

REVIEW

***Campylobacter*: from microbiology to prevention**

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Summary

In last years, *Campylobacter* spp has become one of the most important foodborne pathogens even in high-income countries. Particularly, in Europe, *Campylobacteriosis* is, since 2005, the foodborne disease most frequently notified and the second in USA, preceded by the infection due to *Salmonella* spp. *Campylobacter* spp is a commensal microorganism of the gastrointestinal tract of many wild animals (birds such as ducks and gulls), farm animals (cattle and pigs) and companion animals (such as dogs and cats) and it is responsible for zoonoses. The transmission occurs via the fecal-oral route through ingestion of contaminated food and water. The disease varied from a watery diarrhea to a severe inflammatory diarrhea with abdominal pain and fever and can be burdened by some complications. The main recognized sequelae

are Guillain-Barré Syndrome (GBS), the Reactive Arthritis (REA) and irritable bowel syndrome (IBS). Recently, many cases of *Campylobacter* spp isolated from human infections, showed an important resistance to various antibiotics such as tetracyclines and fluoroquinolones. For these reasons, the prevention of this infection plays an essential role. Many preventive measures exist to limit the transmission of the pathogens and the subsequent disease such as the health surveillance, the vaccination of the poultry and the correct food hygiene throughout the entire production chain. A global surveillance of *Campylobacteriosis* is desirable and should include data from all countries, including notifications of cases and the microbiological data typing of strains isolated from both human and animal cases.

Introduction

The bacteria belonging to the genus *Campylobacter* have long been recognized among the most common responsible agents of enteritis and gastroenteritis in humans, both among adults and in pediatric patients [1-3]. In recent years, in high-income countries, cases of *Campylobacteriosis* have exceeded those caused by classic enteric bacteria. The micro-organism is isolated from patients with infections of the alimentary tract with a frequency of about 3-4 times higher than in *Salmonella* or *Escherichia coli* [4]. In low- and middle-income the data, although poor, suggests that the rate of infection by *Campylobacter* has increased in recent years [5]. It is often difficult to trace the sources of exposure to *Campylobacter*, this is due to the sporadic nature of the infection and the important role of cross-contamination. For these reasons, over the past decade, many countries have put in place a number of important preventive measures to avoid these food-borne infections [6]. In addition, recent scientific advances, such as the complete sequencing of the genome of the microorganism, the new findings on causes of the infection and the recognition of the role of immunity in protecting against *Campylobacter* infections [7], exploitable process for the development point of the appropriate vaccine have led to a better understanding of the pathogenesis [8] and have helped to guide the Assessment and Management Risk along the chain “farm-to-table”.

Nevertheless, *Campylobacteriosis* remains a difficult disease to prevent and infection epidemiological trend continues to remain high throughout the world.

Microbiology

The taxonomy of the genus *Campylobacter* has been extensively revised; currently the immediate family is that of *Campylobacteriaceae*, which includes three distinct genera: *Campylobacter*, *Arcobacter* and *Helicobacter* [9]. The genus *Campylobacter* includes 22 species, of which the best known are *C. jejuni* and *C. coli*, the main responsible of gastroenteritis in humans, although other species such as *C. concisus*, *C. upsaliensis*, *C. ureolyticus*, *C. hyointestinalis* and *C. sputorum*, species now considered “emerging”, have been associated with gastroenteritis and periodontitis [9, 10]. All these species normally colonize different apparatuses of domestic or wild animals and can be found in many foods of animal origin [10]. The genus *Campylobacter* comprises gram-negative microorganisms, non-sporeforming and with variable dimensions, with a length between 0.5 and 5 µm and a width comprised between 0.2 to 0.9 µm [11]. Most of the species is mobile and is characterized by a spiral movement caused by a polar flagellum present on one or both ends of the cell. The only exceptions are *Campylobacter gracilis*, which is motionless, and *Campylobacter showae* that has multiple flagella [12]. The

DNA is around 1.6-1.7 Mbps and is rich in adenine and thymine; GC ratio is, in fact, about 30% [13-15]. From a metabolic point of view it is of micro-aerofili bacteria that, therefore, survive and grow best in an environment characterized by a low oxygen tension (5% O₂, 10% CO₂, and 85% N₂) [9, 16].

All species, except *C. gracilis*, synthesize the oxidase enzyme. Not ferment nor oxidize carbohydrates but they get energy from amino acids or tricarboxylic acid [12]. *Campylobacter* species is able to grow at pH between 6.5 and 7.5 and at temperatures between 37° and 42° C. For this reason is defined, by some authors, "thermophilic". Levin has, however, proposed that these micro-organisms are more correctly referred to as "thermotolerant" since they do not present a real thermophilic, being unable to grow at temperatures equal to or above 55° C [17]. They are also unable to grow at temperatures below 30° C, for the absence of the genes coding for the heat-shock-protein that play a role in the adaptation to low temperatures. Finally, it was shown that the growth does not occur in environments with water activity (aw) concentrations of less than 0.987 (sensitive to sodium chloride of greater than 2% w/v), while it is optimal if equal to 0.997 (about 0,5% w / v NaCl) [18].

