison et al., 2005).

3.1.2 Waste stabilization ponds

When Sheludchenko et al. (2016) studied fecal microbes in remote and rural maturation ponds in Australia, they reported baffled maturation ponds operated more effectively for removal of presumptive *Campylobacter* spp. than other waste stabilization pond systems; as you would expect based on improved residence time control and reduced turbidity. In clear waters, rapid sunlight inactivation or at least the loss of culturability of *C. jejuni* is possible as detected in a survival study where waste stabilization pond effluent in seawater and river water was tested in New Zealand (Sinton et al., 2007).

3.1.2.1 Aerated lagoons

The efficiency of aerated lagoons to remove

Campylobacter spp. remains unknown. Of other enteric bacteria in aerated wastewater lagoons, *Salmonella* reduction of approximately 0.91 log₁₀ and fecal coliform reduction of 1.4 log₁₀ has been reported in Tunisia (Ellouze et al., 2009). However, it is not known if these numbers are indicative for *Campylobacter* spp. reduction.

The VBNC state of *C. jejuni* cells, which are of a coccoid form, can be induced under oxygen-rich aerobic conditions (Oh et al., 2015). Thus, the ability of microaerophilic *Campylobacter* spp. to survive in aerated conditions may impact on *Campylobacter* spp. viability yet be missed if culture-based methods are used. However, the decimal reduction of *C. jejuni* HipO gene has been shown to be significantly faster in aerobic conditions as compared to anaerobic conditions in studies of thermophilic bio-waste sludge treatment in Austria (Wagner et al., 2009).

In addition to the oxygen content, also temperature affects *Campylobacter* culturability. This was seen for example in anaerobic swine manure lagoon samples in Mississippi, USA, where the rising lagoon water temperature was found to decrease viable *Campylobacter* counts (McLaughlin et al., 2012).

3.1.3 Wetlands

UK data demonstrated that wetlands constructed as horizontal reed beds may remove approximately 3 log₁₀ of

the *Campylobacter* spp. from poultry manure wastewater (Duggan et al., 2001). While sequential and continuous loading had little effect on *Campylobacter* removal, a rising water level seemed to leach *Campylobacter* from the reed beds back into the effluent (Duggan et al., 2001). In Germany, Alexandrino et al. (2007) used qualitative PCR assay targeting *C. jejuni*, but non-detects for treating primary sewage by vertical and horizontal subsurface flow constructed wetlands hampered their estimate of removals.

3.1.4 Wastewater treatment and resource recovery facilities

As a common cause of gastroenteritis worldwide, *Campylobacter* spp. are often present in high numbers in untreated sewage (Table 2). While wastewater treatment generally reduces the number of campylobacters, combined sewer overflows (CSO) release untreated sewage to the receiving surface waters. It has been reported that during and after heavy rainfall when CSO is discharged, the rivers become highly contaminated with *Campylobacter* spp. and other kinds of pathogens (Rechenburg and Kistemann, 2009).

3.1.4.1 Primary/preliminary treatment

The reported log₁₀ reduction of *Campylobacter* by primary treatment ranges from 0.7 to 1.4 (Table 6; Arimi et al., 1988; Höller, 1988).

