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Towards understanding clinical campylobacter infection and its transmission: time for a different approach?

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ABSTRACT

Campylobacter spp. are among the most commonly diagnosed causes of human infection. Methods for detection of the 29 campylobacter species have mainly focused on cultivation of the thermophilic species. More than 99% of clinical campylobacter isolates notified in the UK in the recent past have been from faecal samples and associated with gastroenteritis. Campylobacter enteritis notifications in temperate zones show a seasonal increase during the summer months with a sharp decrease in the winter months, a pattern which remains incompletely understood. The striking seasonality in the expression of many human genes, some concerned with inflammation and immunity, suggests a need for further study of the host regarding the temporal distribution of many human infections, including campylobacteriosis. A tendency for campylobacter to enter a non-cultivable state under adverse conditions effects a reduction in the number of isolations. A Polymerase Chain Reaction (PCR)-based screening approach for the presence of the *Campylobacter* genus and followed by speciation has provided some insight into the limitations of cultivation for campylobacter, also allowing the discovery of new species. The increased sensitivity of the PCR-based approach over culture-based methods may make it difficult for the laboratory to differentiate asymptomatic campylobacter carriage from clinical campylobacter infection in non-sterile body sites. Campylobacter infection depends on a combination of host factors, and on acquisition of a suitably virulent strain with a tropism for human epithelium. The possibility of persistence of campylobacter in a viable but non-culturable latent form in the human body may also require further investigation. The scope of this review includes a discussion of current methods for diagnosing acute campylobacter infection and for detecting campylobacter in water and foodstuffs. The review also questions the prevailing view that poultry is the most common source of campylobacteriosis.

KEYWORDS

Campylobacter; gastroenteritis; detection methods; challenges; growth conditions; poultry; VBNC state

Introduction

Key challenges in clinical and food microbiology include the provision of appropriate laboratory conditions for the cultivation of the causative infectious agents of disease. Cultivation remains the standard approach in many cases [1]. This challenge is exemplified in the fastidious nature of campylobacter and in differing growth requirements among different species in this genus. This is accepted to have caused under-reporting of contamination in both foodstuffs and in cases of campylobacter-caused clinical disease. This review lists all of the species of the *Campylobacter* genus, along with recommended conditions for their growth and an indication of their reported role in disease, when previously reported. The review also examines conditions that may predispose to clinical campylobacter infection, including host factors.

Campylobacter is a genus of Gram-negative rod or spiral-shaped bacteria of the Campylobacteraceae family [2]. While it is believed that campylobacter was first discovered in 1886 by Theodor Escherich, who discovered non-culturable spiral form bacteria in stool specimens and large intestinal mucous associated with diarrhoea in neonates and also in kittens [3], it has only been since about 1980 that it has been recognised as a cause of illness in humans [4]. The *Campylobacter* genus currently comprises 29 species which are listed in Table 1, along with their reported role in human illness. Note that this list of associated clinical disease is not exhaustive. However, attention has been paid to the role of these species in gastroenteric disease, in bacteraemia and in oral disease in particular.

Campylobacter spp. as a cause of illness

Despite challenges with isolating campylobacter in the clinical laboratory, the thermophilic *Campylobacter* spp. (having the capability of growing at 42 °C) are the leading reported cause of bacterial gastroenteritis in humans worldwide [4]. In the UK in 2012, for example, the Public Health Agency reported an incidence rate of

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Table 1. Campylobacter species (2016) and their reported principal clinical associations.

Campylobacter species	Associated clinical illness	References
C. coli	Gastroenteritis, Bacteraemia, Reactive arthritis	[5–7]
C. concisus	Gastroenteritis, Oesophageal disease, Inflammatory bowel disease, Potential oral pathogen, Abscess	[8–11]
C. curvus	Abscess, Potential oral pathogen	[11,12]
C. fetus	Gastroenteritis, Bacteraemia, Meningitis, Abortion/neonatal infection	[13–15]
C. gracilis	Bacteraemia, Potential oral pathogen, Abscess	[11,16,17]
C. hyointestinalis	Gastroenteritis	[14,18]
C. insulaenigrae	Bacteraemia	[19]
C. jejuni (subspecies *jejuni* and *doylei*)	Gastroenteritis, Inflammatory bowel disease, Reactive arthritis, Guillain-Barré syndrome and variant, Meningitis/encephalomyelitis, Bacteraemia, more commonly with subspecies *doylei,* Abortion/neonatal infection	[5,6,20–27]
C. lari	Gastroenteritis, Bacteraemia	[14,28]
C. rectus	Abscess, Potential oral pathogen	[14,15,17,29]
C. showae	Bacteraemia with cholangitis, Potential oral pathogen, Abscess	[17,29,30]
C. sputorum biovar *sputorum*	Abscess, Bacteraemia	[31,32]
C. upsaliensis	Gastroenteritis, Bacteraemia, Abortion	[13,33,34]
C. ureolyticu	Gastroenteritis, Putative cause of inflammatory bowel disease	[9,14,35]
C. volucris	Bacteraemia	[36]
C. avium, C. canadensis, C. corcagiensis, C. cuniculorum, C. geochelonis, C. helveticus, C. hepaticus, C. hominis, C. iguaniorum, C. lanienae, C. mucosalis, C. peloridis, C. subantarticus, C. troglodytes	No clinical disease has been associated with these species to date	

