CHAPTER

5

Campylobacter epidemiology—sources and routes of transmission for human infection

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5.1 INTRODUCTION

Following the routine introduction of simple culture techniques (Skirrow, 1977), it was rapidly established by the early 1980s that the thermophilic *Campylobacter* spp. (*Campylobacter jejuni* and its close relative, *Campylobacter coli*), were a significant cause of diarrhea in humans. Few countries at that time had surveillance of intestinal infectious disease (IID). However, IID had been monitored in England and Wales for many years, and accumulated data quickly determined that, even given the uptake of the new laboratory techniques, campylobacteriosis was increasing year on year, becoming a significant public health burden, with substantial costs. As a similar picture appeared in other industrialized countries, major research efforts were directed toward identifying the sources of infection, and the routes of transmission of human campylobacteriosis. Early epidemiological studies looking for reservoirs established that campylobacters were ubiquitous in the environment, and recoverable from most livestock and pets, as well as foods of animal origin. Nevertheless, these early studies suggested that the food production chain was the most likely source of human infections.

In this chapter, we will review the essential aspects of *Campylobacter* epidemiology, with special emphasis on the approaches used for source attribution of human

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campylobacteriosis, and on the contribution of potential reservoirs in the food production chain, and elsewhere, to the burden of the disease.

5.2 SOURCE ATTRIBUTION

The attribution of sources and routes of transmission of zoonotic pathogens is an important public health tool, incorporating a growing number of modern methodological approaches and data types. Quantitative estimates of the relative contributions of different sources to the human disease burden can inform risk managers on the priorities for intervention, and enable the implementation and measurement of the impact of interventions, in order to reduce human exposure. A detailed overview of definitions, terminology, and methodologies for source attribution has been presented elsewhere (Pires et al., 2009). Briefly, animals are usually defined as reservoirs or amplifying hosts; the environment and water as potential sources; food and direct contact with animals as examples of transmission routes; meat, dairy, drinking water, etc. as examples of exposures; and swimming in rivers, consumption, and handling of chicken meat, etc. as examples of risk factors. In practice, however, the term "source" is used to refer to any point across the transmission chain.

In the past decade, various approaches have been used to investigate the sources of human campylobacteriosis. Primarily, these approaches comprise epidemiological methods (e.g., analysis of outbreak investigations, and case-control/cohort studies), microbiological methods (e.g., microbial subtyping, and comparative assessment of exposure), or intervention studies, including "natural experiments." Early epidemiological studies for *Campylobacter* were hampered by the ubiquitous distribution of the organism, and the lack of harmonized and stable strain tracing methods. Even today, when genomic analysis is almost routine, the plasticity of the *Campylobacter* genome prevents the timely tracing of specific strains through zoonotic transmission routes. Thus, our current knowledge is founded on the accumulated data from epidemiological, microbiological and intervention studies.

5.2.1 EPIDEMIOLOGICAL METHODS FOR SOURCE ATTRIBUTION

Given the sporadic nature of *Campylobacter* infections in humans (Kapperud et al., 2003), source attribution based on outbreak investigations has had limited value. This is largely because, unlike for salmonellosis (Wagenaar et al., 2013), campylobacteriosis outbreaks are rarely reported. However, they may be more frequent than initially thought (EFSA, 2010c). Outbreak data in Europe during 2005–06 have been analyzed to infer the likely sources (Pires et al., 2010). Although most outbreaks (~64%) were not attributable to known sources, ~12% were attributed to meat products in general, and ~10% specifically to chicken meat. At the individual case level within these outbreaks, about one-third (36%) remained unattributed, but most (~44%) cases were attributed to foreign travel, 17% to contaminated water, and 10% to chicken meat. Therefore, on the basis of outbreak data, chicken meat was the major foodborne source.

Case-control studies have been the most successful epidemiological approach to identify the sources attributable to sporadic cases of campylobacteriosis. Such studies have been conducted in various countries, and a metaanalysis of the data indicates that the handling and consumption of chicken meat is a major risk factor for human campylobacteriosis (Domingues et al., 2012). Other commonly identified risk factors include the consumption of unpasteurized dairy products, eating at restaurants, direct contact with dogs, especially puppies, and with livestock, as well as foreign travel (Studahl and Andersson, 2000; Mughini Gras et al., 2012, 2013; Stafford et al., 2007; Doorduyn et al., 2010; Friedman et al., 2004; Gallay et al., 2008; Danis et al., 2009; Neal and Slack, 1997). From such extensive case-control studies it is generally considered that 20–30% of campylobacteriosis cases are attributable to the handling, preparation, and consumption of chicken meat (EFSA, 2010c).

Case-control studies can be hampered by a number of factors, including acquired immunity. It is well recognized that repeated exposures (at low doses) to pathogens, such as *Campylobacter*, can lead to the development of specific immunity, sufficient to provide protection against clinically overt disease (Swift and Hunter, 2004), but not necessarily against colonization (Havelaar et al., 2009). This effect may explain why campylobacteriosis is rare in adults in the developing world (given later), and that the regular consumption of chicken (at home) is sometimes identified as a protective, rather than a risk, factor (Friedman et al., 2004).

Treatment with gastric antacids, such as proton-pump inhibitors (PPIs), may also affect case-control studies (Mughini Gras et al., 2012; Doorduyn et al., 2010; Tam et al., 2009). PPIs reduce gastric acidity (increased pH) that could favor the survival of *Campylobacter* during passage through the stomach. Alternatively, a damaged gut lining could predispose individuals toward disease (Neal and Slack, 1997). In the Netherlands, the increase in campylobacteriosis observed during 2003–11 correlated with the number of prescriptions for PPIs in the population (Bouwknegt et al., 2014).