Reservoirs and transmission

Campylobacter spp is a commensal germ of the gastrointestinal tract of many wild animals (birds such as ducks and gulls), farm animals (cattle and pigs) and companion animals (such as dogs and cats). It is, also, predominantly, in all avian species fit for human consumption [19-21]. They are micro-organisms responsible for zoonoses and the transmission occurs through the fecal-oral route through ingestion of contaminated food and water [22-24]. The main environmental niche is represented by the intestinal tract of all avian species, particularly poultry (ie broilers, laying hens, turkeys, ducks and ostriches) which is considered the main route of transmission [25-29]. The consumption of this meat, in fact, represents 50-70% of human cases of Campylobacteriosis [30]. However, even the consumption of raw milk, raw red meat, fruits and vegetables has been identified as a possible cause of transmission [31, 32]. Moore *et al.* have indicated that the prevalence of colonization by *Campylobacter spp* in cattle varies widely, even between 0-80% while it is around 20% in sheep [33].

POULTRY

Eating or handling raw or undercooked meat of chicken would be the main risk factor for contracting campylobacteriosis [34,35,36,37]. It was seen, in fact, that the feces of infected poultry may contain up to 105-108 CFU/g. These high levels permit bacteria to spread easily in the environment, thus allowing the contamination [38]. Bull *et al.* has estimated that the chicken meat retail is contaminated with *C. jejuni* up to 98% of cases in the US and from 60% to 80% of cases in Europe [39]. Contamination occurs between the same

farm animals, where transmission can be vertical in nature (*i.e.* from hen to chick via egg), quite rare event, or horizontally within the environment where the animals are bred [40, 41]. The infection can be contracted in the very first days of life, but the presence of the organism in stool samples is detected no earlier than two or three weeks old [42]. The reason for this lag phase is still unknown, but it could be due to the protective effect of maternal antibodies [43] or to the microbial flora of the animal itself. In the latter situation, the microbial flora residing in the chicken gastrointestinal apparatus could play a competitive role against *Campylobacter*, delaying the colonization [44]. During slaughter, however, the main critical points for contamination of carcasses were identified in plucking, evisceration and final washing. The treatment with water at temperatures above 60° C, causes a decrease of the bacterial load which, however, increases during the plucking operations causing a cross-contamination [45, 46]. The bacterial load also can further increase during the evisceration due to spill of intestinal content rich of *Campylobacter* [45, 47]. Moreover, the spread of the microorganism occurs through the shedding into the environment of wild bird feces [48]. Their presence in playgrounds has been recognized as an emerging environmental source of Campylobacteriosis, especially for children, who frequently put her hands to her mouth favoring the ingestion of germs [49, 50]. Many playgrounds are natural habitat for a variety of wildlife including birds, lizards, dogs and stray cats. New Zealand researchers have analyzed the bird fecal material collected in children's playgrounds and isolated *C. jejuni* in 12.5% of samples [49].

MILK

Unpasteurized cow's milk and dairy products are common vehicles for the transmission of *Campylobacter spp*; to identify them as a source of human Campylobacteriosis is already known since 1978, when four cases of infection by *C. fetus* have been identified in a hospital in Los Angeles County [51]. In 1996 Evans *et al.* has described an outbreak of Campylobacteriosis associated with the ingestion of raw milk occurs in U.K. [52]. Javid, later, led a study of cattle from a dairy, highlighting that 12% of samples of raw milk were contaminated with *C. jejuni* [53]. The likely causes of contamination of milk are possible contact with bovine feces, contaminated water or direct contamination due to bovine mastitis [54, 55].

FRUITS AND VEGETABLES

Numerous studies have shown the presence of *C. jejuni* and *C. coli* in lettuce, spinach, radishes and peas [56-59]. It is likely that the contamination of vegetables to occur as a result of irrigation with contaminated water, use of natural fertilizers or through the same soil contaminated with droppings predominantly avian origin [60-62]. It is also possible to cross-contamination during the handling and packaging or through kitchen utensils used for cutting of other foods such as poultry [63]. Verhoeff-Bakkenes *et al.* have shown that the consumption of fruit and vegeta-

bles, especially packaged, is an important risk factor for Campylobacteriosis: on 5.640 samples of fruit and vegetables analyzed in their survey, 13 (0.23%) were positive to *Campylobacter*, with a higher percentage (0.36%) in packaged products compared to fresh ones (0.07%) [31]. Kirk *et al.* and Blaser *et al.*, in the past published two reports relating to two *Campylobacter* outbreaks caused, respectively, by the consumption of cucumbers served at a buffet [64] and the consumption of salad prepared by an employee of a soup kitchen from whose hands was isolated *Campylobacter* [65].