66.4 per 100,000 population in Northern Ireland [37] and an incidence rate of 132.1 was reported for Wales for the same period [38]. In the Republic of Ireland (ROI), the Health Protection Surveillance Centre (HPSC) reported that during 2014 the number of notifications of campylobacteriosis reported to the HPSC increased by 25.6% compared to 2013 [39]. This gave a crude incidence rate of 57 per 100,000 population in 2014, which is somewhat comparable to the European figure reported of 64.8 per 100,000 population in 2013 [39]. There have been a growing number of clinical laboratories over this period which use Polymerase Chain Reaction (PCR) detection methods for enteric pathogens in the ROI. This may have contributed to the increasing notifications [40]. In the ROI, the highest rate of notification is seen in the 0–4 year age group, although an increase of greater than 45% in the over 65 age group has also been noted recently [39]. It should also be mentioned, however, that there has been a reported rate of asymptomatic carriage of 0.7% in a population study involving adults in the UK [41] when using culture-based detection of campylobacter. It is also possible that the use of more sensitive methods has allowed detection of campylobacter concomitant with, for example, *Clostridium difficile* intoxication in a hospital patient on whose faeces sample a range of investigations are conducted, whereupon asymptomatic carriage of campylobacter may be difficult to distinguish from infection.

The symptoms of campylobacter enteritis include nausea, vomiting, abdominal pain, headaches, fever and/or diarrhoea which may often be bloody [42]. There is an incubation period of between two and five days after infection and symptoms last approximately three to six days [42]. One paper which investigated the incubation periods of campylobacteriosis among people who acquired it outside their country of residence concluded that all thermophilic campylobacter strains have similar incubation periods [43]. This relatively long incubation period means that traceability of the source of the infection is made more difficult. However, campylobacter has also been shown not to multiply on food at room temperature [44], and person-to-person transmission has been stated to be uncommon [45,46]. It has been reported that fewer than 1% of campylobacter infections may be related to an outbreak, after a study by Ebel et al. comparing sporadic and epidemic illnesses in the USA between 2004 and 2011 [47]. In 2011, 16 European countries reported a total of 595 outbreaks of campylobacter infection which accounted for 10.6% of all foodborne outbreaks reported to EFSA [5,48]. It has been widely accepted that there is a degree of under-reporting inherent in these figures, however – for the UK, the multiplication figure to adjust for this may be up to 52, depending on the modelling method used [49].

Table 1 shows the *Campylobacter* spp. which have been associated with gastroenteritis to date. *Campylobacter jejuni* and *Campylobacter coli* have, until recently, been the two most commonly reported species in this type of infection. It is believed that the infectious dose of *C. jejuni* is often as low as 400–500 bacterial cells [50]. *Campylobacter concisus, Campylobacter fetus, Campylobacter hyointestinalis, Campylobacter lari, Campylobacter upsaliensis* and *Campylobacter ureolyticus* have also been associated with gastroenteritis. *C. ureolyticus*, formerly known as *Bacteriodes ureolyticus* has begun to emerge as another significant species in human enteric disease. A paper by our research group in 2011 (in Ireland) described this species as the second most common *Campylobacter* spp. to be detected in patients suffering from acute gastroenteritis (comprising 23.8% of the campylobacter infections, which put it ahead of *C. coli* [14]. An examination of the subset of