5.2.2 MICROBIAL SUBTYPING METHODS FOR SOURCE ATTRIBUTION

Currently, microbial subtyping is the most common approach for source attribution of human campylobacteriosis. *Campylobacter* strains are phenotypically and genotypically highly diverse, and this diversity can be exploited to develop subtyping strategies, with the aim of tracing the organism back to its reservoir. Unfortunately, this approach has been constrained by the high plasticity of the *Campylobacter* genome, and the frequency of horizontal transfer of genetic material between strains, a fact that generates instability in both phenotype and genotype. For many years, these constraints have prevented direct source attribution using serotyping, phage typing, and the simple molecular typing techniques, previously applied to other enteric pathogens.

In 2001, a standardized multilocus sequence typing (MLST) scheme was developed for *C. jejuni* (Dingle et al., 2001). The MLST approach was initially developed to investigate the evolutionary structure of biological populations, but was subsequently applied to source attribution of some zoonotic pathogens. For *Campylobacter,*

MLST depends on the genetic sequence of, usually, seven house-keeping genes. Because of their functions, such genes are highly conserved, but their sequence can vary with time and evolutionary selection pressures. The application of MLST to large *Campylobacter* strain collections has indicated that some sequence types (STs) are associated with specific reservoirs, in particular with poultry and cattle, and even certain environments. By exploiting these associations through the use of stochastic models, it is possible to infer, with some statistical certainty, the origin of those *Campylobacter* strains derived from human cases (Mughini Gras et al., 2012; McCarthy et al., 2007; Wilson et al., 2008; Mullner et al., 2009b; Sheppard et al., 2009; Strachan et al., 2009).

In 2010, MLST-based source attribution models estimated that 50–80% of *Campylobacter* strains infecting humans originate from chickens, 20–30% from cattle, and the rest from other reservoirs (sheep, pigs, and wild animals) (EFSA, 2010c). Similar conclusions have since been drawn from studies worldwide, and the power of using of such approaches for risk management has been exemplified in New Zealand (Muellner et al., 2011). It is interesting to note that source attribution studies in the Netherlands and Luxembourg indicate that both *C. jejuni* and *C. coli* strains isolated from humans are mainly attributable to chicken. However, the proportion of human *C. coli* infections attributed to pigs is far higher than that of *C. jejuni*, whereas the opposite is true for strains attributed to cattle (Mughini Gras et al., 2012; Mossong et al., 2016).

Although MLST has become the most important tool for generating microbial data for source attribution, like other subtyping techniques, it has shortcomings—including the high costs of sampling, isolation, and typing. In addition, the degree of certainty of source attribution from such data is questionable. Many of the strain collections investigated have skewed populations, largely as a result of limited sampling, and poor recovery. Moreover, a significant proportion of *Campylobacter* strains fall into several very large STs (such as ST-21, ST-45, and ST-828) that have a weak host association, thereby reducing the strength of the case for source attribution (Dearlove et al., 2016). As a result, the use of whole genome sequences is increasingly recommended, with even greater cost and, therefore, strain selectivity. The enormous amount of data generated from such approaches makes it increasingly difficult for nonspecialist epidemiologists to analyze and interpret the information in a meaningful way, and apply this information for control and prevention.

5.2.3 COMBINING BOTH EPIDEMIOLOGICAL AND MICROBIAL METHODS

It is known that case-control studies alone do not suffice to attribute human cases to reservoirs (i.e., amplifying hosts) because they can only trace back the sources of human infections up to the point of exposure (e.g., food items consumed, contact with animals, etc.) that may not correspond to the original reservoirs because of, for instance, crosscontamination, or alternative transmission routes (EFSA, 2010c). To overcome this limitation, the MLST of strains isolated during targeted epidemiological studies

has been undertaken (Mughini Gras et al., 2012; Mullner et al., 2009a; Mossong et al., 2016). This combined approach suggests that transmission routes other than foodborne are also important. For example, in a Dutch investigation using strains isolated from a case-control study, infections caused by chicken-associated strains are not only associated with the consumption and handling of chicken meat, but also with contact with individuals suffering from gastrointestinal symptoms (Mughini Gras et al., 2012). This suggests that person-to-person spread of these strains is more frequent than previously thought. Also, ruminant-associated strains are linked not only to the consumption of tripe and barbecued meat, but also to occupational exposure and rural living, suggesting that direct/indirect environmental contact with these animals is important. The consumption of game meat and the use of swimming pools were also significant risk factors for infection with *Campylobacter* strains originating from the environment, especially during springtime. It seems likely that, as more such combined studies are undertaken, the nonmeat-foodborne aspects of campylobacteriosis will become clearer.

5.2.4 NATURAL EXPERIMENTS AND INTERVENTIONS FOR SOURCE ATTRIBUTION

There have been several "epidemiological events" involving poultry that have served as natural experiments of the effect of a major and sudden reduction of the exposure to *Campylobacter* in the human population. In 1999, the crisis following the finding of dioxin in animal feed in Belgium led to the national withdrawal of various poultry products intended for human consumption. Subsequently, there was a drastic reduction in the nationwide consumption of chicken meat, a fact that was associated with a concurrent drop of 40% in campylobacteriosis across Belgium (Vellinga and Van Loock, 2002). Similarly, in 2003, an epidemic of avian influenza (H7N7) hit the Netherlands. To control this epidemic, massive bird culling measures were implemented (~30 million birds culled), and a number of poultry slaughterhouses were closed (Stegeman et al., 2004). The epidemic was associated with simultaneous declines in campylobacteriosis locally and nationally (50 and 30%, respectively) (Friesema et al., 2012). Although sales of poultry meat declined throughout the Netherlands by ~9% during this period, this alone was considered insufficient to account for the reduction in campylobacteriosis. Moreover, this reduction continued far beyond the recovery in poultry meat sales. Overall, the analysis of this natural experiment has suggested that a significant proportion of the observed public health benefit resulted from the reduction in the environmental burden of campylobacters originating from poultry.

