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A Review of *Campylobacter* Infections in Dogs

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ABSTRACT

Campylobacter spp. are important bacterial enteropathogens that cause diarrhea in dogs with the predominant species being *Campylobacter jejuni*, *C. helveticus*, and *C. upsaliensis*. Although campylobacteriosis is subclinical in many dogs, some may develop moderate to mild enteritis. Puppies, kenneled dogs, and dogs with concurrent conditions are highly susceptible to *Campylobacter*-associated diarrhea. Diagnosis of suspected *Campylobacter*-associated diarrhea in dogs poses a challenge to veterinarians as the fecal enteric panels are costly, require technical knowledge, and are time consuming, however molecular approaches for diagnosing the *Campylobacter* spp. infections are becoming readily available and can help in accurate and fast diagnosis. *Campylobacter* spp. are amongst the prioritized human and animal pathogens with regards to antimicrobial resistance. The rise and spread of antimicrobial resistance threatens the effective treatment and control of *Campylobacter* infections. Multi-drug resistance *Campylobacter* spp. have been isolated from dogs therefore treatment should only be administered where necessary. In conclusion, understanding the epidemiology, diagnosis, treatment and zoonotic potential of *Campylobacter* spp. will enable small animal practitioners include them among the differential diagnoses of diarrheic diseases in dogs.

Key words: *Campylobacter* infections, Dogs, AMR, Zoonotic.

INTRODUCTION

Campylobacter spp. are categorized as the motile, rod or spiral-shaped, gram-negative and fastidious bacteria that require a microaerophilic environment for growth (Corry and Atabay 2001; Kaakoush et a1. 2015). They do not form spores and are 0.2-0.8µm by 0.5-5µm in size, and they obtain their energy source from the intermediate cycle of tricarboxylic acid or amino acids (Vandamme et al. 2005). The *Campylobacter* genus belongs to the family *Campylobacteraceae*, order *Campylobacterales*, class *Epsilonproteobacteria* and phylum *Proteobacteria* (Fitzgerald and Nachamkin 2011).

Campylobacter spp. are important bacterial enteropathogens that cause diarrhea in dogs (Guilford and Strombeck 1996; Cave et a1. 2002) with the predominant species being *Campylobacter jejuni*, *C. helveticus* and *C. upsaliensis* (Parsons et al. 2010; Marks et a1. 2011). Human pathogens like *C. coli*, *C. lari*, *C. gracilis*, *C. mucolas*, *C. showae*, *C. fetus*, *C. concisus*, and *C. sputorum* have also been isolated in dog feces (Chaban et al. 2010).

Campylobacter spp. are primary zoonotic pathogens that cause gastroenteritis globally (Havelaar et al. 2015; FSA 2017). Most infections are caused by thermotolerant *C. jejuni*, *C. upsaliensis*, *C. coli* and other species (Lastovica and Skirrow 2000; Labarca et al. 2002; Lastovica and le Roux 2003; Galanis 2007; Noreen et al. 2019; Gahamanyi et al. 2020).

Although dogs of all ages can be affected, puppies one year and below have a high prevalence (Hald et al. 2004; Chaban et al. 2010) which varies depending on the study design, sampled population and the detection method (Iannino et al. 2017). Consuming or handling undercooked/contaminated meat (poultry to be specific) is the key source for human *Campylobacter* infections (Adak et a1. 2005; Strother 2005; Hermans et a1. 2012; Sahin et a1. 2015). Contact with dogs has also been implicated as a risk for *Campylobacter* infections in children (Tenkate et a1. 2001; Mughini et a1. 2013).

Diagnosis of suspected *Campylobacter*-associated diarrhea in dogs is based on fecal panel test results (Marks et a1. 2011). However, the use of these fecal panels (culture and direct PCR) poses a challenge to veterinarians as the fecal enteric panels are costly, time-consuming and require technical skill (Marks and Kather 2003). The methods of detection used in *Campylobacter* spp. diagnosis of the infections in laboratories are biased towards the

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pathogenic *C. jejuni*, resulting in the under-diagnosis of the other *Campylobacter* spp. This challenge has confirmed the importance of culture-based research advances and *Campylobacter* spp. molecular detection (Chaban et al. 2010; Kaakoush et al. 2015; Bojanić et a1. 2016; Buss et al. 2019).

