Campylobacter infections, a significant issue of veterinary urban hygiene: dog-related risk factors

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Summary

Campylobacter spp. are ubiquitous bacteria and campylobacteriosis is the most frequently reported zoonotic disease in humans in Europe, since 2005. Handling or consuming contaminated/undercooked meat (especially poultry) are the most important sources of human campylobacteriosis. However, in recent years, the role of dogs as a source of infection for humans has been amply demonstrated. Approximately 6% of human campylobacteriosis cases are due to contact with pets. This review analyses the current literature related to risk factors at the dog-human interface.

Introduction

The members of Campylobacter genus are ubiquitous bacteria, frequently found in the alimentary tracts of wild and domestic birds and mammals, commonly contaminating the environment, including water. The genus Campylobacter belongs to the family Campylobacteraceae and includes 34 species that are known as human and animal pathogens (Newell 2001)\(^1\).

Campylobacter spp. are non-spore-forming, catalase and oxidase positive with a single polar flagellum, bipolar flagella, or no flagellum, depending on the species (Lengerh et al. 2013, Bolton 2015). Campylobacter gracilis has not flagella, while Campylobacter showae has multiple flagella, and all other Campylobacter species have a single polar flagellum at 1 or both ends of the cell (Bolton 2015).

Generally, the optimum growth temperature is 37°C. Thermophilic Campylobacter species (Campylobacter jejuni, Campylobacter coli, Campylobacter lari, Campylobacter upsaliensis, and Campylobacter elveticus) do not grow below 30°C and have an optimum growth temperature at 42°C. Visible colonies of C. jejuni usually appear within 48 to 72 hours (Hindiyeh et al. 2000).

\(^1\) LPSN-2015 List of Prokaryotic names with Standing in Nomenclature www.bacterio.net/campylobacter.html.
Most Campylobacter species grow under microaerobic conditions (but some can also grow aerobically or anaerobically), and have a respiratory type of metabolism. However, several species (Campylobacter concisus, Campylobacter curvus, Campylobacter rectus, Campylobacter mucosalis, Campylobacter showae, Campylobacter gracilis, and, to a certain extent, Campylobacter hyointestinalis) require hydrogen or formate as an electron donor for microaerobic growth. In addition, certain species prefer anaerobic conditions for growth. Campylobacter is sensitive to many external conditions, like low water activity, UV light, salt, and heat. In contrast to Salmonella, Campylobacter does not multiply outside a warm-blooded host (e.g., on meat samples), given the absence of microaerobic conditions and the non-permissive temperatures (EFSA 2011).

Campylobacteriosis identifies infectious diseases caused by members of the genus Campylobacter and mostly characterised by acute enteritis, extraintestinal infections, and post-infectious complications. Currently, the high global prevalence and the socioeconomic burden associated with Campylobacter infection pose major challenges for public health in the 21st century.

**Campylobacteriosis in humans**

Since 2005, campylobacteriosis has been the most frequently reported zoonotic disease in humans in Europe, with the annual number of notified cases increasing in recent years (EFSA 2012, Tam et al. 2003). However, considering the high number of campylobacteriosis cases, the severity in terms of reported fatalities is low (0.05%) (EFSA 2015).

Campylobacteriosis in humans is caused by thermotolerant Campylobacter spp., although other species including the non-thermophilic C. fetus, are also known to cause human infection.

The most commonly associated species with human infection are C. jejuni, followed by C. coli, C. lari, and C. upsaliensis (Wieland et al. 2005, Leonard et al. 2011, EFSA 2012). The infective dose of these bacteria is generally low. However, recent studies highlighted the potential limitations of routine culture methods. While selective temperature and media are well established for C. jejuni or C. coli, they are not for other species and so the infective doses for these species are likely underestimated (Koziel et al. 2014). At the same time, the high concentration of antibiotics used in Campylobacter selective media to suppress commensal gastrointestinal flora may also inhibit the growth of less common species (Maher et al. 2003).