WATER

European legislation provides that the natural mineral water obtained from springs and, occasionally, by drilling sources is free from parasites and pathogens. Unlike the water distributed through the taps, it cannot be subjected to any type of treatment that could alter its chemical composition [66]. A variety of organisms, including coliforms, can be found in mineral waters, in particular non-carbonated water supplied in plastic bottles and bottled by hand [67]; Gillespie *et al.*, reported a case in which the bottled water has been identified as a possible vehicle for *Campylobacter* infection [68].

SWINE AND CATTLE

It is important not to underestimate the role of cattle and pigs that are often colonized with *C. jejuni* and *C. coli* [69-74].

A study carried out by Taylor *et al.* in the US has revealed that 5% of outbreaks of Campylobacteriosis in the period from 1997 to 2008, was due to the consumption of contaminated meat pork, beef and game [75]. Multiple studies have also shown that *Campylobacter*, preferentially, colonize the lower gastrointestinal tract of cattle [72] but has also been found in the liver, gall bladder and bile juice [69, 73, 74]. Moreover, there is a higher prevalence of *Campylobacter* in cattle from intensive farming [68%] than in adult cattle grazing (7.3%) [71]. This could be explained by the greater density of animals that are constantly in contact with their own faeces and the sharing of areas including drinking water and food distribution [76, 77].

As for the pigs, these appear to be predominantly colonized by *C. coli* and, less frequently, by *C. jejuni* [74]; some studies have shown, however, the possible co-existence, in these animals, of both the microorganisms [78, 73]. As for cattle, colonization by *Campylobacter* in pigs was particularly notable among animals in intensive farming [69] than those reared in traditional agricultural systems [79].

SHELLFISH

Wilson and Moore have shown the presence of *Campylobacter* also in molluscs, colonization due, probably, to the contamination of the waters in which stalling and are collected [80]. In this study, have been isolated thermotolerant *Campylobacter spp* in 42% of samples analyzed. The majority of these (57%) were urease-positive thermophilic *Campylobacter* (UPTC) [81, 82], with a

clear predominance of *C. lari* [80]. In particular *C. lari* colonizes the intestine of seagulls that contaminate the water with their feces [83].

PETS

A number of domestic animals have been identified as hosts for *Campylobacter spp* [84-86]. In European and Asian research many Authors have reported the isolation of *C. jejuni*, *C. coli*, *C. upsaliensis*, *C. helveticus*, and *C. lari* from canine fecal samples [87-91]. In the United States, Stehr-Green *et al.* referred that 3% of all cases of salmonellosis and 15% of Campylobacteriosis can be attributed to contact with pets [92]. Chaban *et al.* reported the isolation of *C. jejuni* in 5 dogs feces of 70 (7%) at concentrations up to 10⁶ CFU/g [86]. Seeing as the infective dose of *C. jejuni* is estimated at 500 microorganisms [93], these high concentrations present in feces represent a possible risk factor for environmental contamination and human infection by accidental exposure. Veterinarians assume that the animal contamination could occur following the ingestion of raw meat [94, 95].

The transmission mode of *Campylobacter* from animals to humans have not yet been described; however, pet the animals and manipulate objects came into contact with them could transfer pathogens from the fur or object contaminated to the human hands and lead to infection [96].

FLIES

It has been shown that even the flies represent an important carrier for *Campylobacter* and they are, therefore, able to contaminate both humans that animals [97-99]. Gordon *et al.* have shown that some cases of diarrhea increased especially during the summer season when the larvae grow and mature by increasing the number of adult insects [100]. Some studies support this theory. Layton *et al.* and Neal *et al.* have reported the reduction of cases of diarrheal syndromes following the application of measures for fly control [101, 102]. They have assumed that the transmission of the disease occurs by direct contact of foods with the paws, proboscis and body fur of the insects that were contaminated with fecal or regurgitated material contaminated [100]. Contamination can occur at any stage of the food chain.

Epidemiology

UNITED STATES

In the US, there is an active surveillance system called FoodNet, which constantly monitors the spread of food-borne diseases. In particular, the surveillance program is concerned of control of 7 bacterial infections confirmed in the laboratory (*Campylobacter*, *Listeria*, *Salmonella*, *Escherichia coli* O157 and non-O157 of shiga-like toxin producers [STEC], *Shigella*, *Vibrio* and *Yersinia*), 2 parasitic infections (*Cryptosporidium* and *Cyclospora*) and cases of HUS. FoodNet system to belong, currently, 10 states (Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, California, Colorado

and New York), which together make up 15% of the US population (48 million people in 2011) and is the result of a collaboration between CDC, the Departments of Health of the 10 Member States, the UFSA-FSIS (US Department of Agriculture's Food Safety and Inspection Service) and the FDA (Food and Drug Administration). According to this network [103], in 2012 were 19,531 reported infections, 4,563 hospitalizations and 68 deaths associated with foodborne diseases. For most infections, the incidence was higher among children aged < 5 years, but the percentage of people hospitalized and died was highest among persons aged ≥ 65 years. In 2012, compared to the period 2006-2008, the overall incidence of infections was unchanged but increased cases of infections caused by *Campylobacter* and *Vibrio*. *Campylobacter*, in particular, ranked second, after *Salmonella*, as a cause of food-borne infections. The number of *Campylobacter* infections (incidence per 100,000 population) was 6.793 [14, 30] and, of them, were typed 2.318 (34%) isolates of which 2.082 (90%) were *C. jejuni*, and 180 (8%) were *C. coli*. Estimated incidence of infection was higher in 2012, compared to the period 2006-2008 (up 14% CI: 7%-21%). The percentage of hospitalized subjects was 31% while the percentage of patients who died ranged from 0.2%.