The emergence and dissemination of multidrug-resistant (MDR) *Campylobacter* spp. is a result of the widespread use of antimicrobials in the treatment of *Campylobacter*- associated diarrhea (Fitzgerald 2008) and therefore the prudent use of antibiotics (enrofloxacin and the macrolides (erythromycin and azithromycin)] is advised, as many cases resolve with supportive therapy (Marks 2003).

This paper reviews epidemiology, diagnostic work-up, treatment, antimicrobial resistance, and the public health importance of *Campylobacter* infections in dogs.

Epidemiology of *Campylobacter* Infections in Dogs

In animals, *Campylobacter* spp. transmission is through indirect or direct fecal-oral route, with infection sources from raw or undercooked food, fresh feces from fomites, infected animals, environment, and when in-contact with animals that are infected (Acke 2018). Numerous studies on the prevalence of *Campylobacter* spp. have been conducted in different countries (Table 1) and it varies depending on the age, the housing, geographic region, study design, diarrheic versus healthy dogs, the method of diagnosis, and the presence of infection or concomitant disease with enteropathogenic bacteria.

Pintar et a1. (2015) conducted a meta-analysis research considering 34 publications that described the prevalence of *Campylobacter* spp. in pet animals and an average prevalence of 24.7% was reported in household cats and dogs. The study noted that lack of standardization of methods significantly complicated the interpretation of results thus confirming gaps in the knowledge which exists in the significant *Campylobacter* spp. infections and its effect on humans.

Factors Associated with *Campylobacter* spp. Prevalence in Dogs

Signalment

Several studies reported that puppies had a high prevalence in comparison with the adult dogs (Parsons et al. 2010; Leonard et al. 2011; Rahimi et al. 2012; Kumar et al. 2012; Holmberg et al. 2015; Selwet et al. 2015; Thépault et al. 2020), this may be an indication that they lack prior exposure to the immune system. There is no proof of any sex or animal breed predispositions in the literature published so far.

Intensive Housing

Kenneled dogs that are immunocompromised are more likely to be positive to *Campylobacter* spp. on culture than pet dogs (Marks 2003; Workman et al. 2005; Parsons et al. 2011; Acke 2018). This is probably due to the stress, dietary variation, and close interaction with other animals (Marks et al. 2011; Parsons et al. 2011; Giacomelli et al. 2015; Leahy et al. 2016). Poor kennel conditions provide an environment conducive to the propagation of *Campylobacter* spp.

Presence of Intestinal Disease

Chaban et a1. (2010) reported that diarrheic dogs had a higher *Campylobacter* spp. prevalence in comparison with the healthy dogs. However, some studies demonstrated an insignificant difference in the prevalence of *Campylobacter* spp. from diarrheic or healthy dogs (Duijvestijn et al. 2016; Suchodolski et al. 2010; Leahy et al. 2017), which may be an indication of the frequency of subclinical infections.

In a study conducted by Olson and Sandstedt (1987), dogs were infected experimentally with *C. upsaliensis* and *C. jejuni*, one out of the three dogs infected with *C. upsaliensis* passed soft feces whereas one out of the three dogs infected with *C. jejuni* developed diarrhea. In addition, puppies orally inoculated with *C. jejuni* developed mild symptoms of enteritis (Macartney et a1. 1988).

Parasitic or viral enteritis has also been suggested to predispose dogs to *Campylobacter* spp. infection (Brown et a1. 1999). Clinical signs may develop in dogs with pre-existing or concurrent diseases (Brown et a1. 1999).

Diet

Majority of the pet owners feed purchased or homemade raw meat diets which pose a high risk for the dogs and humans handling the meat as it is frequently contaminated with these organisms (Fredriksson-Ahomaa et al. 2017). Puppies fed on a homemade food diet are also at a risk for *Campylobacter* infection (Leonard et a1. 2011). This increases the fecal shedding of the organism thus increases the risk of spread to other pets, domestic animals and humans especially those who are at a high risk due to age or immunosuppression. To reduce this risk, raw meat diets should not be fed to dogs. When handling the meat prior to cooking, handwashing is encouraged together with disinfecting any surfaces that have come in contact with the raw meat.