Developments in molecular diagnostics have revealed that infections caused by non-thermophilic Campylobacter have higher prevalence than previously thought. Bullman (Bullmann et al. 2012) reported that approximately half of the species detected by polymerase chain reaction (PCR) were non-C. jejuni/C. coli, with C. ureolyticus being the second most common. In the same study, the less common species C. lari, C. fetus, C. upsaliensis, and C. hyointestinalis, collectively account for 10% (Chaban et al. 2010, Hald et al. 2004). Lastovica and Engel (Lastovica and Engel 2001) reported that C. upsaliensis is the third most prevalent Campylobacter species isolated from the diarrhoeic stools of paediatric patients representing 23% of the 4,260 Campylobacters isolated during a period of 10 years. The most significant reservoir of C. upsaliensis is the canine species (Hald et al. 2003, Carbonero et al. 2012).

The most common sources of human campylobacteriosis are handling or consumption of contaminated/undercooked meat (especially poultry), handling or consumption of contaminated or unpasteurised milk and dairy products, drinking contaminated water, person-to-person contact, direct contact with carrier farm animals, direct contact with pets and insect as flies (Saied et al. 1993, Adak et al. 2005, Mazick et al. 2006, Strother et al. 2005).

The majority of Campylobacter infection cases are sporadic, and outbreaks are not common, although reported cases have the highest peak in Summer (Tenkate and Stafford 2001).

After ingestion, C. jejuni colonises the lower gastroenteric tract (ileum, jejunum, and colon), sometimes without symptoms. Colonization requires motility, adhesion, invasion, and toxin production (Bolton 2015).

In most symptomatic cases, campylobacteriosis is manifest as mild and self-limiting gastroenteritis characterised by 1-3 days of low fever, vomiting, myalgia, and headaches, followed by 3-7 days of abdominal pain with watery or bloody diarrhoea. Acute infection sometime begins with a high fever, peaking during the first days of illness. Excretion ends within 10-14 days (Newell 2001) and severe complications are uncommon (Altekruse et al. 2003, Blaser 1997, Bolton 2015).

Campylobacteriosis does not require antimicrobial treatment. However, in some cases such as septicaemia, or other invasive forms of the disease, antibacterial therapy may be needed. Chronic infections or extra-intestinal infections may occur with fatal bacteraemia, hepatitis, pancreatitis, meningitis, recurrent colitis, acute cholecystitis and cystitis, cardiovascular complication, abscesses and complications of the reproductive system (Goossens et al. 1987, Manfredi et al. 1999, Acke et al. 2009, Keithlin et al. 2014). Epidemiological studies indicate that a prior exposure with subsequent host-immunological
Colonization with *Campylobacter* may predispose dogs to infection with parvovirus, or with a secondary infection, and may increase the severity of the primary infection (Workman *et al.* 2005, Olson and Sandstedt 1987).

Dogs can carry a wide range of *Campylobacter* species. Some studies report *C. jejuni* as the most common species, although others indicate *C. upsaliensis* as predominant (Table I), which has dogs as main reservoir. Simultaneous presence of multiple species with various pathogenicity characteristics can also occur. Koene and colleagues (Koene *et al.* 2004) describe multiple *Campylobacter* species in 26% of the positive-testing stools.

All consulted studies show that dogs should be regarded as potential sources of exposure for humans. The prevalence of dogs carrying *Campylobacter* spp. varies widely (Table I), depending on the sampled population, on the design of the study, and on the detection method. The main factors affecting prevalence and recorded in literature are: environment, age, presence of diarrhoea or enteric disease, high-density housing (e.g., kennels), food, contact with other animals, walking outdoors, and season.