The 2013 data confirm that the food-borne infections continue to be an important public health problem in the United States and emphasize the importance of preventive measures. In particular, infections due to *Campylobacter spp* accounted for 35% of the total, preceded by those due to *Salmonella spp* (38%).

In 2014, FoodNet has identified 19,542 cases of infection with 4,445 hospitalizations and 71 deaths. The crude number and incidence was 6,486/100,000.

EUROPE

All the data concerning the epidemiology of foodborne infections in the European Union (EU) are published, annually, by the European Food Safety Authority [EFSA]. EFSA's headquarters is located in the city of Parma (Emilia Romagna, Italy). The data shows that in Europe Campylobacteriosis is, since 2005, the foodborne disease most frequently notified with over 190,000 cases reported each year in humans. However, it believes that the actual number of cases to be about nine million/year. In addition, according to the EFSA, the Campylobacteriosis cost for health systems, in terms of lost productivity, is approximately 2,4 billion euro per year.

In 2011 Campylobacteriosis has established itself as the most frequently reported zoonotic disease in humans, with a continuous increase of the reported cases [104]. In particular, a total of 220,209 cases of infection were reported, 2.2% more than in 2010. The food where *Campylobacter* was most found was the chicken meat. Despite the significant decrease in recent years, salmonellosis was again the second reported zoonotic disease with a total of 95,548 cases. Altogether *Campylobacter* was the most frequently reported cause, it is mentioned less often as the cause of outbreaks of food-borne. The most common food sources of outbreaks were eggs and

egg products, composite foods, fish and seafood products.

For the first time in five years, in 2012 human Campylobacteriosis decreased slightly, but is still the most commonly reported zoonotic disease, responsible for 214,268 cases of infection with a 4.3% decline compared to 2011 [105]. The notification rate was 55.49/100,000 inhabitants. Considering the high number of cases, the gravity (reported deaths) was low (0.03%). Overall, 23.6% of fresh chicken meat samples tested were positive for *Campylobacter spp*, less than in 2011 in which was positive for 31.3% of the samples.

Campylobacter spp in 2013 continued to be the most commonly reported gastrointestinal pathogen in the European Union (EU). The number of confirmed cases reported was 214,779, with an EU notification rate of 64.8/100,000, the same level of 2012 [106]. Mortality was low (0.05%). Overall, 31.4% of fresh chicken meat samples checked were positive for *Campylobacter spp*. In the period 2012-2013, this increase in *Campylobacter*-positive samples was mainly due to the placing of the data coming from Croatia, which reported results for the first time in 2013. *Campylobacter* it was also detected less frequently in the flesh of turkey and other foods. In 2013, moreover, they have been reported from 414 *Campylobacter* outbreaks. The sources of these outbreaks were, in order of importance, chicken meat and dairy products and other foods such as milk and mixed foods.

ITALY

In Italy, the latest data available on Campylobacteriosis concern 2006 with 476 isolations of *Campylobacter spp* from clinical specimens that have been reported by the laboratories of the Enter-net network. In 73,9% of cases the laboratories carried out the identification of species. *C. jejuni* was the most frequently isolated species. 35,5% of strains were isolated from pediatric patients under the age of 6 years, especially in the summer months. The presence of antimicrobial-resistant strains is high in particular for quinolones and fluoroquinolones [107].

Pathogenesis and virulence factors

Colonization and intestinal epithelium adherence are the first and indispensable stages of the disease pathogenesis. For this reason, the characteristic motility of the bacterium by polar flagella that the cell possesses is fundamental [108]. The flagella determine a rotational propulsive movement of the cell body while the helical shape determines a typical movement like a corkscrew [109]. The intestinal epithelium colonization is secondary to a chemotaxis process in which the main chemoattractors are the mucins and glycoproteins constituting the intestinal mucus [110]. The main bacterial chemoceptors are represented by proteins called What A, B, R, W, Y and Z [111].

The subsequent bacterial adhesion to the intestinal epithelial surface is mediated by several adhesins placed

on the surface of the bacterium [112]. In particular, a key role is played by an external membrane protein that binds the fibronectin named CADF [113] and by a protein called CapA or protein of *Campylobacter* Adhesion [112]. The consequent cell damage is related to the production of various cytotoxins [114, 115]. The most studied Cytotoxin is the CDT or Cytolethal Disintegrating Toxin [116]. This toxin has desoxyribonuclease activity and determines the cell cycle block in the G2 phase [116] and fragmentation of the nucleus resulting in cell death [117].