Infection with Multiple *Campylobacter* spp.

Multiple *Campylobacter* spp. identified from fecal samples by direct polymerase chain reaction (PCR) and/or bacterial is highly common (Kulkarni et al. 2002; Chaban et al. 2010; Kaakoush et al. 2015; Bojanić et al. 2017). The complex epidemiology of canine campylobacteriosis has been demonstrated by the confirmation of genetic heterogeneity within several *Campylobacter* spp. (Koene et al. 2009; Parsons et a1. 2010; Amar et a1. 2014; Bojanić et a1. 2017; Thépault et al. 2020). Introgression among multiple *Campylobacter* spp. may lead to pathogen adaptation, higher pathogenicity and antimicrobial resistance.

Concomitant *Campylobacter* spp. Infection with Other Organisms

Concomitant *Campylobacter* spp. infection with organisms such as parvovirus (Olson and Sandstedt 1987; Workman et al. 2005) and *Helicobacter* spp. (Rossi et al. 2008) has been reported. Direct PCR performed on fecal samples in healthy dogs from dog parks revealed *Campylobacter* spp. co-infection with coronavirus, circovirus, *Clostridium* spp., as well as *Cryptosporidium* spp. (Hascall et a1. 2016). Co-infection of *Campylobacter*

Table 1: Prevalence of Campylobacter spp. in dogs across the globe from different studies

Study/Reference	Prevalence (%)	Species isolated	Country
Fernandez and Martin (1991)	42.5	*C. jejuni, C. coli*	Chile
Baker et al. (1999)	43	*C. jejuni, C. upsaliensis, C. coli*	Australia
Hald et al. (2004)	76	*C. jejuni, C. upsaliensis, C. coli, C. lari*	Denmark
Tsai et al. (2007)	2.8	*C. jejuni, C. upsaliensis, C. coli*	China
Rossi et al. (2008)	30.2	*C. jejuni, C. upsaliensis, C. helveticus, C. lari*	Italy
Parsons et al. (2010)	38	*C. jejuni, C. upsaliensis*	UK
Chaban et al. (2010)	56	Multiple *spp.*	Canada
Carbonero et al. (2012)	35.2	*C. jejuni, C. upsaliensis*	Spain
Holmberg et al. (2015)	37	*C. upsaliensis, C. jejuni, C. helveticus*	Sweden
Begum et al. (2015)	60	*C. jejuni, C. coli*	Ethiopia
Rodrigues et al. (2015)	19.4	*C. jejuni, C. coli*	Brazil
Bojanić et al. (2016)	36	*C. jejuni, C. upsaliensis, C. helveticus, C. lari*	New Zealand
Karshima and Bobbo (2016)	23.8	*C. jejuni, C. coli*	Nigeria
Conan et al. (2017)	10	*C. jejuni, C. coli*	Kenya
Ahmed et al. (2018)	28.4	*C. jejuni*	India
Komba (2018)	9.9	*C. jejuni*	Tanzania
Polzler et al. (2018)	3.7	*C. jejuni, C. upsaliensis*	Austria
Karama et al. (2019)	47.8	*C. jejuni, C. coli, C. upsaliensis*	South Africa

spp. with other organisms may impact on the progression on *Campylobacter* infection in dogs from mild to severe and may increase the incidence of multidrug resistant organisms.

Intermittent Shedding and Transient Infection

The shedding of *Campylobacter* spp. is either intermittent or transient. A study by Hald et al. (2004) reported a high shedding prevalence in puppies less than a year old. They also reported that the shedding of *C. upsaliensis* was more continuous over a long period in comparison to *C. jejuni* suggesting the commensalism nature of *C. upsaliensis* and a transient *C. jejuni* infection (Hald et al. 2004). A similar finding was noted in kenneled dogs by Parsons et al. (2011). The intermittent shedding of homogenous *Campylobacter* spp. strains in several dogs has been determined by pulsed-field gel electrophoresis (Hald et al. 2004).