**Risk factors at the dog-human interface**

Dogs are the most popular companion animals in many countries. Ownership of dogs is beneficial to the owner’s psychological and physical health, promoting a less sedentary lifestyle, emotional protection, and social interaction (McNicholas *et al.* 2005). They increasingly participate in animal-assisted therapy. For this reason the high prevalence of *Campylobacter* infection, recorded in many studies is an important topic of public health (Table I); especially as there is evidence of increased risk of *Campylobacter* infections associated with dog ownership (Tenkate and Stafford 2001, Parsons *et al.* 2010). The genotyping of isolates has aided the identification of contamination sources and the tracing of the transmission routes, e.g. the environment, reservoirs, and human beings, thus increasing the understanding of the epidemiology of this disease (Keller *et al.* 2007). Mughini Gras and colleagues (Mughini Gras *et al.* 2013) hypothesise the following scenarios: humans and pets become infected from the same source; humans become infected from dogs; dogs become infected from humans.

**Environment**

Poor hygiene conditions may represent an important source of *Campylobacter*. It can resist in the environment, especially when protected from
Table I. Prevalence of Campylobacter infections in the relevant literature according to species isolated, type of sample, population sampled, country, and detection methods.

<table>
<thead>
<tr>
<th>Bibliography</th>
<th>Population</th>
<th>Samples</th>
<th>Total dogs</th>
<th>Prevalence</th>
<th>Detection</th>
<th>Species</th>
<th>Identification method</th>
<th>Geography</th>
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<td>Culture and direct PCR</td>
<td>PCR</td>
<td>UK</td>
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dryness. These bacteria can survive on dry surfaces for at least 7 days (Ullman and Kischkel 1981). Most surface water sources are contaminated by animal manure, containing Campylobacter. In slurry and dirty water, Campylobacter can survive for up to 3 months (Nicholson et al. 2005).

Some studies have shown the presence of Campylobacter on flies. The common houseflies (Musca domestica) and other flies thrive in excreta and other filth. They could act as mechanical vectors, by carrying bacteria on the hairs and surface of their bodies, or on their feet. In this respect, it is worth noting that fly control has shown to be effective in preventing childhood diarrhoea (Wright 1983, Ekdahl et al. 2005). Campylobacter spp. has been isolated from sewage contaminated water (Bolton et al. 1987), contaminated soil (Brown et al. 2004), and aquatic sediments (Jones et al. 1990).

Age
The majority of studies found that younger dogs were more likely to be carriers of Campylobacter spp. and to shed the organism more commonly than older dogs (Sandberg et al. 2002). This suggests an age predisposition and age-immunity development (Sandberg et al. 2002, Workman et al. 2005, Acke et al. 2006, Parsons et al. 2010). In a study conducted in Barbados (Workman et al. 2005) over 70% of Campylobacter-positive dogs were under 1 year old, and 32.8% were younger than 9 week old (Workman et al. 2005).

Diarrhoea and enteric disease
As previously written, this topic is controversial, but the precautionary approach requires counting the presence of diarrhoea among the risk factors.

High-density housing
The prevalence in dogs living in groups (for example in kennels or shelter) is higher than in household dogs (Workman et al. 2005, Acke et al. 2006). This is probably due to the stress, increased prevalence of gastrointestinal disease, close contact with other animals, and dietary variation (Table I).

Contact with other animals
The contact of dogs with other animal species could be a factor of great importance. Natural reservoirs for Campylobacter spp. include chicken and other poultry, wild birds, pigs, cats, sheep, cows (Workman et al. 2005), and exotic pets, such as turtles (Harvey and Greenwood, 1985), and hamsters (Fox et al. 1983). The high prevalence (39.3%) reported by Workman and colleagues (Workman et al. 2005) in wild birds is of particular interest, as dogs can easily meet bird faeces in parks.

Food
Homemade cooked food or leftover food in the dog’s diet (or added to the dog’s diet) may increase the odds of Campylobacter carriage (Leonard et al. 2011). Westgarth and colleagues (Westgarth et al. 2009) report that feeding dogs with leftover human food in its bowl greatly increases the risk of infection; whereas feeding dogs directly from a person’s plate reduces the risk of its being positive. It is possible that owners would be more likely to put ‘spoiled’, undercooked or contaminated food in the dog’s bowl, whereas food from a plate would be less likely to be contaminated. It is also possible that the food may become contaminated while it is waiting to be fed to the dog. Raw food, especially meat, is generally considered to be a source of Campylobacter species (Westgarth et al. 2009).

