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Campylobacter species in animal, food and environmental sources and relevant testing programs
in Canada

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ABSTRACT

Campylobacter species, particularly thermophilic campylobacters, have emerged as a leading cause of human foodborne gastroenteritis worldwide, with *Campylobacter jejuni*, *C. coli* and *C. lari* responsible for the majority of human infections. Although most cases of campylobacteriosis are self-limiting, campylobacteriosis represents a significant public health burden. Human illness due to infection with campylobacters has been reported across Canada since the early 1970s. Many studies have shown that sources including food, particularly raw poultry and other meat products, raw milk and contaminated water have contributed to outbreaks of campylobacteriosis in Canada. *Campylobacter* spp. have also been detected in wide range of animal and environmental sources including water in Canada. The purpose of this article is to review the prevalence of *Campylobacter* spp. in animals, food and the environment, and relevant testing programs in Canada with a focus on potential links of campylobacters with human health in Canada.

Key words: *Campylobacter* in animal, food and environment, *Campylobacter* testing programs, Canada

39 Introduction

40 Microaerophilic Gram negative bacteria that are now classified as *Campylobacter* species were first
41 isolated from samples collected from aborting ewes in 1906 in the United Kingdom (UK) (Skirrow,
42 2006). Since that time, the development and use of selective culture media (Skirrow, 1977; Blaser et
43 al., 1979) has led to the recognition of *Campylobacter* as a leading cause of human diarrhoeal illness
44 in many countries, including Canada. *Campylobacter* spp. are commensal organisms in the intestinal
45 tract of a variety of animals including birds, and thus animals are a potential source for
46 contamination of food, water and the environment (Humphrey et al. 2007). Currently, 18 species are
47 recognized in the genus *Campylobacter* (Debruyne et al. 2008), with additional species including
48 *Campylobacter canadensis* sp. nov being recently proposed (Inglis et al. 2007). *C. jejuni*, *C. coli* and
49 *C. lari* are responsible for the majority (95%, 4% and less than 1% respectively) of the human
50 gastrointestinal-related *Campylobacter* infections worldwide (Moore et al. 2005). Other species
51 including *C. fetus*, *C. hyointestinalis*, *C. upsaliensis* and *C. sputorum* have also been recognized as
52 causal agents of human gastroenteritis (Humphrey et al. 2007) and prenatal infection (Simor et al.,
53 1986), and *C. rectus*, *C. gracilis*, *C. concisus*, *C. curvus*, and *C. showae* as pathogens responsible for
54 human periodontal disease or abscesses (Humphrey et al. 2007).

55
56 Most cases of campylobacteriosis are self-limiting; however, some infections require hospitalization,
57 and may lead to death and life-threatening post-infection sequelae. Gastroenteritis due to *C. jejuni* is
58 recognized as the most common antecedent infection associated with Guillain-Barré Syndrome, an
59 acute peripheral demyelinating polyneuropathy causing limb weakness and, in severe cases, total
60 paralysis (Rees et al. 1995). *Campylobacter* infections, particularly those caused by *C. jejuni* and
61 infrequently by *C. coli*, have been linked to reactive arthritis and other reactive musculoskeletal

symptoms, enthesopathy or bursitis, postinfectious irritable bowel syndrome and abortion (Blaser and Engberg, 2008). *C. jejuni* infection may occasionally cause meningitis in infants (Goossens et al. 1986), and *C. jejuni* bowel infection can rapidly lead to fatal myocarditis (Pena and Fishbein, 2007).

Campylobacter infections are classified as notifiable diseases and are recognized as the leading bacterial cause of human enteric infections in all provinces and territories in Canada (Public Health Agency of Canada, 2009a; Rajda and Middleton, 2006; Thomas et al. 2013). From 2001 to 2004, 32,702 cases of campylobacteriosis were reported in Canada with an average of 34.9 cases per 100,000 population (Ruzante et al. 2011). Similar to other developed countries, campylobacteriosis in Canada peaks in the summer months (June to September), with lowest numbers reported in February and March (Keegan et al. 2009; Lal et al., 2012; Public Health Agency of Canada, 2009a). *C. jejuni* is the most frequently isolated *Campylobacter* spp. in Canadian human clinical samples, with *C. coli* being the second most common and *C. upsaliensis*, *C. fetus* and *C. lari* isolated only in a small number of cases (Bowman et al., 2003; Public Health Agency of Canada, 2009a). The predominant identification of *C. jejuni* in Canadian human clinical samples may reflect bias in the microbiological methods used for isolation (Lastovica, 2006), which may have led to false-negative results for cases of human campylobacteriosis. However, a study by Inglis et. al. (2011), conducted in Alberta, found that while current methods did underestimate the presence of other species of *Campylobacter*, these species were observed in both healthy and diseased individuals and only *C. jejuni* and *C. coli* were significantly associated with diarrheic individuals.

Most cases of human campylobacteriosis in Canada and internationally are sporadic. According to a report of the National Enteric Surveillance Program (NESP), which collects and reports the national

incidence of laboratory confirmed enteric disease cases in Canada (Public Health Agency of Canada, 2011a), for the period between 2000 and 2004, 99.8% of the total (8353) confirmed *Campylobacter* cases were sporadic with only 177 cases related to 11 outbreaks and case clusters (Public Health Agency of Canada, 2009a). An investigation of 138 outbreaks of campylobacteriosis in Canada between 1978 and 2005 found that most were associated with poultry (56%) and dairy products (25%) (Ravel et al. 2009). Waterborne outbreaks due to *Campylobacter* spp. have also been reported in Canada, with the largest outbreak occurring in May 2000 in Walkerton, Ontario (Public Health Agency of Canada, 2000; Hrudehy et al., 2003; Schuster et al. 2005; Clark et al. 2003).

The purpose of this article is to review the prevalence of *Campylobacter* spp. in animals, food and the environment, and the relevant testing programs in Canada with a focus on the potential links between sources of campylobacters and human health. The review will provide useful information for further coordinated studies to determine prevalence and baseline levels of this organism throughout the country. A review of the extensive Canadian studies with investigation of *Campylobacter* epidemiology, basic bacteriology and pathogenesis at the cellular and molecular levels and antimicrobial resistance is beyond the scope of this article.

Overview of testing programs for campylobacters in food and related sources in Canada

According to Raji et al. 2007, Canada's vision for the agri-food industry in the 21st century is the establishment of a national food safety system employing hazard analysis and critical control point

(HACCP) principles and microbiological verification tools, with traceability throughout the gate-to-plate continuum. Voluntary on-farm food safety programs, based in part on HACCP principles, provide producers with guidelines for good production practices focused on general hygiene and biosecurity. Mandatory HACCP programs in federal meat facilities include microbial testing for generic *Escherichia coli* and specific testing of ground meat for *E. coli* O157:H7 and raw products for *Salmonella* (Raji et al. 2007). In Canada, there have been no mandatory federal or national policy and testing programs for campylobacters in food or food animals, water or the environment, however, there have been studies by provincial and federal governments and academics to determine the prevalence of campylobacters in food and related sources. In several provinces including Alberta, Ontario and Quebec, efforts have been made to determine the prevalence of campylobacters in poultry farms and products, and risk factors associated with *Campylobacter* contamination of various sources (Arsenault et al. 2007a, 2007b and 2007c; Bohaychuk et al. 2006, 2009a, 2009b, and 2011; Johnson et al. 2003; Ministry of Ontario Agriculture, Food and Rural Affairs, 2004). FoodNet Canada (formerly known as C-EnterNet) is a multi-partner initiative facilitated by the Public Health Agency of Canada to support activities that will reduce the burden of enteric diseases in humans, by comprehensive sentinel site surveillance and testing for pathogens in potential sources including retail foods, agriculture operations and local water sources (Public Health Agency of Canada, 2014). Currently, there are three sentinel sites within the program, one in each of the provinces of Ontario, British Columbia and Alberta. A national Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was established in 2002 by the Public Health Agency of Canada in collaboration with Health Canada, the Canadian Food Inspection Agency and provincial partners (Public Health Agency of Canada, 2011b). This program monitors trends in antimicrobial use and antimicrobial resistance in selected bacterial organisms

including campylobacters from human, animal and food sources across Canada. A pilot study examining antimicrobial resistance in human *Campylobacter* isolates from Saskatchewan was initiated in 2005.