Table 6. Wastewater treatment reductions of *Campylobacter*

Area	Bacterial Type	Treatment	Description of the Treatment Train	Concentrations Influent MPN ^a or CFU ^b /L	Concentrations Effluent MPN or CFU/L	Average Log ₁₀ Treatment Reduction	Reference
Australia (Queensland)	Presumptive <i>Campylobacter</i> spp.	Wastewater stabilization ponds	Maturation pond with baffles in the subtropical climate	7.0E+02 CFU	60 CFU	1.0	Sheludchenko et al., 2016
Australia (Queensland)	Presumptive <i>Campylobacter</i> spp.	Wastewater stabilization ponds	Maturation pond in the tropical climate	9.6E+04 CFU	8.0E+04 CFU	0.1	Sheludchenko et al., 2016
Finland (Siilinjärvi)	<i>Campylobacter</i> spp.	Primary flotation with disinfection	Peracetic acid disinfection PAA; 3 mg/L after dissolved air flotation	>5.0 CFU	<1.0 CFU	> 0.7 to 1.3	Pradhan et al., 2013
Germany(Kiel)	<i>Campylobacter</i> spp.	Primary treatment	8 to 27 °C	3.7E+04 CFU	1.5E+03 CFU	1.4	Höller 1988
Germany (Kiel)	<i>Campylobacter</i> spp.	Secondary treatment	Activated sludge (measured after primary treatment)	1.5E+03 CFU	2.0E+02 CFU	0.9	Höller 1988

Area	Bacterial Type	Treatment	Description of the Treatment Train	Concentrations Influent MPN ^a or CFU ^b /L	Concentrations Effluent MPN or CFU/L	Average Log ₁₀ Treatment Reduction	Reference
			Activated sludge 1 to 7 days retention			1.41 to 2.26	
Germany (North-Rhine Westphalia)	Campylobacter spp.	Secondary treatment	Activated sludge +trickling filters 1 to 7 days retention	1.1E+06 CFU	1.0E+03 CFU	3.01 to 3.51	Rechenburg and Kistemann, 2009
			Tertiary treatment (sand filtration) 1 day retention			2.81 to 2.90	
Germany (Weisse Elster)	Campylobacter spp.	Primary treated sewage	Oxidation pond	15 CFU	Non-detect	NR ^e	Stelzer and Jacob 1991
Germany (Weisse Elster)	Campylobacter spp.	Primary treatment	4 Emcher tanks, flow 1100 m ³ /day	5.1E+02 CFU	15 CFU	1.5	Stelzer and Jacob, 1991
			Grit separating and sedimentation tanks + activated sludge	1.1E+04 CFU	5.8E+02 CFU	1.3	
Germany (Weisse Elster)	Campylobacter spp.	Secondary	Final sedimentation	5.8E+02 CFU	1.3E+03 CFU	-0.2	Stelzer and Jacob, 1991
			Total reduction	1.1E+04 CFU	1.3E+03 CFU	0.9	
Italy (Bologna)	Thermophilic campylobacters	Secondary effluent Chlorine disinfection	2 ppm chlorine dioxide (2 mg/L)	40 CFU	Non-detect (<10 CFU)	>0.6	Stampi et al., 1993
Italy (Bologna)	Thermophilic campylobacters	Secondary	Oxygen based activated sludge	1.7E+04 CFU	50 CFU	2.4	Stampi et al. 1993
Italy (Bologna)	Thermophilic campylobacters	Secondary	Activated sludge Secondary air insufflation	1.7E+04 CFU	2.1E+02 CFU	2.0	Stampi et al. 1993
Italy (Bologna)	Thermophilic campylobacters (C. jejuni and C. coli)	Secondary effluent disinfection	3 ppm chlorine dioxide (3 mg/L), 15 min	3.0E+02 MPN	non-detect (<10 MPN)	>1.5	Stampi et al. 1992
Italy (Bologna)	Thermophilic campylobacters (C. jejuni and C. coli)	Secondary	Activated sludge	1.6E+04 MPN	3.0E+02 MPN	1.7	Stampi et al. 1992
The Netherlands (Gelderland)	Campylobacter spp.	Secondary	Activated Sludge 46,000 citizen equivalents	1.0E+04 MPN	1.0E+03 MPN	1.0	Koenraad et al 1994
The Netherlands (Gelderland)	Campylobacter spp.	Secondary	Trickling filter	Influent: 4.0E+03 MPN	NR	0.6	Koenraad et al 1994