Unfortunately, such natural experiments only allow for the retrospective observation of effects. The implementation of national intervention programs to reduce *Campylobacter* on poultry meat, however, allow prospective opportunities to study the effect of reduced exposure to poultry-associated campylobacters. For instance, following interventions in Iceland and New Zealand, the total number of campylobacteriosis cases decreased by 72% (Stern et al., 2003) and 54% (Sears et al., 2011),

respectively. In New Zealand, MLST-based source attribution analyses showed that this reduction was largely due to a fall in the cases of poultry-associated campylobacteriosis (Muellner et al., 2011).

5.2.5 TRANSMISSION ROUTES FOR POULTRY-ASSOCIATED CAMPYLOBACTERS NOT INVOLVING THE HANDLING AND CONSUMPTION OF POULTRY MEAT

By 2010, it was apparent that there was a significant discrepancy between the attribution of the handling and consumption of poultry meat as a source of human campylobacteriosis based on case-control studies (20–40%), and that of the chicken reservoir as a whole (50–80%) based on MLST (EFSA, 2010c). This observation has been more recently supported by the analysis of the avian influenza outbreak in the Netherlands (given earlier), and by combining molecular and epidemiological approaches to source attribution (Mughini Gras et al., 2012). Overall, current opinion accepts that, although poultry are the major livestock reservoir for campylobacteriosis, there are various routes of transmission to humans, and the handling and consumption of poultry meat may not be the cause of the majority of such infections.

5.3 FOODBORNE SOURCES OF CAMPYLOBACTERIOSIS

5.3.1 *CAMPYLOBACTER* IN CHICKEN AND OTHER POULTRY

Although the relative importance of poultry meat as the primary cause of campylobacteriosis may be debatable, the importance of chicken as a major reservoir of infection is indisputable. Reflecting this, most public health activities aimed at the reduction of campylobacteriosis have focused on poultry production.

C. jejuni and *C. coli* appear to have evolved to preferentially colonize the avian gut. Most domestic poultry reared for consumption or egg production, including chicken, turkey, geese, ducks, pigeons, and even ostriches, as well as wild birds, are frequently colonized with these *Campylobacter* species. This colonization appears to be asymptomatic, except perhaps in young ostriches, and thus has no obvious cost implication for farmers. The epidemiology of *C. jejuni/coli* colonization in broilers has been comprehensively reviewed elsewhere (Wagenaar et al., 2008). Therefore, only references that contribute additional information will be indicated in this section.

5.3.1.1 Campylobacter *in live broiler chicken*

The most significant data on *Campylobacter* colonization of poultry has been derived from the conventional broiler industry, because this sector has been easily investigated, and is the largest of the poultry industry. Over the past three decades, many studies throughout the industrialized world have reported on the prevalence of broiler colonization with *Campylobacter*, and a huge variation has been observed. However, comparisons between studies and countries are hampered by differences in sampling,

culture methodology, and flock management factors. In 2008, a European Union baseline survey of *Campylobacter* colonization of broiler flocks was undertaken, using standardized sampling procedures and detection methods on 10,132 batches of broilers from 561 slaughterhouses, in 28 European countries (EFSA, 2010a). The average prevalence of *Campylobacter*-positive broiler batches was 71.2%, but this varied between countries from 2% to 100%, with the lowest levels being reported in Scandinavian countries. This European study remains the most comprehensive to date, and confirms that each country, and maybe even geographical region, needs to establish their own baseline prevalence before undertaking any intervention.

There are several interesting common features in the outcomes of broiler epidemiological studies. First, prevalence is directly related to the age of the flock. It is now generally accepted that campylobacters are rarely, if ever, vertically transmitted, so that chicks are hatched *Campylobacter*-free. Positive flocks are rarely detected until 2–3 weeks of age. The reason for this is unclear, but maternal immunity (Cawthraw and Newell, 2010) and/or lack of exposure are likely explanations. All birds in a flock rapidly become colonized following the first detection. Experimental challenges with fresh chicken strains show that the dose for successful chicken colonization can be very low (less than 10 cfu), and colonization levels in the cecum increase to over 10^9 cfu/g of cecal contents within 3 days. Coprophagy and feed/water contamination ensure rapid spread of the organism, so that the with-flock prevalence is about 100% within days of the first detection. Effective bird-to-bird transmission is also enhanced by bacterial adaptation to efficient colonization of the chicken gut.

Flock prevalence is also seasonal, with a summer peak that is also geographically distributed, with countries of high latitudes, that is, Scandinavia, having a peak later in the season (EFSA, 2010b). Explanations for these effects include weather patterns (i.e., rainfall and sun/UV levels), allowing/preventing *Campylobacter* survival in the environment, and fly seasons enabling *Campylobacter* transmission from the environment. Flock management systems, such as levels of containment and biosecurity, certainly have an effect on prevalence (Newell et al., 2011). Some farming practices can significantly increase the horizontal transmission of campylobacters from the environment into the flock. Factors such as thinning (the structured reduction of bird numbers prior to flock harvesting), multilivestock farming, and the extensive rearing of flocks can all increase the risk of flock positivity. *C. jejuni* is the most prevalent species recovered from broilers, but *C. coli* prevalence may increase in older birds. Most intensively reared flocks are colonized with a limited number of strains. Frequently, only a single strain can be detected, suggesting limited exposure, competitive exclusion and/or differences in strain colonization potential. Finally, there is increasing evidence that the genetic lineage of broilers can affect susceptibility to colonization with *Campylobacter* (Psifidi et al., 2016), though this has yet to be confirmed in the field situation.