Since the 1980s, there have been many ad hoc studies of different scales to determine the prevalence of campylobacters in food, food animals, water and the environments in Canada. However, the testing methods, seasons of the year, sampling size, sample type, geographic location, and sampling methods varied greatly between these studies. Thus the wide range of results between studies is difficult to compare and interpret. Nevertheless, the results obtained from these studies provide useful information regarding the distribution of campylobacters in Canada, and form a basis for further coordinated studies to determine prevalence and baseline levels of this organism throughout the country. Recently, a Pathogen Reduction Initiative was established by federal, provincial and territorial governments in Canada, to reduce the four main foodborne bacterial pathogens in meat, including campylobacters in chickens (Canadian Food Inspection Agency, 2012). The first task of the initiative has been to establish a national baseline of the four key pathogens in meat and poultry, including *Salmonella* and *Campylobacter* in raw chicken, *E. coli* O157:H7 in raw ground beef and trim, and *Listeria monocytogenes* in ready-to-eat meats. Since poultry has been shown to be an important source of campylobacters for human infection, nationwide coordinated baseline surveys for poultry will facilitate the implementation of control and prevention programs in Canada.

Campylobacters in animals

Due to the fastidious nature of *Campylobacter* organisms, propagation generally occurs only within animal hosts, where optimal temperature (37 to 43°C) and microaerobic environments favour their

growth (reviewed in Moore et al. 2005). For *in vitro* isolation, this organism requires modified atmospheric conditions, a longer growing time relative to other enteric pathogens, a narrow temperature range, and specialized media. Campylobacters are commensal colonizers of almost all bird and mammal species investigated, including farm animals, pets and wildlife. Most cases of human illness due to *Campylobacter* infection are likely to be attributed to poultry (Nadeau et al. 2002; Ravel et al. 2009, Wingstrand et al. 2006). The 42°C body temperature of poultry is closest to the optimal growth temperature of the thermophilic campylobacters most frequently associated with disease. Zoonotic *Campylobacter* reservoirs are important sources of contamination for food (meat, produce), water and the environment (Silva et al. 2001).

Farmed species.

Campylobacter species have been identified in all types of farm animals that have been investigated in Canada. Residents or workers on farms or living in proximity to high densities of farm animals including cows, pigs and chickens or in rural and agricultural areas have a higher incidence of campylobacteriosis than that among rural non-farm or urban residents in Canada, indicating the potential risk of campylobacters in animals to human health (Thompson et al., 1986; Green et al., 2006; Ellis et al. 1995; Levallois et al. 2013).

Cattle and sheep. *Campylobacter* spp. have been frequently detected in fecal or tissue samples of Canadian cattle at the farm level in studies conducted from to present (Table 1). The prevalence in cattle ranges between 19 and 93% using cultural methods, and between 21 and 90% with culture-independent PCR methodology (Table 1). While *C. jejuni* and *C. coli* were reported to be the dominant species in cattle *manure* in most of the culture-based studies (Munroe et al. 1983;

178 Waltner-Toews et al. 1986; Van Donkersgoed et al., 1990b; Inglis et al., 2006; Van Donkersgoed
179 et. al. 2009; Hannon et al. 2009a), studies using culture-independent methods indicate that *C.*
180 *lanienae* is the dominant species (Inglis et al., 2003; Inglis et al., 2004; Guevremont et al., 2008).
181 This latter species is currently considered to be an emerging cause of enteritis with unknown
182 significance. Campylobacters were also isolated from cattle undergoing abortion or with mastitis in
183 Saskatchewan, Canada (Van Donkersgoed et al. 1989; Van Donkersgoed et al. 1990b;
184 Gudmundson and Chirino-Trejo, 1993). Studies have shown a link between campylobacters in
185 cattle and human clinical isolates (Garcia et al. 1985; Hannon et al., 2009b; Public Health Agency
186 of Canada, 2000). These studies indicated a role of cattle as a potential source of
187 campylobacteriosis in Canada. Sheep have also been shown to be an important reservoir for
188 campylobacters. (Sutherland et al. 2009) isolated *Campylobacter* in 8.7% of 102 feces collected
189 from 10 Ontario sheep farms, and (Scott et al. 2012) isolated campylobacters in 62% of 275 fecal
190 samples from a total of 51 sheep flocks in Ontario. In another study in British Colombia
191 campylobacters were isolated from 5 of 42 (11.9%) and 3 of 13 (23.1%) of domestic sheep and
192 goats respectively (Jokinen et al. 2010).

193
194 **Poultry.** As in other countries, poultry is frequently colonized with campylobacters in Canada
195 (Table 2). The last national baseline survey of poultry, conducted from 1983 to 1986, found
196 *Campylobacter* in 73.7% and 38.2% of turkey and chicken carcasses, respectively (Lammerding et al.
197 1988). Estimates of the current prevalence of campylobacters in poultry flocks are based on results
198 of provincial baseline surveys and smaller Canadian studies. Overall the results of these studies are
199 variable, with prevalence in poultry flocks between 10 and 88% (Table 2) with the exception of one
200 survey of poultry fecal samples where no *Campylobacter* was detected (Public Health Agency of

Canada, 2011c). In addition, a recent study showed that 50% of 10 production lots from 6 organically raised chicken production units were positive for *C. jejuni* in Quebec (Thibodeau et al. 2011) suggesting a prevalence similar to that of a conventional poultry farm. Results from studies using fecal samples from farm or intestinal samples in slaughter houses (Table 2) varied. It is not clear which sampling method would best represent the true prevalence, although studies of feces may under-represent true prevalence due to rapid die-off of campylobacters in this matrix (Shanker et al. 1990). Overall, the variability of results reported between these surveys is likely due to differences in methods used for sampling and microbiological analysis (as discussed at the summary of detection method later on), as well as temporal and geographical factors. Without standardization of methodologies, or regular national baseline surveys, it is difficult to assess whether progress has been made in reduction of campylobacter in this food commodity.

Swine. *Campylobacter* spp. are commonly detected in Canadian pigs (Table 3). A nationwide survey (1983-86) indicated a prevalence of 16.9% (Lammerding et al. 1988). Provincial surveys using culture methods have found prevalence up to 99% (Mafu et al. 1989; Varela et al. 2007) with *C. coli* as the most common species detected. Using a PCR method, a survey in Quebec found a prevalence of 25% (Guévremont et al. 2008) with 40% for both *C. lanienae* and *C. coli* among the positive samples (Guévremont et al. 2008). Variability in prevalence appears to be largely associated with source of the samples, with rectal and cecal samples showing higher prevalence than tissue or fecal samples.

The risk of swine as a source for human campylobacteriosis remains unclear. One investigation in Quebec using pulsed-field gel electrophoresis (PFGE) and phenotypic assay revealed no

epidemiological relationship between isolates from pigs and humans in the same geographic area, suggesting a low risk of contamination of humans by *Campylobacter* associated with swine production in Quebec (Guévremont et al. 2004). A study in Switzerland showed 1.2% of 730 human campyobacterosis cases were attributed to pigs, although lower than that of 70.9, 19.3 and 8% attributed to chicken, cattle and dogs (Kittl et al., 2013). Further study for a better understanding of the risks of campylobacters from pigs and pork products to human health in Canada is required.