non-culturable campylobacter from this population was conducted using genus-specific PCR. Samples which were PCR-positive were then cultured using campylobacter-selective medium and microaerobic conditions at 42 °C. After incubation, the following results were recorded: species-specific PCR identified *C. jejuni* (50·7%) *C. ureolyticus* (41%) and *C. coli* (5·7%) as the most prevalent species while *C. fetus*, *C. upsaliensis*, *C. hyointestinalis* and *C. lari* accounted for 10% of culture-negative samples; mixed *Campylobacter* spp. were detected in 11% of samples [51]. A different approach to exploring the role of *Campylobacter* spp. in gastroenteritis has been shown in a Canadian paper from Inglis et al., in which populations of persons with and without gastroenteritis were studied for the presence of different species of campylobacter [52]. The authors found that the DNA of *C. concisus* was present in a higher number ($P < 0.001$) of healthy than diarrheic humans and that the prevalence of *C. curvus*, *C. fetus*, *C. gracilis*, *C. helveticus*, *C. hominis*, *C. hyointestinalis*, *C. mucosalis*, *C. showae*, *C. sputorum*, and *C. upsaliensis* was either not significantly different ($P > 0.05$) or it was significantly lower ($P \leq 0.05$) for diarrheic compared to healthy individuals [52]. Admittedly, a larger healthy population than 58 subjects would be preferable on which to base findings; and a matched study nearer to home might be more reflective of the population of Western Europe.

Many *Campylobacter* species have been reported to cause illness other than gastroenteritis, as shown in Table 1. Sepsis can occur particularly in patients who already have underlying conditions. One such case study described *C. jejuni* fatal sepsis in a patient with Non-Hodgkin's Lymphoma. The patient suffered from fever associated with neutropenia and thrombocytopenia after chemotherapy, however did not suffer from abdominal pain or diarrhoea. The difficulty in identifying *C. jejuni* using biochemical phenotyping methods combined with its slow growth in the blood culture system resulted in the sepsis being fatal [27]. A study by Fernández-Cruz et al. reviewed the records of any patients who presented with campylobacter bacteraemia to a 1750-bed tertiary teaching hospital between 1985 and 2007 [53]. The study reported that 82% of the patients were male, 32.8% of the patients had liver disease, 23.4% had HIV infection, 10.9% had malignancy, 3% had had organ transplantation, 15.6% had hypogammaglobulinemia and 31.2% had other underlying pathologies; a number of these patients presented with more than one of the previously mentioned underlying health conditions. It was also reported that 66% of cases were caused by *C. jejuni*, 19% were caused by *C. fetus* and 12% were caused by *C. coli* [54]. *C. fetus* subsp. *fetus* tends to target the human vascular endothelium and as a result causes bloodstream infection and haematogenous spread.

Routine surveillance of approximately one million campylobacter cases [54] in the UK between 1989 and 2009 showed that they were predominantly from gastrointestinal sites (99.65%) and were associated with diarrhoea. A further 0.25% of cases were from areas from which isolations are not normally made except in the case of infection, and most of the *Campylobacter fetus* cases were invasive.

Long-term sequelae of campylobacter illness

After campylobacter infection a minority of patients develop complications from the initial infection, the most widely reported of which have been Guillain-Barré syndrome, reactive arthritis and bacteraemia or sepsis.

Guillain-Barré syndrome (GBS) is an autoimmune condition which affects the nervous system as well as causing acute flaccid paralysis [55]. It can often be preceded by gastrointestinal illness caused by *Campylobacter* spp. and it can occur anywhere from 10 days to 3 weeks after infection [56]. *Campylobacter jejuni* has been known to trigger GBS and between 25 and 40% of patients with GBS worldwide have reported *C. jejuni* infection 1–3 weeks prior to illness [57,58]. However, GBS is relatively uncommon, having previously been estimated to affect one in every 5000 patients with a notified campylobacter infection [59]. A study in the Netherlands of a family outbreak of *C. jejuni* enteritis followed by GBS in one family member, demonstrated that the GBS patient's serum reacted much more strongly with several gangliosides than was the case for his siblings, reacting also with the lipopolysaccharide fractions from the *C. jejuni* strains isolated from his family members. Results of Histocompatibility Leukocyte Antigen (HLA) typing of this patient did not show a type associated with auto-antibody production. The authors concluded that ganglioside mimicry is necessary but not sufficient for the induction of anti-ganglioside antibodies, and that other susceptibility factors were needed to induce an anti-neural immune response [60].