Clinical manifestations and related complications

The clinical spectrum of *Campylobacter* varied from a watery diarrhea without blood to a severe inflammatory diarrhea with abdominal pain and fever. The disease appears to be less severe in developing countries than in industrialized countries [118, 119]. In detail, in industrialized countries, the clinical picture is generally characterized by bloody stools, fever and abdominal pain and is often more severe than that caused by *Salmonella* and *Shigella* spp, in developing countries, instead, the symptoms are generally represented by watery stools with leukocytes, fever, abdominal pain, vomiting, dehydration [120, 121]. The *Campylobacter* spp infection can be burdened by some major complications. The main recognized sequelae are Guillain-Barré Syndrome (GBS), the Reactive Arthritis (REA) and irritable bowel syndrome. The Miller Fisher Syndrome, a variant of GBS, can also be associated with a previous *Campylobacter* infection. Evidence suggest a possible association with Inflammatory Bowel Disease (IBD), and there is evidence that other functional gastrointestinal disorders may be related to gastroenteritis in general (not specifically caused by *Campylobacter*). This aftermath, of course, they contribute significantly to the burden of disease [122].

GUILLAIN-BARRÉ SYNDROME

The role of *Campylobacter* spp has now been extensively studied in triggering an autoimmune response that leads to damage of the peripheral nervous system and the development of GBS. The *Campylobacter*-induced GBS is now considered a real disease and it seems that the basis of its unleashing there is the phenomenon of molecular mimicry. There are quite comprehensive data on the incidence of GBS in Europe and North America [123, 124]. The disease has also been well studied in China [125] and Japan [126], but the population incidence data are still scarce. The data on the worldwide incidence of GBS are limited with regard to low-income countries standards. In Bangladesh a recent publication reports that the disease has a higher incidence, and the presence at a young age, compared to high-income countries [127]. The lack of a common definition of GBS hampers the comparability of data and uniformity in the notification. Recently, there has been proposed guidance for a standardized definition of the clinical

case of GBS, the so-called “Brighton criteria” that are receiving broad international support [128]. Globally, around a third of cases of GBS have been attributed to a previous *Campylobacter* spp infection [129]. A link between reduced incidence of Campylobacteriosis and reduced incidence of GBS has been reported in New Zealand [130]. A link between reduced incidence of Campylobacteriosis and reduced incidence of GBS has been reported in New Zealand [130]. Some researchers have studied the clinical course of GBS and have shown that cases of GBS preceded by *Campylobacter* spp infection are more severe and are characterized by poorer therapeutic results with long-term possibility of disability [131, 132]. Treatment of the disease includes a general multidisciplinary assistance and specific treatment with plasmapheresis and/or intravenous immunoglobulin. Approximately 20% of patients is hospitalized in an intensive care unit to support ventilation and to monitor the autonomic dysfunction. Access to optimal treatment, however, varies greatly around the world, especially in less developed countries, where the GBS remains a serious and potentially fatal disease. The fatality rates vary widely and range between 3% and 10% in high-income countries while the lethality in countries developing is assumed to be higher. A recent meta-analysis concluded that as many as 31% of GBS cases could be attributed to *Campylobacter* spp [129]. This meta-analysis was based on studies conducted mainly in high-income countries and China and India, while it was only considered a study conducted in a country classified by the US as “less developed.” A more recent study in Bangladesh showed that 57% of cases of GBS could be attributed to *Campylobacter* spp [133].

REACTIVE ARTHRITIS

Available data suggest that reactive arthritis occurs 1-5% of individuals infected with *Campylobacter* spp. The annual incidence of REA after *Campylobacter* spp infection is estimated at 4.3 per 100,000 inhabitants in high-income countries [134]. In a study, in 5% of subjects the resulting reactive arthritis to *Campylobacter* spp infection is found to be chronic or recurrent [135]. There is evidence that musculoskeletal disorders can be triggered by *Campylobacter* and other enteric infections. In a US study published in 2008 and conducted by Townes *et al.* [136] in Minnesota and Oregon, the individuals with positive stool culture for *Campylobacter* spp, *Salmonella* spp, *Shigella* spp, *Yersinia* spp and *Escherichia coli* O157 were followed for 8 weeks. In particular, they were monitored 6379 patients with a confirmed infection; of these, 70% have completed screening and 575 (13%) have developed reactive arthritis. Other studies have reported a long-term disabilities resulting reactive arthritis. Hannu *et al.* [137] have estimated that 25% of patients with reactive arthritis can develop a chronic spondylo-arthritis, with different manifestations.

IRRITABLE BOWEL SYNDROME [IBS]

The infectious gastroenteritis is one of the major predisposing factors for the development of IBS [138, 139].