Pets, game and non-farmed species

Dogs. Only a limited number of studies of campylobacters in dogs in Canada have been conducted (Table 4). The prevalence of campylobacters in dogs ranged from 0 to 97% depending on the study, health status and methods used. Multiple species including *C. fetus*, *C. gracilis*, *C. helveticus*, *C. jejuni*, *C. showae*, and *C. upsaliensis* have been detected in healthy dogs (Chaban et al. 2009; Himsworth et al. 2009). In diarrheic populations, *C. coli*, *C. concisus*, *C. fetus*, *C. gracilis*, *C. helveticus*, *C. jejuni*, *C. lari*, *C. mucosalis*, *C. showae*, *C. sputorum* and *C. upsaliensis* have been detected at levels significantly higher than those in healthy dogs (Chaban et al., 2010).

Game animals. Few studies of *Campylobacter* species in game animals in Canada have been conducted (Table 4). One study, aimed at investigating the sources of campylobacters on a watershed in British Columbia conducted small scale surveys (5 to 42 samples) of various animals including horses, llamas, alpacas, geese, ducks, cows, chickens, pigs, sheep and goats (Jokinen et

al., 2010). Prevalence of *Campylobacter* species in these animals ranged from 9-80%. Typing of animal isolates using *flaA* gene restriction fragment length polymorphism (RFLP) analysis provided evidence that untreated water, sewage, and some domestic and wild animals share the same *flaA*-RFLP profiles indicating that campylobacters could spread to multiple domestic and wild animal species sharing the same water source and environment.

Aquatic species. Shellfish from contaminated water have been recognized as a source of campylobacters (Moore et al. 2005). *Campylobacter* spp. were isolated from 47.2% of 72 *Mya arenaria* clams from the north shore of the St. Lawrence River in Quebec (Lévesque et al. 2006 in Table 4). Contaminated seafood was responsible for 6.3% of the Canadian campylobacteriosis outbreaks detected between 1976 and 2005 (Ravel et al. 2009). In 262 outbreaks of campylobacteriosis reported in USA from 1997 to 2008, 12 cases (1.9%) were due to consumption of seafood (Taylor et al. 2013). Further studies in Canada will be required to identify water sources contributing to higher risk for seafood contamination by campylobacters, and types of seafood with higher risk of causing human campylobacteriosis.

Wild birds and animals. Wild birds in Canada are frequently colonized with *Campylobacter* spp. and may play a role in the transmission of this organism to animals and humans by contaminating the environment, feed and food. *Campylobacter* spp. were isolated from ring-billed gulls (*Larus delawarensis*) in Quebec, from double-crested cormorant chicks in Prince Edward Island, and from waterfowl including seagulls, Canada geese and ducks in Ontario and British Columbia (Table 4). A novel species (*Campylobacter canadensis* sp. nov.) was recovered from captive whooping cranes (*Grus americana*) in Alberta (Inglis et al. 2007). The disease-causing potential of this species

requires further investigation. *C. jejuni* was not isolated from faecal samples of pronghorn and mule deer in southern Saskatchewan during fall hunting season (Van Donkersgoed et al. 1990a). There have been no other studies to investigate the prevalence of campylobacters in other common wildlife species such as elk and moose in Canada.

It is generally believed that wild animals and domestic pets, including dogs, are a potential reservoir of campylobacters for humans (Whiley et al. 2013). A study in Switzerland showed that 8% of 730 human campylobacteriosis cases were attributed to dogs (Kittl et al. 2013) and one of 262 outbreaks of campylobacteriosis reported in USA from 1997 to 2008 was due to contact with kittens (Taylor et al. 2013). A recent study in USA established a laboratory-confirmed link between a campylobacteriosis outbreak in human and consumption of contaminated raw peas with birds as a source of produce contamination (Gardner et al. 2011; Kwan et al. 2014). Although the above indicates generally low risks of non-farmed animals and wildlife as a source of campylobacters for humans, the role of migratory birds and wild animals in transmission of pathogenic campylobacters to humans in Canada requires further investigation.

Campylobacters in the environment including water

Campylobacteriosis in Canada is commonly associated with contaminated water. A review of waterborne outbreaks occurring between 1974 and 2001 in Canada indicated that among 150 of 288 outbreaks when a single causative agent was identified, 24% were caused by *Campylobacter* (Schuster et al. 2005). The waterborne *Campylobacter*-associated enteritis outbreaks included: 1) 6 patients infected with *C. jejuni* in a small rural community of Quebec in 1987 due to the consumption of unfiltered and unchlorinated potable water (Alary and Nadeau, 1990), 2) a large

outbreak with 45 laboratory-confirmed and 241 suspected cases in Ontario in 1985 due to the contamination of well water by spring run-off and heavy rains (Millson et al. 1991), 3) an outbreak in Walkerton, Ontario in May 2000, due to the contamination of well water serving the town by surface water carrying livestock waste immediately after heavy rains that resulted in an estimated 2,300 illness (167 cases confirmed as *E. coli* O157:H7 and 116 cases confirmed as *Campylobacter* spp.) (Public Health Agency of Canada, 2000; Hrudehy et al. 2003; Schuster et al. 2005).

Several recent Canadian studies have further indicated that the contamination of water at different locations and seasons by campylobacters is common in Canada. These studies at different scales with numbers of samples between 42- 2471 using either cultural methods or combination of cultural and molecular (PCR) methods showed prevalences between 0 and 100% (Table 5). Water can be contaminated from various fecal sources including farm animals, wildlife and humans (Jokinen et al. 2010). A study using a probabilistic estimation model indicated that while cattle are responsible for the largest amount of manure produced and manure from farm animals such as poultry contain higher concentrations of *Campylobacter* spp. and are likely to contribute significantly to contamination of water in agricultural environments (Dorner et al. 2004). A survey in Quebec (Arsenault et al. 2007a) showed that there were positive associations between the prevalence of intestinal colonization of campylobacters in chickens and turkeys and environmental risk factors such as distance of manure heap from the poultry/turkey houses, and the use of unchlorinated drinking water.

By examining extreme rainfall and spring snowmelt in association with 92 Canadian waterborne disease outbreaks caused by *Campylobacter* and two other organisms between 1975 and 2001,

Thomas et al. (2006) suggested that warmer temperatures and extreme rainfall are contributing factors to waterborne disease outbreaks. (Galanis et al. 2014) examined the association between drinking water, agriculture and 2992 cases of sporadic human campylobacteriosis in 2005–2009 in one region of British Columbia (BC), and found that the odds of campylobacteriosis compared to 4816 other enteric disease controls were higher for individuals serviced by private wells than municipal surface water systems, and the campylobacteriosis risk, compared to other enteric diseases, seems to be mediated by vulnerable drinking water sources and rural factors. These studies indicate that *Campylobacter* outbreaks in Canada have been associated with inadequate treatment of small water systems. Because *Campylobacter* prevalence is generally high in surface water, the protection of groundwater and proper treatment of drinking and irrigation water is very important.

Contamination of other environmental sources, such as soil, has also been implicated with campylobacteriosis in Canada. (Stuart et al. 2010) reported a large campylobacteriosis outbreak associated with a mountain bike race that took place in muddy conditions in June 2007 in British Columbia. Among 537 racers, 225 had diarrheal illness after the race. Fourteen *C. jejuni* clinical isolates were obtained and found to be identical by MLST indicating a common source of infection, with contaminated mud identified as the most likely source (Stuart et al., 2010). Campylobacters were isolated from 0-71% of 44 soil samples in a feedlot environment of Alberta with manure applications in spring and fall (Van Donkersgoed et. al. 2009). Sheep have also been shown to be an important cause for environmental contamination with campylobacters. (Sutherland et al. 2009) isolated *Campylobacter* with subsequent PCR confirmation in 22.3% of 279 soil samples, and 8.7% of 102 feces collected from 10 Ontario sheep farms.

These waterborne and environmental-borne outbreaks have shown the need for attention to broad issues of drinking water security and safety management to prevent such tragedies in the future. Special efforts should be taken to avoid contamination of agricultural products, such as vegetables, from irrigation practices.