A rapid change of diet can predispose to enteric dismicrobism, which could favour the onset of acute diarrhoea. In this condition, pathogens, like silent Campylobacter spp., could take over, multiply, and exacerbate the gastroenteric symptoms.

Season
The season can affect the patterns of infection and shedding of Campylobacter spp. by dogs. Some authors reported a higher number of isolations during Summer and Autumn (Lopez 2002, Torre and Tello 1993, Mohan 2015). For example, Carbonero and colleagues (Carbonero et al. 2012) reported in a study performed in Cordoba (Spain) that C. upsaliensis peaks in Spring, while C. jejuni peaks in Summer. This agrees with other studies performed on other species, like cattle and sheep, where the highest prevalence rates were also found in Spring-Summer (Grove-White et al. 2010).

Walking outdoors
Housed dogs have less chance of becoming infected. Dogs that escaped from home in the past, or that are free to access the external environment, have higher odds of being positive (Westgarth et al. 2009).

Antimicrobial resistance
An important aspect, from a public health and urban hygiene point of view, is the frequent anti-microbial resistance of zoonotic agents. Increasing antimicrobial resistance in both medicine and agriculture, is recognized as a major emerging public...
health concern by various authors and national authorities, including the World Health Organization (WHO) (Moore et al. 2002, Di Giannatale et al. 2014, Ozbay and Tasdemi 2014, WHO 2001). The growing number of household pets and their growing health care standards have led to an augmented number of geriatric animals accompanied by extensive medical histories, including antimicrobial drug administration. Companion animals may play an important role as a reservoir of resistant bacteria or resistance genes. Furthermore, human beings may be a reservoir of pathogens for their pets (Rutland et al. 2009).

The spread of antimicrobial-resistant bacteria can occur by direct contact (petting, licking, etc.) or indirectly, via the household environment, contamination of food, furnishings, etc. (Guardabassi et al. 2004).

Antimicrobial drugs exert a selection pressure not only on pathogenic bacteria, but also on commensal microorganisms of the intestinal tract of humans and animals. This may constitute a reservoir of resistant genes for potentially pathogenic bacteria (Guardabassi et al. 2004, da Costa et al. 2013).

Anti-microbial resistant enteric infections are highest in the developing world, where the use of anti-microbial drugs in humans and animals is largely unrestricted (Lengerh et al. 2013). In Campylobacter isolated from animals and food, the highest levels of resistance were reported for ciprofloxacin, nalidixic acid and tetracyclines (EFSA 2015).

The major issue of concern is the resistance to aminoglycosides such as gentamicin, macrolides, and quinolones which are the drugs of choice for serious campylobacteriosis in humans. In particular macrolides and quinolones are the antibiotics most frequently used in dogs (Marks et al. 2011).

Resistant Campylobacter infection in dogs may pose a hazard for owners, especially if they have increased susceptibility to infection.

**Laboratory diagnosis**

Isolation of causative agent is conducted using selective media under microaerophilic conditions (Marks et al. 2011). Rectal swab specimens or fresh faeces should be collected (Fox 1990), paying particular attention to prevent faeces from drying. When swabs are used, a transport medium (like Amies, Cary Blair or Stuart) must be used (OIE 2008). Transport to the laboratory and subsequent processing should therefore be as rapid as possible. It should be conducted preferably by the same day of collection, and, in any case, no later than 2 days from the collection. No recommendation on the ideal temperature for transportation can be made, but it is clear that freezing or high temperatures can reduce viability. High temperatures (> 20°C), low temperatures, and fluctuations in temperature must be avoided. When the time between sampling and processing is longer than 48 hours, storage at 4°C (± 2°C) is advised (OIE 2008). Currently, 2 ISO International Organization for Standardization (ISO) procedures are available: a horizontal method for detection of thermo-tolerant Campylobacter in food and animal feeding stuffs (ISO 2006), and a procedure for the isolation of Campylobacter from water (ISO 2005).