Recent evidence suggests that *Campylobacter* spp. may be associated with Inflammatory Bowel Disease (IBD). IBD is described as a series of relapsing inflammatory episodes of the gastrointestinal tract. Two of the most well-known diseases include Crohn's Disease (CD) and Ulcerative Colitis (UC) [9]. The first published investigation of the relationship between IBD and *Campylobacter* spp. was conducted in 1984, however there was insufficient evidence at the time to confirm an association between the two [61]. In 2009 another study showed an association between *Campylobacter concisus* and newly diagnosed paediatric CD [9]. A study by Mukhopadhya et al. in 2011 found that there was a positive association between UC in adults and the presence of *Campylobacter* spp, specifically naming *C. concisus* and *C. ureolyticus*, which appear to have the most significant association with UC [62]. PCR-based methods are most commonly used in determining the relationship

between *Campylobacter* spp. and IBD. When cultivation has been used in the past, very few isolates have been recovered [63]. This low isolation rate might be expected because of the challenges inherent in isolation of variable numbers of campylobacter from the mixed flora of the large intestine. In addition to *Campylobacter* spp., non-typhoidal *Salmonella enterica* serotypes and *Shigella* spp. are also known to be associated with IBD [55].

Sources of thermophilic Campylobacter spp

There are many known sources of thermophilic campylobacter. These include poultry, water and raw milk as well as a wide range of both farm animals (cattle, pigs and sheep) and wild animals [64]. In particular, *C. jejuni* has been described as a commensal bacterium in many animals which makes the risk of consuming contaminated animal products high for humans [65]. Owing to the rapid decrease in campylobacter viability, infection due to contamination is most likely to occur when consumption occurs shortly after contamination [66]

Colonisation of chickens by campylobacter takes place at poultry farms, usually within seven days after hatching [67]. At the pre-slaughter stage, it is understood that the prevalence of campylobacter in broiler chickens ranges from 3–90%, however this depends on the country [68,69]. Cross contamination often occurs at the processing plant with the processes of scalding, defeathering, evisceration and carcass chillers being reported as known sites [70]. However further contamination routes remain to be elucidated regarding the contamination of poultry carcasses throughout the complete production chain [67]. An Expert Opinion Assessment by the European Food Safety Authority has estimated that the consumption of chicken meat accounts for 20–30% of campylobacteriosis in the EU, while 50–80% of cases may be attributed to the chicken reservoir as a whole [71]. However, a large UK study has also reported that campylobacter prevalence is not directly linked to the amount of chicken consumed [54], which appears not to support the Expert Opinion Assessment. The suggestion that poultry is the major source of human infection has previously been supported by a reported seasonality of campylobacter in poultry flocks along with a corresponding seasonality in the human population. However, evidence from a paper by Nylen et al. when studying seasonality of human infection and poultry carriage rates in nine European countries and New Zealand found a number of epidemiological mismatches, and they also suggested that there was a need to reconsider the predominant view that poultry is the main source of human infection throughout the year [72]. In New Zealand, however, there have been reports of a 50% reduction in the number of campylobacter notifications following the implementation of a strategy by both the NZ food safety authority and the poultry industry to reduce campylobacter in the food chain [73]. There were two major Multilocus Sequence Types (MLST) of *C. jejuni*, namely ST-474 and ST-48 associated with poultry and clinical infections, which have been reduced [73]. Confusingly, in Finland the converse is true; overlaps between poultry and human MLST types have decreased during a recent decade, despite a simultaneous increase in the consumption of chicken [74]. Overall, however, prevalence of campylobacter among poultry tends to be lower in Finland than in many other countries. In any case, and given that successful transmission of campylobacter to a susceptible human requires an inoculum at least equal to the infectious dose for that individual, it is prudent that measures to reduce, if not to eradicate, campylobacter from potential food sources are to be encouraged.

A further potential transmission route is through contact with household pets. Acke et al. investigated household pets as carriers of *Campylobacter* species in Ireland. As part of the study, 60 isolates were collected from Irish cats and dogs in private households and animal shelters. *C. upsaliensis* was the most commonly isolated *Campylobacter* spp. from household pets in Ireland, being found to account for 65% of these isolates, while *C. jejuni* followed with a prevalence of 22.5%. Overall, the study concluded that even though there were indistinguishable Pulsed-Field Gel Electrophoresis (PFGE) profiles of *C. jejuni* detected from healthy pets and human clinical cases, pets are not a substantial risk for human infection [75].