5.3.1.2 Campylobacter *in other birds*

As indicated previously, *C. jejuni/coli*, appear to have a preference for the ecological niche provided by the avian gut. It is not surprising, therefore, that all other poultry

can be colonized, including turkeys, ducks, geese, guinea fowl, ostrich, and pigeon, as well as game birds and caged pet birds. The rearing and management of such birds in intensive or enclosed conditions may contribute to bird-to-bird transmission. The issue of wild birds will be addressed later.

5.3.1.3 Campylobacter *in poultry meat*

During processing, campylobacters that have been colonizing the chicken gut can contaminate the carcass surface to a variable extent, but frequently at levels of over 1×10^6 cfu per carcass (EFSA, 2010a). Quantitative risk assessment models indicate that higher levels of contamination generate the greatest risk to consumers (EFSA, 2010c). This contamination arises from fecal leakage, gut tissue damage, or feathers soiled during rearing or transport to the abattoir (reviewed by Jacobs-Reitsma and Lyhs, 2008). Several stages of processing at the abattoir contribute to this contamination, including scolding, defeathering, and evisceration. Although the risk of carcass contamination is higher from colonized birds, processing also contaminates the abattoir equipment, potentially transferring organisms, usually at low numbers, onto the carcasses of uncolonized birds subsequently entering the processing line. Surveys of contaminated broiler carcasses have been undertaken at the abattoir, after processing, and at retail. Comparison of the data from such surveys is confounded by unstandardized approaches to sampling and culture. Analysis of the 2008 baseline survey undertaken in Europe (EFSA, 2010b) clearly showed that levels of contamination varied between countries, and between slaughterhouses.

Because of their fastidious nature, high numbers of campylobacters contaminating poultry carcasses do not multiply, but can survive through to retail, and constitute a risk to customers from handling and crosscontamination in the kitchen. *C. jejuni/coli* are fragile organisms, highly susceptible to the toxic effects of atmospheric oxygen, dehydration, temperature, and many chemicals, so the numbers of organisms surviving on carcasses to retail is highly dependent on the initial level of contamination, and the postprocessing methods employed. Washing in appropriately chlorinated water, or water containing other decontaminating chemicals, such as organic acids, significantly reduce the surface contamination levels, and can be used for public health interventions. However, regulations for the postprocessing chemical treatment of poultry meats vary between countries. Freezing and air-drying can reduce levels on the carcass by factors of several hundredfolds. However, survival is enhanced if the organisms are located within the feather follicles. Unfortunately, the poultry industry approach, which increasingly reduces the time between slaughter and retail, and introduces storage and packaging methods aimed at improving meat shelf life (cool, dark, and moist conditions wrapped in plastic largely excluding the normal atmosphere), enables long term survival of *Campylobacter* on poultry meat, ensuring the viability of the organism at the point of sale. It should be also noted that the surface of poultry meat packaging can itself be contaminated with campylobacters (Harrison et al., 2001), constituting a risk to customers at the retail shelf level.

The greatest proportion of campylobacters are located on the poultry skin or carcass surface, and easily destroyed by cooking. Organisms can be detected in poultry

muscle, but the levels are generally low. However, chicken liver can be highly contaminated (Jacobs-Reitsma and Lyhs, 2008), and constitute a particular risk if consumed undercooked.

5.3.1.4 *Campylobacter* in laying hens and eggs

Like other commercial breeds of *Gallus gallus*, table-egg laying hens are frequently colonized with campylobacters. Viable organisms are recoverable from the oviducts of laying hens, and egg shells can be contaminated, presumably from feces, but culturable campylobacters have been rarely recovered from egg contents (Wagenaar et al., 2008), suggesting that eggs are rarely, if ever, a source of campylobacteriosis. This is consistent with outbreak data, the age-related flock colonization data, and the lack of colonization in experimental control groups of chicken.

5.3.2 *CAMPYLOBACTER* IN RUMINANTS

Source attribution studies indicate that domestic ruminants (cattle, sheep, and goats) are the second most important reservoir of *C. jejuni/coli* strains, causing up to 33–38% of human campylobacteriosis cases (Jonas et al., 2015; Mossong et al., 2016; Sheppard et al., 2009).

Campylobacter-colonized ruminants pose a risk through the consumption of contaminated meat, offal, and dairy products and, surprisingly, studies in Luxembourg (Mossong et al., 2016) and the Netherlands (Doorduyn et al., 2010) report that consuming beef (both in and out of the home) is a particular risk factor for *C. coli* infection. However, the main risk of campylobacteriosis from ruminants seems to be through environmental contamination, which will be discussed later.