However, neither of these standard methods is optimal for the isolation of Campylobacters from live animals (OIE 2008). Many media can be used in the recovery of Campylobacter spp., such as modified charcoal, cefoperazone or desoxycholate agar (mCCDA). The latter is the recommended medium, although alternative media may be used (OIE 2008). A detailed description on Campylobacter spp. detection by culture and the variety of existing media has been provided by Corry and colleagues (Corry et al. 2003, Corry et al. 1995). Isolation is enhanced by selective filtration of faeces to be cultured through a 0.45 μ filter (Goossens et al. 1991). Microaerobic atmospheres of 5-10% oxygen, 5-10% carbon dioxide are required for optimal growth (Corry et al. 1995, Vandamme 2000). Incubation is frequently carried out at 42°C to select for thermophilic Campylobacter, but a temperature of 37°C should be used to ensure isolation of variable or non-thermophilic species (Marks et al. 2011). Campylobacter jejuni and C. coli usually show growth on solid media within 24-48 hours at 42°C. As the additional number of positive samples obtained by prolonged incubation is very low, 48 hours of incubation is recommended for routine diagnosis (Bolton et al. 1988).

Biochemical and thermo-tolerance testing are used to differentiate Campylobacter spp. Hippurate hydrolysis test is the most common phenotypic test used in routine diagnostics (Engvall et al. 2002). Several molecular techniques have been described to identify and differentiate Campylobacter spp. PCR, multiplex PCR, immuno-PCR, Realtime PCR, Random Amplified Polymorphic DNA (RAPD)-PCR, DNA microarrays, and probes are widely used (Wieland et al. 2005, Engvall et al. 2002, Dham et al. 2013). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) has become commonly used in diagnostic laboratories for species identification (Bessede et al. 2011, Seng et al. 2010, Fagerquist et al. 2005).

Serological assays are not routinely in use for the detection of C. jejuni and C. coli colonization (OIE 2008). Unfortunately, no systematic studies have
been conducted to ascertain the importance of antibody titers in dogs and cats as an indicator of infection in animals with or without diarrhoea (Fox et al. 1990).

**Conclusions**

The “One Medicine” concept as described by Schwabe (Schwabe 1964) has seen unprecedented revival in the last decade and has evolved towards “One Health” conceptual thinking, which further emphasises epidemiology and public health (Zinsstag et al. 2009). Rabinowitz suggests that, as humans, we should change our “us versus them” perspective of the entire environment, including animals as determinants of ‘risk to human health’ towards a perspective of “shared risk” between humans and animals (Rabinowitz et al. 2008). Initially, “One Health” research focused on zoonoses from farm animals and wild animals. There are, however, numerous specific examples of human diseases for which small companion animals may play a significant role in the transmission or by acting as reservoirs of infection.

Measures to control human campylobacteriosis should focus on preventing transmission from dogs in addition to other sources, such as undercooked poultry, contact with livestock, raw milk and untreated surface water. The relationship established among companion animals, human beings, and the risk of zoonotic infection from pets varies with the given context (cultures, economics, religions, etc.). The most common factors that affect the odds of infection in dogs, as reported in the relevant literature, are: environment, age, presence of diarrhoea or enteric disease, high-density housing, food, contact with other animals, walking outdoors, and season.

The awareness of the risk of *Campylobacter* spp. infection from dogs may be an important way to reduce the transmission from dogs to humans, in particularly infants, young children, and immune-compromised individuals. In this context, veterinarians bear a crucial role in protecting dogs and their families. In order to provide targeted education and recommendations to high-risk patients, veterinarian practitioners need to be aware of their clients’ immune status and of the presence of high-risk household members or others who frequently enter in contact with dogs, including those outside of the home (e.g., dogs involved in animal-assisted activities or therapy). To this end, communication between physicians and veterinarians must be improved. Further research in risk factors is also necessary to better understand the epidemiology of *Campylobacter* spp., as well as to develop a correct human-dog relationship and healthy lifestyles.

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Campylobacteriosis and dog-related risk factors