Some studies have reported that up to 36% of individuals with acute Campylobacteriosis develop IBS within 1-2 years [140]. There seems to be a close correlation between risk of developing IBS and the severity of the acute illness. Following an outbreak of infection with *Campylobacter spp* and Enteromorrhagic *E. coli* (EHEC) caused by contaminated water, Marshall *et al.* have reported an increased risk of IBS among those who had had a greater length of diarrhea, dysentery and abdominal cramps during the acute phase of the disease [141]. The studies carried out on patients with IBS post-*Campylobacter* infection have shown an increase of intraepithelial lymphocytes and upregulation of cytokines in colon-rectal mucosa, typical of a persistent immune activation [142-145]. The intestinal inflammation and hyperplasia of enterochromaffin cells in IBS post-infection are also accompanied by an increase in intestinal permeability resulting in an increase in the antigenic load and further activation of the immune system [146].

OTHER FUNCTIONAL GASTROINTESTINAL DISORDERS RELATED TO *CAMPYLOBACTER*

Scientific evidence linking infectious diarrheal syndromes with other functional gastrointestinal disorders such as functional dyspepsia. Mearin *et al.* [147] and Porter *et al.* [148] have reported an association between infectious diarrhea invasive, respectively *Salmonella spp* and from all causes, and post-infectious functional dyspepsia (OR 5,2 and 5,0, respectively). Similarly, Parry *et al.* showed an increase of 2,9 times of functional dyspepsia resulting in bacterial gastroenteritis (including *Campylobacter*) compared to non-exposed controls [149]. A further study also identified a link between acute enteric infection and functional dyspepsia in children [150]. It seems that there is lastly a relationship between the presence of *Campylobacter* and other functional gastrointestinal disorders such as diarrhea, functional constipation and gastro-oesophageal reflux disease [151, 152].

INFLAMMATORY BOWEL DISEASE (IBD)

In recent years, it has strengthened the hypothesis of an association between IBD and acute diarrheal infection caused by *Campylobacter*. The first studies that described the possible association between acute infection and inflammatory bowel disease date back to the '90s; Schumacher, for example, observed that cases of traveler's diarrhea were associated with a first attack of IBD in 62% of patients [153]. *Campylobacter* was isolated from 10% of cases of IBD relapsing [154]. Recent cohort studies have shown a higher risk of developing IBD following an acute infection with *Campylobacter* [155-156]. A study by Garcia-Rodriguez *et al.*, Published in 2006, showed that the risk of developing IBD has increased after a year by an episode of acute gastroenteritis, with an incidence of 60 cases per 100,000 inhabitants-year [157]. The pathogenesis of post-infectious IBD remains unclear. At the base there appears to be an enhanced host immune response to intestinal microbiome [158] due to

an increased absorption of bacterial antigens secondary to increased intestinal permeability that residual, as damage, after the infectious episode [157].

Laboratory diagnosis

The conventional method for the isolation of *Campylobacter* species in stool is represented by seeding the sample on selective media followed by incubation at 42° C in microaerophilic environment (5% O₂, 10% CO₂, 85% N₂). Some species (*C. sputorum*, *C. concisus*, *C. mucosalis*, *C. curvus*, *C. rectus* and *C. hyointestinalis*) for isolation may require the additional presence of hydrogen [159]. Media used, made selective in order to suppress the competitive bacteria and promote the growth of *Campylobacter spp* may be added to blood or coal, both of which contain one or more antibiotics.

The most commonly used culture media between those that contain blood, are the selective medium of Butzler (sheep blood agar to 10% with bacitracin, novobiocin, colistin, cephalothin and actidione), the Blaser media (agar-blood sheep to 10% with vancomycin, trimethoprim, polymyxin B, cephalothin, and amphotericin B) and the Skirrow media (horse blood agar lysate to 7% with vancomycin, polymyxin B and trimethoprim). Among media with coal the most used is certainly the Preston medium, containing cefoperazone. Merino *et al.* [160] have shown that the latter is the best in the recovery of the higher number of germs from fecal material.

It recently launched a selective chromogenic medium for *Campylobacter* (CASA Agar), which greatly facilitates the isolation and detection of these bacteria. On CASA agar, there is a strong inhibition of the growth of competitive intestinal flora while the colonies of *Campylobacter spp* are red and easily recognized [161]. Often the various species of *Campylobacter* isolated from human samples are not easily identifiable. Only *C. jejuni* can be identified with the use of phenotypic markers such as the morphological appearance of the colonies, biochemical reactions and optimal growth temperature; the other species require a polyphasic approach, using a combination of phenotypic and molecular markers.

In most clinical laboratories, even the identification of *Campylobacter spp* is performed only at the level of genus. The MALDI-TOF MS (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry) represents a new and interesting means of identification of *Campylobacter spp*, despite not provide information on bacterial resistance and lacks a computer system able to suggest other tests additional [162]. They were finally developed molecular assays using species-specific reactions or multiplexes, based on 16S rRNA gene sequences, or other species-specific gene sequences and identification systems based on microarray. All these systems can provide valuable support in official laboratories for Public Health and Food Safety [163, 164].