Area	Bacterial Type	Treatment	Description of the Treatment Train	Concentrations Influent MPN ^a or CFU ^b /L	Concentrations Effluent MPN or CFU/L	Average Log ₁₀ Treatment Reduction	Reference
The Netherlands (Gelderland)	<i>Campylobacter</i> spp.	Secondary	Trickling filter + chlorination chlorine: 15 min 0.7 to 1.7 mg/L	2.0E+03 MPN	non-detect (<3.2 E+02 MPN)	>0.8	Koenraad et al 1994
United Kingdom (Reading)	Thermophilic campylobacters	Secondary	Trickling filter Measured after primary sedimentation 5 h retention	3.9E+04 MPN	1.7E+02 MPN	2.4	Arimi et al. 1988
United Kingdom (Reading)	Thermophilic campylobacters	Primary sedimentation	2 h retention	1.8E+05 MPN	3.9E+04 MPN	0.7	Arimi et al. 1988
United Kingdom (Northamptonshire)	<i>Campylobacter</i> spp.	Manure slurry	Constructed wetlands Experimental reed beds Manure Slurry	1E+03 to 1 E+07 MPN	1E+03 to 1 E+04 MPN	3.0	Duggan et al., 2001

^aMPN: Most probable number, ^bCFU: Colony forming units; ^cNR: Not reported.

3.1.4.2 Secondary treatment

Even though *Campylobacter* cells are reduced by aerobic, biological treatment of wastewater, these processes do not completely eliminate *Campylobacter* spp. (Koenraad et al., 1997).

3.1.4.2.1 Trickling filters

Trickling filters have been reported to reduce the *Campylobacter* counts (Table 6; Arimi et al., 1988; Rechenburg and Kistemann, 2009), yet in other studies, only minimal reduction has been reported (Table 6; Koenraad et al., 1994); thus large numbers of *Campylobacter* spp. might be released into the environment daily from sewage treatment plants if no other treatment is used.

3.1.4.2.2 Activated sludge

In studies from late 1980's, more than 0.8 log₁₀ reduction of culturable *Campylobacter* in secondary treatment (including the activated sludge process) were reported (Table 6; Höller, 1988; Stelzer and Jacob, 1991; Stampi et al., 1992; Koenraad et al., 1994).

More recent reports, often based on molecular detection of *Campylobacter* spp., show discrepancies in reduction and indicate the enhanced ability of this genus to persist and survive in different environments (as reviewed in Whiley et al., 2013). For example, Wéry et al. (2008) study in France using qPCR, concluded that *C. jejuni* VP1 gene survived better during the activated sludge treatment

compared to the *E. coli lacZ* gene. However, due to the lower copy number in the influent, they did not detect *C. jejuni* gene copies in the treated water while the *E. coli* genes were still quantifiable.

3.1.4.2.3 Oxidation ditch

No reports of *Campylobacter* spp. reduction by oxidation ditches were identified. It is presumable that the successful oxidation improves *Campylobacter* reduction. See discussion about the effect of oxygen-rich conditions on *Campylobacter* spp. viability in section 3.1.2.1 (aerated lagoons).

3.1.4.2.4 Membrane bioreactors

In studies reviewed by Hai et al. (2014), almost complete removal of bacteria by membrane bioreactors (MBR) was reported, whereas virus removal was inconsistent. Even though *Campylobacter* was not mentioned, it is presumable that a well-functioning MBR system is efficient in removing *Campylobacter*.

3.1.5 Biosolids/sewage sludge treatment/anaerobic digestion/anoxic digestion

Whiley et al. (2013) suggested in their review that oxygen intolerant *Campylobacter* spp. might survive even better than traditional fecal indicator bacteria during anaerobic digestion. Indeed, it has been observed that *C. jejuni* survives in a viable and culturable state during mesophilic anaerobic digestion (35°C) and the estimated time required for 1 log₁₀ reduction was 793 days under

those conditions (Kearney et al. 1993). Also in a study by Koenraad et al. (1994), campylobacters were isolated from 88% of secondary biosolids, with an average count 2.5×10^3 MPN/L (n=30). Horan et al. (2004), using a culture-based methodology, observed no inactivation of *C. jejuni* within 22 days at 35°C in a primary sludge digester and only 0.4 log₁₀ reduction was observed after the secondary sludge digestion at 15°C for 14 days. Hence, anaerobic digestion appears to be a relatively poor treatment approach to reduce campylobacter numbers.