5.3.2.1 *Campylobacter* in live ruminants

C. jejuni/coli gastrointestinal tract carriage is usually asymptomatic in ruminants. However, both species may cause abortion in sheep and cows. Many prevalence surveys have been undertaken. The absence of standardized approaches to sampling strategy and detection approaches precludes direct comparison of data. However, in Finland and the United Kingdom, structured surveys of fecal samples from cattle at slaughter reported that 31.1 and 54.6% of animals, respectively, carried *Campylobacter* spp. (Hakkinen et al., 2007; Milnes et al., 2008). For small ruminants (sheep and goats), a structured survey undertaken at slaughter in the United Kingdom reported that 43.8% of sheep carried thermophilic campylobacters (Milnes et al., 2008), a fact that was significantly different from carriage in cattle, and is similar to data (32.8%) from sheep and goats at slaughter in Greece (Lazou et al., 2014). However, carriage on farms may be lower for both cattle and sheep (22 and 25%, respectively) than at the abattoir (Rotariu et al., 2009), perhaps reflecting the effect of stress of transport and lairage on the gut microbiome. Several studies have indicated that carriage is generally higher in calves than older animals (Sasaki et al., 2013; Sato et al., 2004; Nielsen, 2002), and may be slightly lower in dairy cows (Johnsen et al., 2006; Merialdi et al., 2015). However, fecal shedding is intermittent (Jones et al., 1999; Stanley et al., 1998a,b), and may be increased by management factors

such as holding animals in feedlots (Besser et al., 2005). In cattle and small ruminants, the reports of the ratio of *C. jejuni* to *C. coli* recovered vary widely. These differences are probably a reflection of many factors, but especially the sample handling, the isolation, culture and detection methods used, and possibly the age of the animals. In the structured national survey of Milnes et al. (2008), 81% of cattle and 65% of sheep isolates were *C. jejuni*, and the remainder primarily *C. coli*.

The application of manure with broadcast spreaders, indoor housing, feed composition, herd size, private water supply, presence of horses in the farm, and the accessibility of feed to birds, have all been identified as significant risk factors for *C. jejuni* carriage in dairy cattle (Ellis-Iversen et al., 2009; Wesley et al., 2000), while a high number of female animals on the farm has been identified as a risk factor in beef cattle (Hoar et al., 2001).

There are generally two seasonal peaks in *Campylobacter* shedding in cattle: one in late spring, and the other in late autumn, with some variation among countries (Hakkinen and Hänninen, 2009; Milnes et al., 2008; Sato et al., 2004; Stanley et al., 1998a). These periods roughly coincide with traditional milk flushes and calving periods, as well as with spring transition from winter housing to summer grazing, and the autumn return to winter housing, suggesting that shedding patterns might reflect either hormonal and stress influences to the gut flora, or changes in diet and water sources. However, nowadays most cattle farmers in developed countries calve all year round, so the association of the seasonal peaks with calving requires further clarification (Merialdi et al., 2015). In small ruminants, the pattern of *Campylobacter* shedding is better defined (Jones et al., 1999), at least in the United Kingdom, with a seasonal low in November–December, when sheep are fed on hay and silage, rather than grazing, and a seasonal high coincident with lambing, weaning, and movement onto new pasture.

5.3.2.2 Campylobacter *in foodstuffs of ruminant origin*

As indicated earlier, milk and dairy products are frequently implicated as vehicles of human campylobacteriosis outbreaks. It is generally assumed that this is the result of fecal contamination, but outbreaks have been occasionally traced back to asymptomatic *C. jejuni* mastitis in dairy cattle (Orr et al., 1995). Effective conventional pasteurization kills campylobacters, but raw or incompletely pasteurized milk may contain viable organisms (Fernandes et al., 2015). In developed countries, contamination in bulk-tank milk is usually low, below 1% (Hill et al., 2012; Muehlherr et al., 2003; Ruusunen et al., 2013), but can be as high as 12% in some instances (Bianchini et al., 2014).

Fecal contamination of carcasses can occur during evisceration, removal of the hide, or because of crosscontamination within the abattoir. However, the prevalence of campylobacters on beef at retail is generally low, for example, 4.5% in the United Kingdom (Little et al., 2008), 3.2% in Ireland (Whyte et al., 2004), and 3.5% in New Zealand (Wong et al., 2007). In the same surveys, slightly higher prevalences were found for lamb/mutton at 12.6, 11.8, and 6.9%, respectively. However, *Campylobacter* contamination of ruminant offal, in particular liver, is substantially higher

(Little et al., 2008). The lower *Campylobacter* contamination of red, compared with poultry, meats is assumed to reflect the wetter conditions during poultry processing that would enable bacterial survival on product surfaces.

5.3.3 *CAMPYLOBACTER* IN PIGS AND PORK PRODUCTS

Only about 0.4% of all human campylobacteriosis cases can be attributed to pigs (Mossong et al., 2016), but for *C. coli* infections this contribution increases to 4.4–6% (Sheppard et al., 2009; Mossong et al., 2016). Since pigs are rarely considered an important reservoir for campylobacteriosis, there are few initiatives to control *Campylobacter* in the pork production chain. However, in some countries the high antimicrobial resistance observed in strains from pigs is considered a particular risk to humans (Quintana-Hayashi and Thakur, 2012). Only under exceptional conditions, such as production in areas remote from other pig producing facilities, might *Campylobacter*-free pork production be feasible (Kolstoe et al., 2015).

5.3.3.1 Campylobacter *in the live pig*

Few large surveys on the prevalence of *Campylobacter* in live pigs have been undertaken, but based on cultured rectal samples at the farm or abattoir, about 38.1–63% of pigs carry these organisms (Nathues et al., 2013; Carrique-Mas et al., 2014; Milnes et al., 2008). *Campylobacter* colonization in pigs is asymptomatic. The concentration of *Campylobacter* in porcine fecal samples is up to 1.2×10^7 cfu/g (Abley et al., 2012). In most studies, the vast majority of these bacteria (>90%) are *C. coli*, and the remainder being *C. jejuni* (Milnes et al., 2008; Quintana-Hayashi and Thakur, 2012), so it seems that the porcine gut is a preferred niche for this species. However, one study from Vietnam found *C. jejuni* as the predominant species in pigs (Carrique-Mas et al., 2014). Whether this reflects a geographical, or methodological, difference is unknown. Little is currently known about the risk factors for *Campylobacter* colonization in pigs.