Campylobacters in food

Raw and ready-to-eat meat and poultry

The prevalence of *Campylobacter spp.* in raw retail food in Canada is similar to that in other countries including USA, Spain, Ireland and Wales (Park et al. 1981; Park et al. 1983; Bohaychuk et al., 2006; Suzuki and Yamamoto, 2009). Prevalence of *Campylobacter spp.* in raw retail chicken varied significantly depending on the studies, ranging from 0 – 83% (Table 6). Surveys between 2007 and 2013 in the Waterloo region of Ontario showed a stable prevalence of campylobacters in raw chicken (Public Health Agency of Canada, 2013, Table 6) indicating a consistent source(s) of contamination. *Campylobacter jejuni* was isolated from one of eight raw beef liver samples, but not from eight pork and eight beef raw meats in a study conducted in Ontario over a 2-year period (Medeiros et al. 2008). Similarly a low prevalence was found in raw veal meat (1/438) in Ontario (Cook et al., 2011), ground beef (1-12.5%) in Ontario between 2007 and 2009, and 0-18% in pork chops in Ontario (2006-2009) (Table 6, Public Health Agency of Canada, 2009). In addition, a study using carcass swab samples of cattle and hogs stored in coolers at slaughter houses in Alberta showed low contamination prevalence for cattle (1.5%) and pig (8.8%) samples (Bohaychuk et al., 2011). These studies indicate that the prevalence of campylobacters in raw beef and pork at slaughter house and in retail meats is lower than that in fecal samples from cattle and pigs (Table 1

and 3). However, the prevalence in retail chicken meat was similar to that in fecal samples (Table 2 and 6), indicating that raw retail poultry meats present a higher risk to humans than beef and pork meats. *Campylobacter* was not detected in processed, ready-to-eat meat products (Bohaychuk et al. 2006; Medeiros et al. 2008).

A seven-year study in Belgium by (Ghafir et al. 2007) supported the above Canadian results of lower prevalence of *Campylobacter* on pork and beef compared to poultry meat. Although the reason for a higher load of campylobacters in chicken meat is not well studied, it may be due to the different meat processing procedures between poultry, cattle and pigs, such as the different types of carcass surfaces or techniques influencing the dryness of carcasses (Ghafir et al. 2007; Whiley et al., 2013) or a higher number of organism in chicken intestines and feces. The common association of *Campylobacter* spp. with poultry products supports studies indicating that the consumption and handling of contaminated poultry meat appears to be the most common risk factor for human campylobacteriosis (Keener et al. 2004; Humphrey et al. 2007).

Raw milk and raw milk cheese

Raw milk continues to be an important source of foodborne campylobacteriosis in Canada. Milk and associated products have been associated with 31% of 138 campylobacteriosis outbreaks with agent and source identified and occurring in Canada between 1978 and 2005 (Ravel et al., 2009). Particularly between 1996 and 2005, 3.1% and 22% of 32 campylobacteriosis outbreaks were due to milk, and dairy products other than fluid milk respectively (Ravel et al. 2009). For example, in 1980, 14 people in Alberta became ill with enteritis caused by *C. jejuni* after consumption of unpasteurized cow's milk (McNaughton et al. 1982), and consumption of raw milk from goats and

cows was associated with campylobacteriosis in 63 farm residents in Ontario between 1978 and 1985 (Thompson et al., 1986). In a survey of 1,720 raw milk bulk tanks in Ontario, 8 isolates of *Campylobacter* spp. (0.5% of milk samples) were detected (Steele et al. 1997). However in another study, *Campylobacter* spp. were not isolated from 34 raw milk cheese samples from Ontario, or from 126 cow, sheep and goat milk samples collected from raw milk cheese manufacturers from across Canada (Medeiros et al. 2008). Further work in determining prevalence of campylobacters in raw milk across Canada is necessary to provide useful relevant risk information.

Produce

The prevalence of *Campylobacter* spp. in fresh produce has not been investigated frequently in Canada (Table 6). Park and Sanders (1992) analyzed 10 types of vegetables taken from farmers' outdoor markets and supermarkets in Ottawa, Ontario for the presence of thermotolerant campylobacters. In this study *Campylobacter* spp. were isolated from nine samples of spinach, lettuce, radish, green onions, parsley, and potatoes from the outdoor markets, but not from vegetables washed with chlorinated water. A recent follow-up study of 410 samples from the same market found no *Campylobacter* (Carrillo et al., 2013). In other studies, campylobacters were not isolated from retail vegetables in Ontario (Park and Sander, 1992; Medeiros et al. 2008; Public Health Agency of Canada, 2009b) or Alberta (Bohaychuk et al. 2006).

The results in the above studies using cultural methods indicate a low prevalence of campylobacters in fresh vegetables in Canada, which is similar to results of other studies in industrialized countries (Jacobs-Reitsma et al. 2008). There has been no report of campylobacteriosis in human due to consumption of produce in Canada. This could be due to the difficulty in determining source of

typically sporadic cases of the campylobacteriosis in humans or to difficulties in isolating the organisms that could be stressed in a viable but non-culturable (VBNC) state or dead due to poor survival under unfavourable environmental conditions. Produce-associated outbreaks have been reported in the USA, with 5% of 262 outbreaks due to produce from 1997-2008 (Taylor et al. 2013). Further studies of commonly consumed vegetables with larger sample sizes would be useful to better understand the contribution of produce to campylobacteriosis in Canada. It would also be worthwhile to further investigate procedures for isolation of campylobacters, that are stressed or in the VBNC state in vegetables and fruits in Canada.

Summary of detection and molecular typing methods and testing programs for *Campylobacter* spp. in Canada

Detection methods

Traditionally campylobacters have been detected using culture procedures, and cultural isolation remains the gold standard for confirming the presence of live bacteria in a sample. Molecular and immunological methods, particularly PCR, have also been used for detection of *Campylobacter* spp. in different sources (Moore et al. 2005). For the culture methods, three types of culture procedures are commonly used: direct plating, enrichment in broth followed by plating on selective agars, and filtration before direct plating or before plating enrichment broths (Corry et al., 1995; Jokinen et al. 2012). Many different culture media are available for the isolation of campylobacters. However, to date there is no generally accepted single 'standard' method of isolating and detecting all *Campylobacter* species due to different requirements of temperature, microaerobic conditions,

nutrients and susceptibility to selective antibiotics (Corry et al. 1995; Moore et al. 2005). In Canada, an official reference test method for *Campylobacter* spp. in food published in Health Canada's Compendium of Analytical Methods is currently in place, namely MFLP-46 (Health Canada, 2014) using Park and Sanders enrichment broth followed by selective plating using mCCDA and Preston agars. Another method, the Agriculture and Agri-Food' Canada Food Safety Procedures Manual-10 (FSPM-10) (Agriculture and Agri-Food Canada, 1997) uses Rosef broth followed by plating on Mueller-Hinton Blood agar with antibiotics. Both methods have been used in a number of Canadian surveys (Table 7). Other methods commonly used in Canada and internationally include the International Standard Organisation (ISO) 10272-1:-2006 standard on detection of *Campylobacter* (International Standard Organisation, 2006) and the method published in the Bacteriological Analytical Manual of the USDA (United States Department of Food and Drug Administration, 2001). Both of these methods enrich samples in Bolton broth followed by plating on mCCDA selective agar, along with a variety of secondary agars. A similar method, MLG 41.03 published by the USDA Food Safety and Inspection Service (FSIS), uses direct plating on Campy-Cefex agar and enrichment in Bolton broth, with plating of enrichment broths on Campy-Cefex agar (United States Department of Agriculture, 2014) has recently been used for a baseline survey of poultry coordinated by the Canadian Food Inspection Agency.

There were at least 67 published surveys by 2014 in Canada, including either a single publication or continuing multi-year projects such as the surveillance by FoodNet Canada, which is considered as one survey in this review. Among these surveys, 61 primarily used cultural methods (Tables 1 - 6), 6 primarily used PCR methods, and 2 additional surveys used culture and conventional or real-time PCR methods in parallel (Tables 1, 3, 4 and 5).