However, treatment conditions have a major impact on *Campylobacter* spp. reductions. The absence of campylobacters was noted when biosolids are digested aerobically (Betaieb and Jones, 1990; Stelzer and Jacob, 1991) and many reports have indicated that culturable *Campylobacter* spp. in sludge are eliminated by digestion processes (Jones et al., 1990; Stampi et al., 1999). In a Swedish study in 2000 to 2001, thermophilic anaerobic digestion at 55°C, mesophilic anaerobic digestion at 34 to 42°C, composting and sedimentation all seem to remove *Campylobacter* spp. from sewage sludge (Sahlström et al., 2004). The sanitation of sludge using composting was also demonstrated in a study from France, where the thermophilic phase of composting was capable of reducing the *C. jejuni* VP1 gene copy numbers from 3.9×10^5 /g of compost wet weight to non-detectable (Wery et al., 2008).

After the digestion of dewatered biosolids, storage temperature plays an important role in further reducing *Campylobacter* spp. Ahmed and Sorensen (1995) reported more than 4 log₁₀ reduction of culturable *C. jejuni* counts within a day at 50°C while only 2 log₁₀ reduction was observed within 62 days at 5°C.

3.1.6 Tertiary treatment post secondary

When further removal of campylobacters is required to minimize human exposure, i.e. for wastewater reuse purposes, in close proximity of recreational areas or raw water intake sites for drinking water production (Levantesi et al., 2010), then various tertiary wastewater treatments options should be considered, as introduced below.

3.1.6.1 Lagooning

See 3.1.2.1.

3.1.6.2 Coagulation

The use of PIX (FeCl₃) and PAX (AlCl₃) coagulants was shown to efficiently reduce the microbial load in wastewater effluents in Finland (Pradhan et al., 2013). However, the reduction of *Campylobacter* spp. was not evaluated due to non-detects in the secondary treated influent.

3.1.6.3 Filtration

Slow sand filtration has long been used for drinking water purification. The removal efficiency of slow sand filtration has been studied at full-scale drinking waterworks

in The Netherlands, where 3.4 ± 0.6 log₁₀ reduction of *Campylobacter* spp. was reported (Hijnen et al., 2004). The culture counts of *Campylobacter* spp. may reduce in sand filtration more efficiently than *E. coli* counts (Hijnen et al., 2004). Sand filtration has more recently been used as a tertiary treatment option (Rechenburg and Kistemann, 2009), however, no campylobacter removal rates for this unit process for wastewater were identified.

3.1.6.3.1 Membranes

Microfiltration with a nominal porosity of 0.1 micron (meaning there are larger pores present but poorly defined) has a moderate efficacy in removing *Campylobacter* spp., while ultrafiltration and nanofiltration filters (with less than 0.05 micron pore sizes) as well as reverse osmosis are expected to provide very high removals of *Campylobacter* spp. (http://www.cdc.gov/healthywater/drinking/home-water-treatment/household_water_treatment.html, accessed 29th June 2016). For example in Belgium, the ultrafiltration permeate contained no fecal bacteria (Levantesi et al., 2010). The real concern is with absolute membrane removal processes is the day-to-day performance, or ability to maintain integrity (see chapter Sewered System Technologies).

3.1.6.3.2 Mono, dual and tri media

No research reports were identified for these types of media. However, it is expected that *Campylobacter* spp. reductions in mono, dual and tri media follow the patterns observed using fecal indicator bacteria, especially *E. coli*.