Antibiotic-resistance

In 2010 in the United States, only 1% of the strains of *C. jejuni* isolates from human infections were resistant to erythromycin, while 43% were resistant to tetracycline and 22% to ciprofloxacin [165]. In the same year, the FDA has reported almost overlapping data observed in strains of *C. jejuni* isolates from chicken meat [166].

In 2010 In the European Union 2% of *C. jejuni* from humans were resistant to erythromycin, 21% to tetracycline and 52% to fluoroquinolones; in strains isolated from chicken meat values were 2%, 22% and 50%, respectively. Both in the US than in Europe, the antibiotic resistance is greater in *C. coli* than in *C. jejuni* [167].

The cause of high resistance to fluoroquinolones appears to be the habitual use of veterinary antibiotics (enrofloxacin and danofloxacin) in the pharmacological treatment of poultry [168]. Because of this, in the United States the use of fluoroquinolones in poultry authorization was withdrawn in 2005 [169]. In Australia, where this use has not been approved, the resistance to these drugs is rare [168]. It was noted that infections resistant to fluoroquinolones are often associated with travel in both developed countries and in developing countries [170-172].

A recent study published in 2015 by Ghunaim *et al.* [173] shows an erythromycin resistance of *C. jejuni*, relatively low: only 8,6% of the isolates were resistant, while 63.2% were resistant to ciprofloxacin. A high rate of resistance to ciprofloxacin was also reported in the UAE, where 85,4% of the isolates were resistant [174]; in Poland they were published [175] lower rates of resistance (40%) and only 2% in Australia [176].

Prevention

There are numerous ways to prevent this infection, including vaccination and poultry control.

HEALTH SURVEILLANCE

According to Thacker S. the "Health Monitoring" is the systematic collection, analysis and interpretation of data on specific diseases within a determinate population in order to guide the actions and decisions in the field of Public Health [177]. A well-structured surveillance program for Campylobacteriosis can provide crucial information about the importance and the presence of the disease than other enteric infections contributing, also, to identify the most common routes of pathogen transmission. A complete surveillance of Campylobacteriosis should be carried out at national level, with data from all regions, including notifications of cases and the microbiological data typing of strains isolated from both human cases and from cases animals. Alternatively, they could be monitored specific sentinel sites, adequately resourced, and broadly representative of the whole country. In New Zealand, a hybrid approach unifies the national data of reported cases, and the epidemiological information related to supervision of sentinel sites [178].

VACCINATION

The WHO recognizes a considerable potential in anti-*Campylobacter* vaccines for both humans and animals. In humans, in particular, this potential concerns the prevention not only of acute infection but also of sequelae, with a remarkably reduction of patients. It is unlikely that a vaccine can be used for preventive purposes on a large scale, but it could be important for those who are at greatest risk. However, you still need considerable research before this can be achieved. Currently there are still no approved vaccines against diarrhea associated with *Campylobacter*. The development of effective vaccines against *C. jejuni* is limited by incomplete understanding of the pathogenic mechanisms of the disease and the strong association of this with GBS. Most strains of *C. jejuni* produces lipo-oligosaccharide (LOS) that contain sialic acid (Neu5Ac) with a structure very similar to human gangliosides. Antibodies directed against these molecules can cross-react with human peripheral nerves causing the establishment of GBS. This association between *C. jejuni* and GBS preclude many vaccination approaches. However, the recent discovery that *C. jejuni* (unlike other enteric pathogens) expresses a capsular polysaccharide (CPS) has favored the creation of a CPS-conjugate vaccine similar to those that have been developed for other pathogens [179]. Although few details are known of the molecular pathogenesis of the disease, the invasion of the intestinal epithelial cells appears to be a critical stage, and the PSC appears to play an important role in adherence to the cells. Thus, antibodies against the CPS may induce a protective immune response. Also, unlike the LOS, there is no evidence of molecular mimicry of the PSC with human gangliosides and, therefore, the antibodies should not trigger autoimmune reactions. There is no reason to think that vaccination could prevent, in the future, the development of *Campylobacter*-associated GBS. A common problem associated with capsular vaccines is the poor immunogenicity particularly in infants, a population to which many of these vaccines are directed. This occurs because polysaccharides are cells T-independent antigens and are able to stimulate only the mature B cells. However, the conjugation of polysaccharide with proteins transforms the immunological response from T-cell-independent to T-cell-dependent leading to the development of immunological memory both in children and in adults [180].

CONTROL MEASURES IN POULTRY

All types of poultry can be colonized with *Campylobacter spp* [181]. Vertical transmission of the bacterium through eggs is an extremely rare event [182]. The vast majority of broiler chickens are free of *Campylobacter* in the day of egg hatching, which means that at the beginning, each new group of chickens is *Campylobacter*-free. Once the *Campylobacter* is introduced into a group, it spread with the faeces rapidly colonizing almost all animals (up to 10^8 *Campylobacter*/gr faeces). The colonization rate remains almost at the same level until the age of slaughter (42 days in conventional production systems).