454
455 The culture methods used in 61 Canadian surveys included 23 surveys using direct plating surveys,
456 39 using enrichment followed by selective plating, 1 using either direct or enrichment depending on
457 sample types, and 5 using filtration or centrifugation followed by direct plating or enrichment
458 Eleven different agars were used for direct plating with mCCDA, Campy-BAP and Karmali being
459 used most frequently (Table 7). Among the seven different enrichment broths used, Bolton broth
460 was the most frequently used followed by Rosef, and Park and Sanders broths (Table 7).

461
462 The significant variability among surveys of the same or similar types of samples (Tables 1 - 6)
463 may in many cases reflect the use of different methods. For example, in a comparative analysis of
464 two methods commonly used in surveillance of poultry samples, the MFLP-46 method using Park
465 and Sanders broth was found to detect 5% more campylobacters than that by the ISO 10272-1:2006
466 method using Bolton broth (Carrillo et. al., 2014), and it was suggested that the incorporation of the
467 antifungal agent, cycloheximide, in the less sensitive method may be responsible for this difference.
468 There have been a number of reports in Canada indicating that antibiotics in selective media inhibit
469 growth of some *Campylobacter* species, particularly *C. coli* (Ng et al., 1985, Brooks et al., 1986;
470 Ng et al., 1988; Carrillo et. al., 2014).

471
472 Several different selective agars were used for plating after enrichment in Canadian surveys. Due to
473 the different antibiotics used in the various agars, the usage of selective agars would likely have
474 affected the isolation of different species and strains. For example, with addition of 2.0 µg of
475 ciproflaxin to mCCDA, (Inglis et al. 2006) demonstrated a 2-fold increase in recovery of *C. fetus*

from calf fecal samples. The effect of the various selective agars on isolation of different *Campylobacter* species and strains in Canada needs further investigation.

Most *Campylobacter* surveys across Canada employed an incubation temperature of 42°C and microaerobic condition (85% N₂, 10% CO₂, 5% O₂), which are commonly used for isolation of thermophilic campylobacters. Occasionally, additional H₂ was used for isolation of *C. fetus*, *C. jejuni*, *C. coli*, *C. hyointestinalis* and *C. lanienae* in cattle (42°C), and *C. canadensis* sp. nov. in whooping cranes at (37/42°C) (Hoar et al. 2007; Inglis et al. 2006; Inglis et al., 2007). In a large scale water study in Canada, (Khan et al. 2013b) also found higher prevalence (60% of 759 samples) when using 37°C culture incubation temperature than that (34%) at 42°C, indicating incubation temperatures significantly affected the recovery of various *Campylobacter* spp.

In addition, campylobacters are remarkably sensitive to environmental conditions, including dehydration, atmospheric oxygen, sunlight and elevated temperature. Therefore, the sampling methods, freshness of samples, and sample transportation and storage conditions could also have a great impact on the recovery of these organisms. In 60 Canadian surveys that employed cultural methods, the protocols for sampling, type and amount and volume of samples, and sample transportation and storage conditions varied. Samples were collected at various times (immediately after slaughter, in field, or in store), in original form or using swabs, transported with or without transport medium at room temperature or on ice, kept at room temperature or refrigerated before testing, or kept for hours or days before testing. For example, among nine published studies using ruminant fecal material for isolation, the samples were collected and transported in Cary-Blair (Munroe et al. 1983; Lefebvre et al. 2006a) or charcoal (Hannon et al. 2009a) medium in three

studies, whereas in six studies, samples were transported as the original material or as swabs without the use of a transport medium (Waltner-Toews et al. 1986; Van Donkersgoed et al. 1990 (b); Inglis et al. 2006; Van Donkersgoed et al. 2009; Sutherland et al. 2009; Public Health Agency of Canada, 2010). In four of these nine studies, samples were transported on ice or at refrigeration temperature (Inglis et al. 2006; Lefebvre et al. 2006a; Hannon et al., 2009a; Public Health Agency of Canada, 2010 and 2011d), while in the other five studies, the samples were shipped at ambient temperature (Munroe et al. 1983) or under non-disclosed conditions (Waltner-Toews et al. 1986; Van Donkersgoed et al. 1990 (b); Van Donkersgoed et al. 2009; Sutherland et al., 2009). FoodNet Canada surveys also reported negative result for poultry fecal samples in the first year and much lower numbers of positive samples for the second year of the surveillance compared with higher and stable prevalence for chicken meat at the same period of time during, and higher and stable prevalence for the subsequent years conducted by the same program (PHAC, FoodNet Canada annual reports, 2006-2013). The negative or low prevalence results in the first two years for the poultry fecal samples were believed to be due to the dryness of the samples (PHAC 2010). These variations in sampling methods, sample transportation and storage may lead to variable results between studies.

Similar to many other bacterial organisms, campylobacters have also been shown to be able to enter a viable but non-culturable (VBNC) state under harsh conditions (Chaveerach et al. 2003; Li et al. 2014; Magajna and Schraft, 2015). The VBNC cells are characterized by a loss of culturability on routine enrichment and agar, which impairs their detection by conventional culture techniques (Chaveerach et al. 2003; Li et al. 2014; Magajna and Schraft 2015). This could lead to an underestimation of total viable cells in food and environmental samples. *Campylobacter* in VBNC

state may be still infectious to humans (Oliver, 2010; Li et al. 2014). Therefore, VBNC pathogenic bacteria should be considered a threat to public health and food safety due to their nondetectability using conventional food, water and other environmental testing methods. This issue should be considered for further development of new methods and strategies for testing of campylobacters in food and environment in Canada.

Eight surveys employed PCR as a primary tool or in parallel with culture for detection of *Campylobacter* spp. DNA with 3 surveys using conventional PCR (Inglis et al. 2003; Guévremont et al. 2008; Lefebvre et al. 2006b) and 5 surveys using real-time PCR (Inglis and Kalischuk, 2004; Chaban et al., 2010; Himsworth et al., 2010; PHAC 2010; Van Dyke et al. 2010). An additional 9 studies used PCR for confirmation or species identification (Lévesque et al. 2006a; Inglis et al., 2007; Jokinen et al., 2010; Sutherland et al., 2009; Leonard et al. 2011; Khan et al. 2013a; Khan et al. 2013b; Schmidt et al. 2013; Khan et al. 2014). In these studies different primers targeting various gene regions were employed. For studies in which PCR and culture were compared, the PCR procedures showed a higher detection rate than the culture procedure (Van Dyke et al. 2010; FoodNet Canada annual reports 2006 and 2007), indicating that detection methods could make a difference in final results particularly when the contamination level is low. In addition, some *Campylobacter* spp., such as *C. lanienae*, could not be cultured on commonly used media (Inglis and Kalischuk 2003). PCR methods could potentially be used as rapid, quantitative and universal methods for detection of wide range of *Campylobacter* spp. However, in most cases, PCR methods do not distinguish between live and dead cell, and likely overestimate prevalence of viable campylobacters.

Most of the studies in Canadian surveys (Tables 1 – 6) were conducted using available methods without indicating the detection limit and specificity. Only a small number of studies in Canada indicated the detection limit, including those using culture methods (Lammerding et al. 1998; Cook et al. 2012; Schmidt et al. 2013; Khan et al. 2013a and b, 2014; FoodNet Canada annual reports) and those using PCR methods (Inglis et al., 2003 and 2004; van Dyke et al. 2010; Himsworth et al. 2010; Chaban et al. 2010). Even for the commonly used methods, different results could be obtained due to the various detection sensitivities when using methods that had not been validated in the original studies. It is difficult to make judgements on the potential false-positive and negative results without knowing the uncertainties of test methods for various types of samples.