3.1.6.4 Land treatment

Managed aquifer recharge (MAR) with treated municipal wastewater is used for water storage and additional purification in water reuse processes. Levantesi et al. (2010) studied the counts of fecal indicators in MAR processes at three European sites. The aquifer characteristics and the quality of the injectant water both appear to affect the quality of recovered water. It is presumable that *Campylobacter* spp. are reduced while the water passes through the soil at least with the same efficiency as *E. coli*, but no data was identified to confirm this.

3.1.6.5 Other processes

None identified.

3.2 Disinfection as a Tertiary (or Post Primary) Treatment

3.2.1 Chlorine, combined etc.

Chemical disinfectants are applied at a concentration for a specified time to deliver what is known as the Ct (concentration x time), but many reports identified only provided concentration data. For example, tertiary

treatment (disinfection) of treated wastewater at 1 to 3 mg/L free chlorine was shown to reduce *Campylobacter* spp. counts below the culture method detection limit (Table 6; Stampi et al., 1992; Stampi et al., 1993; Koenraad et al., 1994). Based on the plate counting of bacteria, *C. jejuni* is generally considered more susceptible to chlorine than *E. coli* and thus the elimination of *E. coli* is considered adequate measure to confirm the absence of *C. jejuni* (Blaser et al., 1986). However, some caution is needed as it is well known that culture methods may seriously underestimate the *C. jejuni* (and *E. coli*) viable counts in chlorinated waters (Lehtola et al., 2006) and viable bacteria cells may remain inside protozoa under chlorine exposure (Snelling et al., 2005).

Alternative options to chlorine have been searched to fulfill the requirement of post-primary treatment disinfection without the hazards related to disinfection by-products. For example, peracetic acid disinfection (PAA; 3 mg/L) after dissolved air flotation treatment have been shown to reduce the culturable *Campylobacter* spp. counts in wastewater effluent to below the detection limit (Pradhan et al., 2013).

3.2.2 Ultraviolet

UV disinfection can be effective against all waterborne pathogens if turbidity is sufficiently low (Hijnen et al., 2006). *C. jejuni* is considered more sensitive to UV compared to *E. coli* and the culturable cells of *C. jejuni* can be easily removed from water using UV. In clear waters (in sterile distilled water) at room temperature and when the

occurrence of VBNC cells were not considered, a 3 log₁₀ *C. jejuni* reduction may be achieved with a fluence of 1.8 mJ cm⁻² (Butler et al., 1987). The estimated required fluence for 4 log₁₀ reduction of *Campylobacter* spp. is 14 mJ cm⁻² (Hijnen et al., 2006). However, lower quality of the secondary effluent turbidity may hamper the efficiency of tertiary UV treatment at wastewater purification plants. Synergistic effects of PAA or H₂O₂ induced radical formation together with UV have been studied to achieve higher disinfection efficiency with more turbid wastewaters (Koivunen and Tanski, 2005).

3.2.3 Natural processes

It has been reported in purified (distilled) water that about two minutes under conditions of strong natural sunlight is enough for 1 log₁₀ reduction of *C. jejuni* plate counts (Boyle et al., 2008). In sanitation applications, the true reductions of viable *Campylobacter* spp. are presumably somewhat lower mainly due to the occurrence of VBNC cells and differential light penetration properties in wastewater-impacted natural waters. In fresh surface water contaminated by sewage effluent, a 1 log₁₀ reduction in *C. jejuni* took more than 82 h in the dark at 14°C, while the same reduction was achieved in 1 to 2 hours in sunlight (Sinton et al., 2007).

In experimental desiccation conditions, *Campylobacter* strains remained viable for 2 to 10 h (Fernandez et al., 1985). Based on studies in feces on pasture, temperature and diffused oxygen, rather than desiccation increased the inactivation of *Campylobacter* spp. (Sinton et al., 2007).

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