Colonization does not determine the onset of clinical signs or a reduction of the life of infected individuals. The broiler chickens can be colonized with *C. jejuni* and *C. coli*. However, approximately in 6 weeks old chickens, most of the isolated strains is represented by *C. jejuni* while in older animals predominates *C. coli* [183, 184]. In literature there are many articles about the possible actions to be taken to control chickens contamination by *Campylobacter* both on farms than in slaughter and processing houses.

There are considerable differences in the production of poultry in different parts of the world as a type of establishment (indoor or outdoor), the equipment used for the supply of food and water, the type of litter used (new or reused between groups), the microclimate, the method of ventilation and, finally, breed of chickens used. These differences will have a weight on the effectiveness of preventive interventions and determine on which interventions should be emphasized in order to achieve the greatest risk reduction.

Although the *Campylobacter spp* contamination control is a global problem, each country must develop its own strategies for achieving it. The only intervention proved to be effective in preventing the introduction of *Campylobacter spp* in the establishments of production is the application of strict biosecurity measures [185, 186]. They include:

- a strict control of establishment access to minimize the entry of unauthorized persons, birds, rodents or other animals;
- an insect control (e.g., flies and cockroaches) [187, 190];
- a workers' control (such as the introduction of hygiene barriers and the change of footwear before entering in the plant) [191];
- water purifying (chlorination) [191];
- control of litter and waste, avoiding the exchange between the groups [192, 193];
- other animals and rodents control [194];
- disinfection of cleaning tools [195];
- cleaning and disinfection of the whole plant and of all the equipments [190].

There are, moreover, a whole series of other interventions "pre-harvest" that have been successful in the field of research, but that have not yet proven effective when applying. They include the use of bacteriocins, bacteriophages, organic and inorganic acids in the feed or drinking water. However, the advantage of an intervention on the field can be lost if there is no simultaneous action in the transportation from the farm to processing establishments that reduce cross-contamination [195, 196].

Other important phases are selection of the animals, transport to the slaughterhouse, time spent in the slaughter facility [187]. The removal of the feed and water prior to the collection of animals has a significant impact on the *Campylobacter* load because may contaminate the animals during the transport and in the treatment plant. This is considered an integral part of the post-harvest control. Even at this stage, a good hygiene is crucial to the success of the control procedures [188-190]. Appropriate

measures include cleaning, disinfection and drying of transport tools, cages and coops, a proper stocking density, sanitize surfaces and liquids used (heaters, coolers, etc.) that come in contact with each carcass in order to reduce cross-contamination, and the use of specific food safety protocols as the application of good hygiene practices. Carcass decontamination by physical or chemical means is the procedure with the greatest chance of success among all post-harvest interventions proposed [191, 192]. The methods include the use of large volumes of water to wash carcasses, countercurrent flow of water in the heaters and water coolers, freezing of carcasses, treatment of carcasses with heat (steam) or irradiation. The chemical decontamination includes the use of chlorine compounds, organic acids, ozone, peracetic acid, peracetic acid with hydrogen peroxide, trisodium phosphate, as well as some "natural" methods such as the use of bacteriophages and bacteriocins.

Finally, the meat is likely to be contaminated even when it comes on the market. At this time, it is essential that the control measures are extended to distributors, retailers and end-consumers. As with any raw product, good hygiene practices are required during the preparation, storage and distribution of food. These practices include washing hands before and after handling food; the handling raw and cooked food; it is important to keep raw meats separate from cooked or ready to eat food; avoid using the same utensils to handle raw meats and other foods (such as cutting boards and/or other surfaces, knives and dishes); wash and disinfect all surfaces and utensils that have been in contact with raw meat; do not wash with tap water the raw meat in order to avoid further spread of microorganisms in the working surfaces. Because, finally, *Campylobacter* is sensitive to cooking temperatures, cook the food at 70° C will minimize the risk of contracting the infection.

Conclusions

Evaluating the epidemiology of Campylobacteriosis we have revealed an increasingly important role for *Campylobacter* infection in Public Health. While global efforts to control the transmission of enteric pathogens have been effective at reducing the incidence of a number of major foodborne pathogens, the human *Campylobacter* infections have been increasing in the last decade with a prevalence of infection ever increasing across most developed nations. Many progresses have been made in diagnostics that are helping to refine estimates of the acute and long-term burden of disease associated with a broad range of *Campylobacter spp*. We assume that better and efficient applied assays are necessary for an improved understanding of the epidemiology of different *Campylobacter spp* and to allow vaccine development. We also believe that it is necessary, due to growing antimicrobial resistance, implement control strategies on their use. Furthermore, it is now well established that poultry and other domesticated animals, such as cattle and pigs, and environmental sources, such as contaminated water, also

play a vital role in the direct transmission of these organisms to humans. For this reason, it is very important the realization of standardized biocontrol methodologies in the poultry sector, a principal source mediating *Campylobacter* transmission to humans.

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Authors' contribution

PL and AD suggested the argument of the paper, supervised the work and edit the manuscript; AF, RR, EA and GV provided the bibliography and wrote the manuscript.

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