5.3.3.2 Campylobacter *in foodstuffs of porcine origin*

There have been several surveys of *Campylobacter* in pork products, but the approaches and methodologies vary significantly. The European Food Safety Authority (EFSA) annually collects any data submitted from Member States on food contamination. In 2014, it was reported that 6–37% of pig carcasses (from Belgium and Poland, respectively), 2.6–6.8% of meat samples at the processing plant (from Hungary and Portugal, respectively), and 0.27–2% of pork meat at retail (from the Netherlands and Spain, respectively) were *Campylobacter*-positive (EFSA and ECDC, 2015). In surveys from four European countries (United Kingdom, Belgium, Poland, and Italy), the prevalences in pork (meat and chops) at retail was 5.0, 5.0–16.6, 10.6, and 5.7%, respectively (Little et al., 2008; Mattheus et al., 2012; Korsak et al., 2015; Sammarco et al., 2010), but the prevalence was higher in pork offal (Little et al., 2008). Interestingly, the Polish study reported *C. jejuni* as the most prevalent species on pork chops. In the USA, *Campylobacter* prevalence on pork chops was reported to be only 0.3–2% (Noormohamed and Fakhr, 2013; Zhao et al., 2010).

5.3.4 *CAMPYLOBACTER* IN READY-TO-EAT FOODS

Some case-control studies have indicated that ready-to-eat (RTE) foods, such as takeaways, pose a risk for campylobacteriosis (Rodrigues et al., 2000). Most investigations of RTE foods at retail in Europe have failed to find *Campylobacter* spp. (Moore et al., 2002; Meldrum and Ribeiro, 2003). However, a few surveys have identified or recovered campylobacters from cooked meats (Maćkiw et al., 2011; Elson et al., 2004), but at a prevalence of >1%. The likelihood is that crosscontamination of such foods occurs postcooking, rather than survival of the organisms after cooking. Such crosscontamination can easily occur in a kitchen (Luber et al., 2006), and risk managers actively communicate the risks of handling raw and cooked meats together.

Perhaps of more concern is the detection of campylobacters at retail in vegetables produced to be potentially eaten raw. For example, in the Netherlands, 0.83% of endive and 2.7% of oak tree lettuce samples were positive (Wijnands et al., 2014). Campylobacters have also been isolated from leafy greens (Ceuppens et al., 2015), ulam (Khalid et al., 2015), and lettuce (de Carvalho et al., 2013), and have been shown to survive for at least a day and up to 8 days on various fruits and vegetables (Kärenlampi and Hänninen, 2004). An outbreak of campylobacteriosis associated with the consumption of raw peas has been described (Gardner et al., 2011). In some investigations, the eating of raw fruit and vegetables are risk factors for campylobacteriosis (Fullerton et al., 2007; Verhoeff-Bakkenes et al., 2011), although in others (Kapperud et al., 2003; Mughini Gras et al., 2012) their consumption is protective.

The use of irrigation water contaminated by feces from livestock is an obvious candidate source. Another potential transmission route would be the direct contamination of the produce by feces from wild birds. This is supported by the subtyping of isolates from the raw pea outbreak (Gardner et al., 2011). Also, *C. lari* can be one of the contaminating *Campylobacter* species (Losio et al., 2015), and such strains are frequent colonizers of wild birds.

5.4 NONFOODBORNE SOURCES OF CAMPYLOBACTERIOSIS

Source attribution studies have estimated that, relative to poultry, ruminants, and pigs, the general environment may account for up to 5–10% of human campylobacteriosis cases (Mughini Gras et al., 2012; Mossong et al., 2016). Such environmental contribution would comprise all surface water (lakes, rivers, puddles, etc.), soils and air, as well as pets, wildlife, and livestock other than poultry, ruminants, and pigs. The range of environmental sites contaminated with campylobacters has recently been reviewed (Whiley et al., 2013). However, the extent of such contamination has yet to be established, and it should be kept in mind that fecal waste, deposited in the environment, can also be widely disseminated by rain water, wind, animal movement, and flying insects to generate further indirect contact routes. Since *Campylobacter* cannot grow naturally outside the gut of a warm-blooded animal, the general environment can only serve as a vehicle for transmission, and not as an amplification niche. Moreover, the fragility of *Campylobacter* means that survival in the environment will be dependent

on exogenous factors, such as temperature, sunlight, and oxygen exposure, absence of moisture, etc. Therefore, it seems reasonable to assume that airborne infection of humans, at least at any distance remote from heavy contamination sources like broiler houses, is unlikely, but that water and soils are likely to be contaminated.

As indicated previously, the level of shedding of *Campylobacter* from colonized livestock depends on a number of factors, including age, stress, changes in diet, and housing conditions, as well as with season, often with a summer peak. Human exposure to potential environmental sources, for example, swimming in a domestic pool, is largely weather-dependent, so not surprisingly the risk of infection with environment-associated strains is also seasonal (Mughini Gras et al., 2012). However, there is also a risk associated with living in a rural environment, especially in young children (Strachan et al., 2009), who might be exposed to a wider range of strains than urban dwellers.