Clinical Signs

Campylobacter jejuni, C. helveticus, and C. upsaliensis are the main *Campylobacter* spp. in dogs (Workman et a1. 2005; Parsons et a1. 2010). Although majority of dogs may be subclinically infected, some dogs especially puppies less than 6 months of age or those from stressful environments may develop moderate to mild enteritis presenting as mild to watery diarrhea or as bloody or mucoid diarrhea with tenesmus (Brown et al. 1999; Chaban et al. 2010; Weese 2011; Acke 2018). Other reported clinical signs include anorexia, dehydration, lethargy and rarely fever, vomiting and abdominal pain (Brown et al. 1999; Sykes and Marks 2013; Marks et al. 2011).

Extra-intestinal *Campylobacter* infections include cholecystitis and cholangiohepatitis (Oswald et al. 1994; Center 2009; Sykes and Marks 2013). *Campylobacter upsaliensis* has been associated with acute polyradiculoneuritis (APN) (Martinez-Anton et a1. 2018) while *C. jejuni* infection has been linked to abortion (Odendaal et a1. 1994) and perinatal death (Sahin et al. 2014).

Barko et al. (2018) suggested that *Campylobacter* spp. may cause canine inflammatory bowel disease (cIBD) however, prospective studies to determine the importance of *Campylobacter* spp. in IBD pathogenesis are warranted (Maunder et a1. 2016). *Campylobacter* spp. were also found in samples of saliva from dogs with oral disease, a likely indication of these organisms plays a role in the pathogenesis of the condition (Petersen et a1. 2007; Yamasaki et al. 2012).

Diagnosis

Campylobacter spp. are either primary or secondary pathogens therefore isolation of these organisms is not a diagnosis of canine campylobacteriosis and concurrent or underlying diseases should be considered (Marks and Kather 2003; Acke 2018). For complicated cases, blood samples should be collected for hematology and serum biochemistry (Allenspach 2013; Sykes and Marks 2013) and diagnostic imaging done when extra-intestinal signs are present (Mapletoft et al. 2018).

Fecal Examination

In cases of *C. jejuni* enteritis, it is possible to see gram negative, slender, gull-wing rods and leukocytes on fecal gram stains (Marks et a1. 2011) and curved bacteria with a darting motion on phase-contrast or dark-field microscopy (Marks and Kather 2003). However, a microscopic morphological diagnosis alone cannot be validated, and further assessment is required (Marks et a1. 2011). Other infectious causes of diarrhea such as, helminths should also be evaluated (Allenspach 2013).

Bacterial Isolation

In cases where there are signs of enteritis, 2-3g fresh feces or rectal swabs in Cary Blair or Amies transport media are submitted to the laboratory for culture (Marks et a1. 2011). Various selective media for isolation of *Campylobacter* spp. from samples in modified atmospheric environments with a temperature range of 37 to 42^{o}C have been described, for example, modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) medium for the isolation of *C. coli* and *C. jejuni* (Gun-Munro et a1. 1987), an agar with Cefoperazone, Amphotericin and Teicoplanin (CAT) for the isolation of *C. coli, C. lari, C. helveticus, C. upsaliensis,* and *C. jejuni* (Acke et al. 2009; Bojanić et al. 2017), and a filtration

technique with blood agar for *C. sputorum, C. curvus, C. concisus,* and *C. rectus* isolation (Lastovica and le Roux 2000; Lastovica 2006; Kaakoush et al. 2015).

Matrix-Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry is an inexpensive, precise, and rapid method applied in commercial diagnostic laboratories for *Campylobacter* spp. identification and typing, and the detection of antibiotic resistance (Kiehntopf et a1. 2011; Singhal et al. 2015).

Molecular Diagnosis

Detection of *Campylobacter* spp. using Molecular Methods

Molecular methods are the yardstick for the detection, speciating, and typing of organisms and they have enhanced the diagnostic capabilities of veterinary diagnostic laboratories (Adzitey et al. 2013; On 2013; Cai et al. 2014; Kaakoush et al. 2015). Multiplex PCR confirms *Campylobacter* spp. from fecal samples isolated on bacterial culture (Persson and Olsen 2005; Neubauer and Hess 2006) whereas real-time PCR detects these organisms from feces without prior bacterial culture (Chaban et al. 2009, Chaban et al. 2010). *Campylobacter* in saliva from dogs have been detected by PCR-denaturing gradient gel electrophoresis (Petersen et al. 2007). Rapid and accurate detection of *Campylobacter* spp. in dogs is critical for appropriate treatment.