The use of standard or equivalent methodologies capable of detection of all *Campylobacter* species with known detection limit for various types of samples is necessary to obtain a clearer picture of the distribution of *Campylobacter* species in Canadian sources. There is also a need to develop or adopt and validate internationally recognizable or comparable and consistent methods to be used in Canada for rapid detection of thermotolerant campylobacters in foods. To date there is no generally accepted single 'standard' method of isolating and detecting all *Campylobacter* spp. In recent years, several procedures including immuno-based assays (ELISA, PCR-ELISA) and DNA-based detection methods (conventional and real-time PCR are becoming more widely accepted internationally (Moore et al. 2005 and Humphrey et al. 2007) and in Canada.

Typing methods

A number of subtyping methods have been applied to *Campylobacter* surveillance in Canada. These have included phenotypic methods (biotyping, serotyping and phage typing), genotypic methods (e.g. PFGE, flagellin-based subtyping and multi-locus sequence typing (MLST)). Currently, the most widely deployed typing method for national surveillance programs is Comparative Genomic Fingerprinting (CGF) (Taboada et al. 2013).

Valuable information regarding the epidemiology of campylobacteriosis has been obtained by the application of *Campylobacter* sub-typing methods to isolates from human, animal, food and environmental samples from across Canada. In early studies, two serotyping schemes developed in Canada in the 1980s, namely the Penner scheme based on heat-stable antigens (Penner and Hennessy, 1980) and the Lior scheme based on heat-labile antigens (Lior et al. 1982), were widely used to characterize *Campylobacter* isolates. Epidemiological linkages between isolates of the same serotype were demonstrated for sporadic cases and outbreaks of human gastroenteritis in Ontario and British Columbia (Karmali et al. 1983; McMyne et al. 1982), and on isolates from cattle and pigs in Ontario (Munroe et al. 1983; Garcia et al. 1985). While serotyping schemes have historically been extremely effective for classification of *Campylobacter* isolates, more recent studies favor the use of molecular typing methods.

A number of genotypic subtyping methods have been developed for *Campylobacter* spp. (reviewed in Humphrey et al. 2007; Taboada et al. 2013). The methods that are most widely used in Canada include: 1) PFGE (e.g., Steele et al. 1998, Nadeau et al. 2002, Clark et al. 2003, Guévremont et al. 2004, 2014), 2) characterization of the flagellin locus through restriction fragment length polymorphism (*flaA*-RFLP) or DNA sequencing of the short variable region (*flaA* SVR) (e.g. Clark

et al. 2005, 2012, Guévremont et al. 2014) , 3) MLST (e.g., Clark et al. 2005, 2012), and 4) CGF (Taboada et al. 2012; Taboada et al. 2013, Deckert et al. 2014). Heat-stable serotyping and fla-RFLP typing (Clark et al. 2003), and Oxford MLST or flagellin short variable region (fla-SVR) sequencing methods (Clark et al. 2005) helped to identify the *Campylobacter* spp. outbreak strains and their source for a waterborne disease outbreak in Walkerton, Ontario in 2000. In Canadian studies, the choice of a particular technique has depended to a large extent on the level of discrimination desired (Steele et al. 1998; Guévremont et al. 2004; Clark et al. 2005, Clark et al. 2012). The limitation of using different subtyping methods is that data generated using different methods cannot be compared.

New methods based on binary typing or comparative genomic analysis (Hannon et al. 2009b; Clark et al. 2012; Taboada et al. 2004, 2012,) are proving to be very useful for both large scale and small scale population typing. The CGF typing scheme has been widely adopted in Canadian surveillance programs (Clark et al. 2012, Taboada et al. 2013, Deckert et al. 2014). The use of this low cost, high throughput methodology has enabled the characterization of thousands of isolates (E. Taboada, personal communication) which can now be analyzed in aggregate, supporting the study of *Campylobacter* epidemiology in Canada (Clark et al. 2012, Deckert et al. 2014).

Recently, whole genome sequencing (WGS) has emerged as a powerful tool for research and diagnostics. WGS data provides the highest levels of discriminatory power for epidemiologic subtyping since single nucleotide differences can be used to distinguish isolates (Carrillo et al., 2012; Taboada et al. 2013). In addition, typing data for historical methods such as MLST and flaA SVR can be derived from WGS data *in silico* enabling comparison to previous studies (Taboada et

al. 2013). Moreover, WGS data generated in different studies can be easily compared at both the national and international level, regardless of methods used to generate sequence data. With decreasing costs, WGS is rapidly becoming a viable option for *Campylobacter* subtyping.

Surveillance program in poultry

The consumption and handling of contaminated poultry meat seems to be the most common risk factor for human campylobacterosis internationally (Keener et al. 2004; Humphrey et al. 2007) and in Canada (Ravel et al. 2009). Multifaceted intervention approaches are required to successfully control contamination of poultry during the various phases of the growth period and processing procedure of broiler chickens. In recent years, there is an international trend to investigate baselines at raw meat and farm levels to effectively understand the effects of intervention measures. Efforts to eliminate *Campylobacter* transmission via the food chain or to reduce the prevalence of *Campylobacter* in poultry slaughter house or flocks have been made internationally (White et al. 1997; Moore et al. 2005; Krause et al. 2006). Many countries, such as the European Union (EU) member countries, United States of America, Iceland and New Zealand (European Food Safety Authority, 2010; Stern et al. 2003; Department of Agriculture of United States - Food Safety and Inspection Service (USDA-FSIS), 2008 and 2012; Wagenaar et al. 2013) have conducted coordinated baseline studies. These baseline studies are an important step for designing further reduction measures. Reduction measures in Iceland including freezing carcasses from positive flocks have significantly reduced prevalence in chicken meat and have resulted in a 72% reduction in cases of human campylobacterosis (Trustin et al. 2011; Wagenaar et al. 2013). In New Zealand, interventions applied to poultry production resulted in a 54% of decline of human campylobacterosis between 2002 and 2008 (Sears et al. 2011). In 2010, the USDA-FSIS

announced performance standards and a compliance guide for *Salmonella* and *Campylobacter* in young chicken and turkey to help the poultry industry address *Salmonella* and *Campylobacter* (USDA, 2010). In Canada, there have been no mandatory federal or national policy and testing/control programs for foodborne campylobacters in food or food animals; however, there have been some efforts by federal, provincial or municipal governments as described above (Table 1 – 6) and baseline surveys of poultry have been recently conducted under pathogen reduction initiatives in Canada. Although HACCP programs have not applied for campylobacters in food programs in Canada, a recent survey in Alberta (Van Donkersgoed et al. 2009) recovered campylobacters from 76% of cattle manure samples (1590/2100) in 21 Alberta feedlots, but only one isolate of *Campylobacter* (1/1653) from beef carcasses in the cooler following HACCP interventions. These results indicate that HACCP interventions play an important role in controlling the spread of campylobacters during processing of cattle. To date, most of the surveys in Canada used only qualitative isolation methods. More informative Canadian baseline studies and food testing programs would benefit from the application of standardized, simple and rapid quantitative culture (or equivalent) methods.

Summary

Although most cases of gastroenteritis caused by *Campylobacter* infection are mild and only rarely result in death, campylobacteriosis represents a significant public health burden (Ruzante et al., 2011), with costs high enough to justify prevention efforts. The understanding of the distribution and prevalence of campylobacters in food, animals, water and the environment is a crucial step for prevention. It has been shown that campylobacters are widely distributed in retail meats, domestic and wild animals, poultry, pets, water and soil in Canada. Prevalence does not seem to have

changed significantly since testing programs began in the 1980s, however, it is difficult to assess changes in prevalence in different sources due to variability of the small scale studies conducted in Canada since this time. There have been a few surveillance studies at the national, provincial and academic levels in Canada, but more data is needed on a national scale, especially in linking clinical and source data. Continued and further coordinated support with consistent and comprehensive schemes for these initiatives is needed. This will greatly enhance the understanding of the epidemiology of these organisms, virulence factors associated with different strains and importantly their traceability. The lack of coordinated national prevalence studies has resulted in reliance on regional investigations that lack consistency in sampling, sample shipping, culture and molecular procedures and consequently a wide range of results across Canada have been reported. Therefore, there is a need to improve, validate and standardize comparable or equivalent methods and protocols according to international requirements and trends to meet different needs of government and industry testing laboratories. There was a paucity of studies in the 1990s during which almost no testing on campylobacters in animals or the environment was reported in Canada (Tables 1, 2, 3 and 5). Therefore, a long term plan should be established for the continuity of monitoring this organism from various sources. Careful and coordinated implementation of studies to detect campylobacters in various sources will enhance understanding of the sources of transmission of these organisms to humans and inform implementation of effective control and preventive measures.