5.4.1 *CAMPYLOBACTER* IN DOGS, CATS, AND HORSES

Up to 87% of dogs are colonized with *Campylobacter* species (see review by Marks et al., 2011). The most prevalent species in dogs is *C. upsaliensis* (Carbonero et al., 2012; Acke et al., 2009). However, *C. jejuni* also colonizes dogs, with a prevalence of 0–45% (Marks et al., 2011). It remains unclear whether *Campylobacter* causes disease in dogs. One case-control study of diarrheic and healthy dogs showed 97 and 58% *Campylobacter* prevalence, respectively, by PCR, while a culture-based study reported no significant difference between such groups (Chaban et al., 2010; Stavisky et al., 2011). The evidence for an increased risk of campylobacteriosis for dog owners is also debatable, with some studies indicating a risk (Mughini Gras et al., 2013), and others not (Rodrigues et al., 2000).

In cats, up to 42.9% of animals are reported to be *Campylobacter* positive, with *C. helveticus* as the predominant colonizing species (Acke et al., 2009; Wieland et al., 2005). Data from horses is scarce, but the reported prevalence is low (<5%) (Moriarty et al., 2015; Roug et al., 2013). The risk of direct contact between *Campylobacter*-colonized companion animals, especially to people with little prior exposure, at community events such as country fairs, petting farms, and even in residential care, has yet to be understood.

5.5 SURFACE WATERS, SOILS

Surface waters are a particular problem, and can be considered "sinks" that collect *Campylobacter* strains from various animal reservoirs. Recreational and alluvial waters are frequently contaminated with campylobacters (Arnone and Walling, 2007), and water-related activities, such as swimming, and even children's paddling pools (Sawabe et al., 2015), can constitute a risk of infection. The recovery of *Campylobacter* from surface waters is often indicative of recent contamination with wastewater effluents or agricultural runoff (Jones, 2001). For example, in Ontario, Canada,

in 2000, well water serving the town of Walkerton was contaminated by *Escherichia coli* O157:H7, and *C. jejuni* from cattle waste washed out from local farms by heavy rainfall, resulting in at least seven deaths, and over 2000 illnesses (Clark et al., 2003). The presence of wild birds, including water fowl, is also a source of surface water contamination, with an associated risk of campylobacteriosis (Mullner et al., 2009b).

The survival of *Campylobacter* in surface waters is dependent on a number of factors, including the hours of sunlight, temperatures, and water quality (reviewed by Whiley et al., 2013). There is increasing evidence for the presence of environmentally adapted *Campylobacter* strains, such as ST45, known to be particularly widespread in the environment (French et al., 2005; Sopwith et al., 2008). Such strains appear to show an enhanced fitness outside the host, presumably as a result of the evolution of bacterial stress mechanisms to improve survival (Sopwith et al., 2008). Survival in the environment may also be enhanced by uptake of the bacteria in protozoa (Trigui et al., 2016; Olofsson et al., 2015), or by the development of biofilms (Pascoe et al., 2015).

5.5.1 POTABLE WATER

The main evidence for drinking water as a source of campylobacteriosis is from outbreak, rather than sporadic, cases. As indicated earlier, such outbreaks can be very large. Interestingly, many occur in countries of northern latitudes, such as in Scandinavia, where survival might be enhanced by reduced sunlight and cooler temperatures. In such countries, drinking water is also recognized as a risk factor for broiler colonization (Newell et al., 2011).

Campylobacters are rarely isolated from potable water supplies, unless there is a breakdown in treatment plants (Jones, 2001). However, in rural areas where drinking water may be supplied from wells, or by rainwater collection, the opportunities for contamination are likely to be high, especially when water is stored in tanks open to wild birds and animals.

5.5.2 WILD BIRDS

Wild birds, of many families, are frequently colonized with *Campylobacter* strains (Colles et al., 2011; Griekspoor et al., 2013), some of which demonstrate distinct host specificity. It is estimated that about 3.5% of human campylobacteriosis cases are attributable to wild bird strains (Cody et al., 2015), with a peak in the summer months, but, in children under 5 years of age living in rural areas, this risk could be as high as ~24% of infections (Strachan et al., 2009). In addition to surface water, other transmission routes for wild bird-associated *Campylobacter* strains are the exposure to fecal material in children's playgrounds (French et al., 2009), pecking of milk-bottle tops (Neal and Slack, 1997), and outdoor leisure or work activities (Strachan et al., 2009). However, stochastic models based on MLST suggest that *Campylobacter* strains from wild birds pose a lower risk to humans, relative to those from domestic poultry. The reason for this is unknown, but host specialization may play an important role.

5.5.3 INSECTS

There is increasing evidence that flying insects, such as the house fly (*Musca domestica*), are transmission vectors, able to carry campylobacters from fecal material in the environment to another host (Förster et al., 2009) that could be chickens or, possibly, humans. A recent risk assessment (Evers et al., 2016) suggests that the importance of this exposure route to humans should be further investigated.

5.6 *CAMPYLOBACTER* EPIDEMIOLOGY IN THE DEVELOPING WORLD

Much of our understanding of the epidemiology of campylobacteriosis comes from investigations within the industrialized world. Travelers from industrialized countries often have a higher risk of acquiring campylobacteriosis, and this risk seems to be particularly high for Western travelers going to Asia, Africa, Latin America, and the Caribbean (Mughini-Gras et al., 2014). It is assumed, but not proven, that this risk reflects a greater environmental, as well as foodborne, burden in these geographical regions, as well as the possibility of exposure to "exotic" strains previously unencountered in the home environment.

The awareness, and monitoring, of campylobacteriosis in the developing world is generally poor, and this has been highlighted by the World Health Organization (WHO) (http://www.who.int/foodsafety/publications/campylobacteriosis/en/). One well-recognized feature of human epidemiology in the developing world is that colonization with *Campylobacter* is common but, in adults, largely asymptomatic. In young children, however, colonization is often associated with disease. One explanation for this is that adults in developing countries have been frequently exposed to *Campylobacter* through food and the environment, and consequently have developed immunity that protects from disease but not colonization (Havelaar et al., 2009).