Sub-Typing of *Campylobacter* spp. Strains

Multilocus Sequence Typing (MLST) is a "gold standard" sub-typing technique used for the identification of *C. helveticus*, *C. upsaliensis*, *C. coli*, and *C. jejuni* from clinical samples without prior bacterial culture (Dingle et al. 2001; Workman et al. 2005; Taboada et a1. 2013; On 2013). Amplification and sequencing of (seven) housekeeping genes are done for MLST and alleic numbers are allocated in comparison with the PubMLST databases (http://pubmlst.org/campylobacter/) (Dingle et al. 2001; Workman et al. 2005; Taboada et al. 2013; Holmberg et al. 2015). According to Cantero et al. (2018), whole-genome sequencing differentiates strains and also identifies the antimicrobial resistant and virulent determinants in *Campylobacter* spp.

Antimicrobial Therapy and Multidrug-Resistant *Campylobacter* Infections

Prudent use of antibiotics in animals with suspected *Campylobacter*-associated diarrhea is advised for febrile or immunocompromised patients, or where the infection is severe or is extra-intestinal (Marks 2003; Marks et al. 2011). In dogs, erythromycin (10-15mg/kg orally every 8 hours) (Marks et al. 2011; Weese 2011), or azithromycin (5-10mg/kg orally every 24 hours) (Marks et al. 2011) has been recommended in cases where susceptibility results are unavailable.

Multi-drug resistance *Campylobacter* spp. have been isolated from dogs therefore treatment should only be administered where necessary (Fitzgerald 2008; Marks et al. 2011; Kumar et al. 2012; Cho et al. 2014; Rodrigues et al. 2015; Ahmed et al. 2018). In the United States, multistate outbreaks of multidrug-resistant *Campylobacter* infections have been reported in humans linked to contact with puppies from pet stores (Montgomery 2018).

Prognosis of canine *Campylobacter* infections is very good when the treatment is appropriate and in the absence of systemic complications. In cases of treatment failure, reinfection, incorrect diagnosis, the intermittent shedding of *Campylobacter* spp., strain variations, and the development of antimicrobial resistant strains should be considered (Marks et al. 2011; Kaakoush et al. 2015; Acke 2018).

Public Health Importance

Campylobacter spp. are among the leading zoonotic pathogens causing gastroenteritis worldwide (Havelaar et al. 2015; Kaakoush et al. 2015; EFSA 2017). The main *Campylobacter* spp. in dogs: *Campylobacter jejuni*, *C. helveticus* and *C. upsaliensis* are considered sources of human *Campylobacter* infection (Labarca et al. 2002; Gras et al. 2013; Kaakoush et al. 2015; Bojanić et al. 2017). Approximately 9% of human *Campylobacter* infections from pet animals are caused by direct fecal contact (Tam et al. 2009; Kittl et al. 2013; Mughini et al. 2013). The presence of puppies in the household has been implicated as a risk for *Campylobacter* infection in children (Tenkate and Stafford 2001; Mughini et al. 2013).

A study by Wolfs et al. (2001) in Netherlands showed evidence of transmission of *C. jejuni* from a household puppy to a 3-week-old infant. Transmission of the *Campylobacter* spp. can also be from infected humans to dogs. A study done in Denmark by Damborg et al. (2004) showed that *C. jejuni* could occur among pet dogs living with infected children. In humans, the reported clinical signs of *Campylobacter* infections include fever, abdominal pain, diarrhea, extraintestinal infections such as bacteremia and meningitis, and post-infectious conditions such as reactive arthritis and irritable bowel syndrome (Janssen et al. 2008; Fitzgerald 2015; Kaakoush et al. 2015).

Conclusion

Canine campylobacteriosis is a significant bacterial disease of public health importance with varying prevalence rates of the causative *Campylobacter* spp. from country to country. Multi-drug resistance *Campylobacter* spp. have been isolated from dogs therefore treatment is recommended where the infections are severe or are extra-intestinal. As molecular approaches which are fast and precise for diagnosing *Campylobacter* infections have become readily available, improved understanding of the epidemiology, diagnosis, treatment and zoonotic potential of *Campylobacter* spp. will enable small animal practitioners include them among the differential diagnoses of diarrheic diseases in dogs and implement effective preventive and control measures.

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