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Table 1. Prevalence and culture procedures used in detection of campylobacters in samples from cattle.

Sample type	Number of Samples	Province ^a	Test method	Prevalence %	Reference
Feces or intestinal	412	ON	Direct plating	19	Munroe et al., 1983
Tissues	525	ON	Enrichment	50	Garcia et al., 1985
Feces	78 (farms)	ON	Direct plating (unknown agar)	13	Waltner-Toews et al., 1986
Neck muscle, gallbladder	666 (beef), 267 (veal)	National	Enrichment	22.6 (beef), 43.1 (veal)	Lammerding et al., 1988
Samples (8 herds with abortions)	Fetus & placenta 120 & 108	SK	Direct plating	10-19 ^b	Van Donkersgoed et al., 1990b
	Feces 19-140			0-50 ^b	
Abomasal tissues	30	SK	Direct plating	20	Jelinski et al., 1995
Feces	382	AB	Conventional and nested PCR	83	Inglis et al., 2003
Feces	299	AB	Nested Real-time PCR ^c	90	Inglis et al., 2004
Feces	7738	AB	Direct plating	20.5	Inglis et al., 2006
Feces	50 (pooled)	BC	Enrichment	60-80	Pritchard et al., 2006
Feces	80	QC	Direct plating	78.7	Lefebvre et al., 2006
Feces	185	QC	Nested PCR	21.6	Guévremont et al., 2008
Manure, carcass after HACCP	2100, 1653 ^d	AB	Enrichment or filtration/plating	76, 0.06	Van Donkersgoed et al. 2009
Fecal swabs	2776	AB	Direct plating	87	Hannon <i>et al.</i> , 2009a
Feces Dairy	112 – 120 (2006 - 2013) ^{e,f}	ON	Enrichment ^g	21 - 82	C-EnterNet or FoodNet annual reports 2006-2013
Beef	80 - 120 (2007 – 2013) ^{e,f}			13 - 82	

^a: ON=Ontario, SK=Saskatchewan, AB=Alberta, BC=British Columbia, QC=Quebec.

^b: Range of prevalence between 8 herds.

^c: Detection limit: $\approx$ 250 CFU/g of feces.

^d: In the same order as samples.

^e: Individual sample (Year).

^f: 30 farms between 2010 and 2013 with prevalence of 97-100%.

^g: Detection limit: 0.3 -10 CFU/g.

Table 2. Prevalence and culture procedures used in detection of campylobacters in samples from poultry.

Sample type ^a	Number of sample	Province ^b	Test method	Prevalence %	Reference
Feces or intestinal content	398	ON	Direct plating	27.1	Munroe <i>et al.</i> , 1983
Cecal/cloecal of chicken, duck & turkey	235	QC	Enrichment	67.7	Bourque, 1983
Colonic samples	600	ON	Direct plating	46.7	Prescot <i>et al.</i> , 1984
Carcasses	205 (turkey)/410 (broiler)	National	Enrichment	73.7/38.2	Lammerding <i>et al.</i> , 1988
Liver with or without necrotic hepatitis	223 diseased, 50 normal	QC	Direct plating	19.4 (21.1 abnormal livers; 12 normal livers)	Boukraa <i>et al.</i> , 1991
Broiler chickens	2325	QC	Direct plating	40	Nadeau <i>et al.</i> , 2002
Cecal samples	450 flocks	BC	Direct plating	5.6 – 88 ^c	Pritchard <i>et al.</i> , 2006
Chickens and turkey cecal samples	2430	QC	Enrichment	35.8 (chickens)/46 (turkey)	Arsenault <i>et al.</i> , 2007a&c
Chicken & turkey cecal samples	2460 (chicken)/770 (turkey)	QC	Enrichment	69.5/ 36.9	Arsenault <i>et al.</i> , 2007b
Carcass rinse	1234	AB	Enrichment	75	Bohaychuk <i>et al.</i> , 2009
Cecal samples (organic farm)	300	QC	Direct plating	50	Thibodeau <i>et al.</i> , 2011
Feces	36 - 200 (year 2007-2013) ^d	ON, BC	Enrichment	0-46	PHAC FoodNet annual reports 2006- 2013
Turkey feces	112 (2013) ^e	BC		79	

^a: Chicken samples otherwise explained.
^b: ON=Ontario, QC=Quebec, BC=British Columbia, AB=Alberta.
^c:Range of prevalence between 8 herds
^d: ON (2007- 2013), BC (2013). 24-30 farms (ON, 2010-2013, BC, 2013) with prevalence of 7-42%.
^e: 28 farms with 82% prevalence.

Table 3. Prevalence and procedures used to detect campylobacters in samples from pigs.

Sample type	Number of sample	Province ^a	Test method	Prevalence %	Reference
Feces	203	ON	Direct plating	58.1	Munroe et al., 1983
Muscle (neck, diaphragm) & feces	666	MA	Enrichment	26.3	Finlay et al., 1986
Neck muscle and gallbladder	469	National	Enrichment	16.9	Lammerding et al., 1988
Muscle (diaphragm) & feces	400	QC	Direct & enrichment	61.7 (99 for feces)	Mafu et al., 1989
Cecal sample	109	PEI	Direct plating	72.5	Hariharan et al., 1990
Rectal sample	1200	ON	Enrichment	99	Varela et al., 2007
Feces	20	QC	Conventional & nested PCR	25	Guévremont et al., 2008
Feces (manure and individual)	359 /21	ON	Enrichment	37/68	Farzan et al., 2010
Feces	111-120 /30 (2006 – 2011) ^{b c}	ON	Enrichment	10-85/40-100 ^c	FoodNet Canada annual reports 2006-2011, PHAC
Carcass swabs	1070	AB	Enrichment	8.8	Bohaychuk et al., 2011

^a: ON=Ontario, MA=Manitoba, QC=Quebec, PEI=Prince Edward Island, AB=Alberta.

^b: Individual/farm (year).

^c: 2009 data is based on 2010 short report (2009 annual report was not complete for farm information). No test after 2011.

Table 4. Prevalence and procedures used in detection of campylobacters in pets and wildlife.

Sample type	Number of Sample	Province ^a	Test method	Prevalence %	Reference
Pronghorns and mule deer feces	187	SK	Enrichment	0	Van Donkersgoed et al., 1990a
Cloacal swabs from ring-billed gulls	264	QC	Enrichment	15.9	Quessy and Messier, 1992
Tree-nesting Double-crested cormorant chicks, cloacal & pharyngeal swabs	100	PEI	Direct plating	22	Dobbin et al., 2005
Shellfish (Whole clams) (<i>Mya arenaria</i> clams)	72	QC	Conventional PCR/culture confirmation	47.2	Lévesque et al., 2006
Whooping and sandhill cranes	42	AB	Direct plating	5	Hoar et a., 2007
Captive adult whooping cranes (<i>Grus americana</i>)	10	AB	Enrichment/PCR characterization	Isolation of a new species: <i>C. canadensis</i> sp. nov.,	Inglis et al., 2007
Cloacal swabs					
Dog fecal, hair, rectal, aural, nasal, oral & pharyngeal swabs	102	ON	Conventional PCR	0	Lefebvre et al., 2006.
Waterfowl feces (seagulls, Canada geese, ducks)	51	ON	Enrichment/PCR	29(culture)/ 32(PCR)	Van Dyke et al. 2010
Dog feces	75 (healthy), 60 (diarrheic)	SK	Real-Time PCR	58 healthy, 97 diarrheic	Chaban et al., 2010
Dog feces	60	SK	Real-time PCR	75	Himsworth et al., 2010
Feces of dog, horse, llama, alpace, goose, duck, rabbit, rat	17, 23, 10, 9, 13, 23, 11, 1 ^b	BC	Enrichment/PCR identification	5.9, 4.3, 10, 11.1, 38.5, 52.2, 0, 0 ^c	Jokinen et al., 2010
Dog feces	240	ON	Direct plating or Enrichment/PCR identification	22	Leonard et al., 2011
Raccoon feces	50	ON	Enrichment/plating± membrane filtration.	78 (76 with filtration)	Jokinen et al., 2012
Dog feces	251	ON	Direct plating or Enrichment/PCR identification	43	Procter et al., 2014

^a: SK=Saskatchewan, QC=Quebec, ON=Ontario, PEI=Prince Edward Island, AB=Alberta.
^{b, c}: numbers in the order as the order of sample types.