In terms of World Bank classification, developing countries denote all low- and middle-income countries. In such countries, livestock production (especially of poultry and pigs) is largely extensive, frequently on widely distributed small-holder farms, or in backyards (Gilbert et al., 2015), which minimizes labor and cost inputs. The little information to date on potential sources of campylobacteriosis in the developing world comes primarily from poultry. Small and large scale commercial poultry farms mostly use a deep litter open-house system where broilers are constantly in contact with external environment, wild animals, and flies. However, village or family based extensive poultry production is also common, and in these cases biosecurity is rarely feasible or practical (Conan et al., 2012). For example, even 1-day-old chicks are in close contact with adult birds likely to be already colonized with *Campylobacter* (Kalupahana et al., 2013). Extensive farming methods in developed countries are known to increase the risk of *Campylobacter* colonization in broilers (Allen et al., 2011). Therefore, high prevalences of *Campylobacter* colonization of broilers at slaughter in developing countries should be expected. In studies from several developing countries, for example, China (Ma et al., 2014), levels of over

70% have been reported. However, much lower levels have been reported elsewhere, for example, 31.9% in Vietnam (Carrique-Mas et al., 2014). Surprisingly, this range of prevalence levels would seem to be little different from those reported in the developed world, but bird age, sampling strategies, culture methodologies, and microbiological competence could all contribute to this observation.

In developing countries, the production of poultry meat is a combination of mechanized abattoir processing, which is to some extent regulated, and an unregulated informal sector, often using live bird markets (alternatively known as wet markets, or pluck shops). In some countries, for example, India, the proportion of chicken meat sold from freshly culled chickens at live bird markets is over 90% (Parkar et al., 2013) (http://gain.fas.usda.gov/Recent%20GAIN%20Publications/Poultry%20and%20Poultry%20Products%20Annual%202015_New%20Delhi_India_9-30-2015.pdf). Local consumer preference determines the type of retail product sold. In some countries, hand-slaughtered chicken is considered fresher and healthier, but in others, such as Sri Lanka, consumers prefer mechanically processed meat. Evidence is increasing for the lower hygiene standards in the preparation of carcasses produced in wet markets. For example, in Yangzhou, China (Huang et al., 2016), although the prevalence of *Campylobacter*-contaminated carcasses retailed from wet markets and supermarkets was about the same (63.3% vs. 66.7%), the levels of *Campylobacter* contamination on the carcasses was significantly higher from the former.

To date, there has been little further characterization of the campylobacters isolated in the developing world. A few studies have reported a higher proportion of *C. coli* isolates being recovered in developing countries (Ansari-Lari et al., 2011; Salihu et al., 2009), than anticipated from surveys in developed countries. In addition, high levels of antimicrobial resistance have been reported in some *Campylobacter* isolates from developing countries (Ansari-Lari et al., 2011; Parkar et al., 2013; Carrique-Mas et al., 2014).

Overall, the situation regarding *Campylobacter* epidemiology in developing countries remains unclear. Surveillance for infectious intestinal diseases is generally poor in these countries. Nevertheless, recent analysis indicates that *Campylobacter* spp. cause the highest bacteria-attributable burden of diarrhea in the first and second years of life, and that to date this burden has been greatly underestimated (Platts-Mills et al., 2015). The sources of these infections are as yet unidentified, but food, and especially poultry meat, would seem to have an important role.

5.7 CONCLUSIONS

Given the successful source attribution approaches previously applied to salmonellosis, our inability to identify the routes of transmission for campylobacteriosis during the last two decades of the 20th century came as a considerable surprise. The control and prevention of *Salmonella* infection relied heavily on outbreak investigations, case-control studies, recovery of isolates from putative sources, and typing of those isolates to track strains from reservoirs to man. In this chapter, we have reported that

Campylobacter was less amenable to these approaches, for a number of reasons. In particular, outbreaks were relatively infrequent and poorly reflected in case-control studies, which in themselves were hampered by the self-limiting nature and extended incubation period for the disease. Tracing the strains through the transmission route was hampered by the fragility of the organisms in the environment. Moreover, despite the considerable heterogeneity of *C. jejuni/coli* strains, the population structure was very different from that of the salmonellas. This meant that the simple, largely serologically based, typing methods, so extensively and successfully used to trace individual *S. enterica* strains through the environment, were unavailable to *Campylobacter* epidemiology. In an attempt to "force campylobacters to comply with the needs of the epidemiologists," increasingly sophisticated typing methods were developed and applied worldwide to the growing collections of *Campylobacter* strains from humans and their environment. Over the past 15 years, the development and application of MLST has enabled the accumulation of evidence worldwide for overlapping animal and human *Campylobacter* populations, and statistical approaches have allowed disease attribution to specific sources, with some confidence. Today, ever greater detailed information on each strain is being acquired with the use of whole-genome sequences. Unfortunately, it is a consequence of the wealth of such data, and the sophistication of the analytical techniques required, that only experts can now interpret the information obtained.

Overall, *Campylobacter* is widely considered a foodborne pathogen, with the handling and consumption of poultry meat implicated as the major source. As a consequence, risk communication and management have focused on the poultry industry. However, there is growing evidence that alternative nonmeat-foodborne transmission routes, such as the contact with animals directly, or indirectly through the environment (including vegetables) they contaminate, have a major role in campylobacteriosis.

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