Table 5. Prevalence and procedures used in detection of campylobacters in samples from water.

Sample type	Number of sample	Province ^a	Test method	Prevalence %	Reference
Catch basin water	21(spring)/21/(fall)	AB	Enrichment	52/0	Van Donkersgoed et al., 2009
Rivers and private surface wells	2471	QC	Enrichment or PCR ±filtration	43	St-Pierre et al., 2009
Waterways water (sheep pasture)	143	ON	Enrichment/PCR confirmation	14.7	Sutherland et al., 2009
River water	344	ON	Enrichment/PCR	0-23 (culture)/57-79 by PCR	Van Dyke et al., 2010.
River watershed water (4 sites)	186	BC	Enrichment/PCR identification/RFLP typing	31.5-56.7	Jokinen et al., 2010
Water (title drainage)	126	ON	Filtration/MPN ^b /PCR classification	73 (41-100)	Schmidt et al., 2013
Beach and river water	389	ON	MPN/PCR confirmation ^c	13	Khan et al., 2013a
Water (Beach, offshore, wastewater)	597	ON	Centrifugation/enrichment/MPN/PCR confirmation	61-63 ^d	Khan et al., 2013b
Agricultural surface water	699 (2009) ^e	AB, BC, ON, QC	filtration or centrifugation/enrichment	49 (centrifugation)/33(filtration)	Khan et al., 2009 & 2014
	769 (2014)		Semi-quantitative MPN/PCR confirmation	49 ^f	
Various types of water ^g	20 - 134 (2006-2013)	ON, BC	Enrichment/quantitative PCR ^g	9 -52 (58-92, Molecular) ^h	FoodNet Canada annual reports (2006-2013), PHAC

^a: AB=Alberta, QC=Quebec, ON=Ontario, BC=British Columbia.

^b: Practical upper and low detection limit: 1-2419 CFU/100 ml water (theoretical detection limit: 0.02 CFU/100 ml).

^c: Generally ≤30 cells per liter.

^d: Results for incubation at 37°C which had higher prevalence than that incubated at 42°C (data not included).

^e: (Year).

^f: 4 - 4000 minimum probable number (MPN) cells per little (L⁻¹).

^g: Untreated and recreational water for 2006-2013 in ON and BC depending on years, irrigation water in BC for 2013.

^h: Quantitative PCR, referred as molecular test in the reference, was conducted for 2006 and 2007.

Table 6. Prevalence and procedures used in detection of campylobacters in samples from raw and retail foods.

Sample type	Number of samples	Province ^a	Test method	Prevalence %	Reference
Raw Meat					
Chicken carcass	500	ON	Enrichment	62	Park et al., 1981
Chicken carcass	150	ON	Enrichment	84	Park et al., 1983
Chicken carcasses	177	QC	Centrifugation/enrichment	23	Michard et al., 2004
Chicken legs and wieners, turkey breast	100, 100 & 101 ^c	AB	Enrichment	62 (chicken legs); 0 (rest)	Bohaychuk et al., 2006
Chicken & turkey	1200 (chicken), 454 (turkey)	ON	Filtration/enrichment	45.8 (turkey), 62.4 (chicken)	Valdivieso-Garcia et al., 2007
Whole or parts of chicken and turkey	51–78	Atlantic, QC, ON, BC	Enrichment	30.3-49.2	Iugovaz et al., 2009
Chicken, Beef Liver	55	ON	Enrichment	9.7 (chicken)/12.5 (beef liver)	Medeiros et al., 2008
Turkey	412	ON	Enrichment/plating+filtration	46	Cook et al., 2009
Chicken carcasses	1256	ON	Filtration/enrichment	59.6	Deckert et al., 2010
Veal (grain-fed)	438	ON	Enrichment/plating+filtration	1	Cook et al., 2011
Carcass swabs in slaughter house cooler	Cattle 1022 Pig 1070	AB	Enrichment	1.5 8.8	Bohaychuk et al., 2011
Turkey meat	412	ON	Enrichment/plating+filtration	46	Cook et al., 2012
Chicken meat	348	National	Enrichment/direct plating	42.8	Carrillo et al., 2014.
Chicken Skin-on breast	127- 200 (2006-2013) ^d	ON, BC	Enrichment	31-51	FoodNet Canada annual reports 2007-2013, PHAC
Ground Chicken	87-158 (2011-2013)			13-68	
Ground turkey	96-155 (2011)			16-44	
Frozen chicken nuggets	24-129 (2011-2013)			0-59	
Ground beef (not tested since 2011)	187 - 200 (2007-2010)			1-2	
Pork chop	140-200 (2006-2010)			0-18	
Ready-to-eat					
Produce	1564	ON	Enrichment	1.6 - 3.3 in various vegetables ^b	Park and Sanders, 1992
Meat, raw milk cheese, vegetable etc.	261	National	Enrichment	0	Medeiros et al., 2008
Fresh produce	674	AB	Enrichment	0	Bohaychuk et al., 2009
Leafy greens	376 (2009)	ON	Enrichment	0	FoodNet Canada annual reports 2007-2013, PHAC
Produce (Market)	410	ON	Enrichment	0	Carrillo et al., 2013

^a: ON=Ontario, QC=Quebec, AB=Alberta, Atlantic=Atlantic provinces, BC=British Columbia.
^b: Range of prevalence between various types of vegetables.
^c: In the same order as samples.
^d: Range of sample numbers (year) depending on year and location; tests conducted in BC for 2011-2013.

Table 7. Summary of culture media used in Canadian surveys.

Direct plating			Enrichment		
Agar type	Number of studies	Frequency (%)	Broth type	Number of studies	Frequency (%)
mCCDA ^a	5	21.8 ^b	Bolton	19	50
Campy-BAP ^c	3	13	Rosef	6	15.8
Karmali	3	13	Park and Sanders	6	15.8
Preston	2	8.7	Hunt	3	7.9
Skirrow	2	8.7	Preston	2	5.3
Muller-Hinton	2	8.7	Skirrow	1	2.6
Columbia	2	8.7	Weybridge ^d	1	2.6
Campy-line	1	4.3			
MCBB ^e	1	4.3			
Weybridge ^d	1	4.3			
Unknown	1	4.3			
Sub-total	23 ^f			38 ^g	

^a: Modified charcoal cefoperazone desoxycholate;

^b: Approximate number due to the round-up;

^c: Campylobacter agar with 5 antimicrobics and 10% sheep blood;

^d: Weybridge transportation and enrichment medium;

^e: Modified Chocolate blood-based;

^f: These are the numbers of agars used as primary isolation media, and there were also two additional agars used with enrichment broth in parallel with a total of 25. To reflect the accurate numbers of surveys, 23 are used for this review;

^g: These are the numbers of enrichment broth used as primary isolation media, and there were also two additional broth used with direct plating in parallel with a total of 41. To reflect the accurate numbers of survey, 38 are used for this review.