There are also reports that campylobacter infection can be contracted from groundwater. Ground water is often used as drinking water for livestock on farms and as a result has been associated with outbreaks of campylobacteriosis in broiler chickens [76], poultry flocks [77] and on dairy farms [78]. Subsurface groundwater aquifers provide favourable conditions for the survival of *Campylobacter* spp. as they have a constant temperature and offer protection from desiccation as well as UV exposure [79]. Isolation rates for campylobacter in contaminated freshwaters have been shown to be highest at temperatures in the range 2–8 °C and lowest at temperatures above 15 °C [80]. Interestingly, studies on seasonal variation in surface waters in the United Kingdom, for example, have shown that highest recovery rates in surface fresh waters occur in the autumn and winter months, becoming lowest during the spring and summer months, possibly affected by level of sunshine [81]. A study of campylobacter levels in effluent mainly contributed by abattoir and animal processing plants, and to a much lesser extent by the human population, reported that the seasonality of campylobacter in the effluent coincided with the peak season for clinical campylobacteriosis [82]. A number of studies have reported that wild birds frequently harbour *Campylobacter* spp. in the alimentary tract. For example, one Italian study, examining 78 hooded crows for evidence of campylobacter, detected *C. jejuni* in 50% and *C. coli* in 40% of these birds [83]. The extent to which faecal shedding in

Table 2. The Campylobacter spp. with recommended laboratory growth conditions for their cultivation.

Campylobacter species*	Conditions suitable for culture based detection	References
C. avium	37 and 42 °C. Microaerobic conditions; Brucella blood agar and filtration.	[95]
C. canadensis	37 and 42 °C. Campylobacter selective medium; also MacConkey agar. Anaerobic or microaerobic conditions	[96]
C. coli	37 and 42 °C. Microaerobic conditions. Campylobacter selective medium	
C. concisus	37 °C. Anaerobic conditions (for optimal growth requires at least 6% H_2)	[97]
C. corcagiensis	37 °C. Anaerobic conditions NAV Medium	[98]
C. cuniculorum	37 °C. Microaerobic conditions Campylobacter selective medium	[99]
C. curvus	37 and 42 °C. Microaerobic conditions. **Membrane filtration method on blood agar	[100]
C. fetus	25 and 37 °C. Microaerobic conditions. **Membrane filtration method on blood agar	[101]
C. geochelonis	25 and 37 °C. Microaerobic conditions	[102]
C. gracilis	37 °C. Anaerobic conditions. Tryptic soy agar supplemented with sodium formate and sodium fumarate	[103]
C. helveticus	37 °C. Microaerobic conditions. Campylobacter selective medium	[104]
C. hepaticus	42 °C. Microaerobic conditions	[105]
C. hominis	Recovered on blood culture	[106]
C. hyointestinalis	37 °C. Microaerobic conditions. Campylobacter selective medium	[107]
C. iguaniorum	37 °C. Microaerobic conditions. Blood agar	[108]
C. insulaenigrae	37 °C. Microaerobic conditions. Agar base with 5% sheep blood	[109]
C. jejuni	37 and 42 °C NOTE: subspecies *doylei* does not grow at 42 °C Microaerobic conditions; Campylobacter selective medium	
C. lanienae	37 and 42 °C. Microaerobic conditions. Campylobacter selective medium	[110]
C. lari	37 and 42 °C. Microaerobic conditions. Agar base with 5% sheep blood (v/v)	
C. mucosalis	37 and 42 °C. Microaerobic conditions. **Membrane filtration method on blood agar	[111]
C. peloridis	37 °C. Microaerobic conditions. Agar base with 5% sheep blood (v/v)	[112]
C. rectus	37 and 42 °C. Anaerobic environment for optimal growth with at least 6% H_2. Agar supplemented with 5% sheep blood (v/v)	[113]
C. showae	37 and 42 °C. Anaerobic environment for optimal growth with at least 6% H_2. Agar supplemented with 5% sheep blood (v/v)	[86]
C. sputorum biovar sputorum	37 and 42 °C. Anaerobic environment for optimal growth with at least 6% H_2. Agar supplemented with 5% sheep blood (v/v)	[114]
C. subantarticus	37 and 42 °C. Microaerobic conditions. Agar supplemented with 5% sheep blood (v/v)	[115]
C. troglodytes	37 or 42 °C. Microaerobic atmosphere	[116]
C. upsaliensis	37 °C. Microaerobic conditions. Selective agar	[117]
C. ureolyticus	37 °C. Anaerobic or H_2 enriched atmosphere. NAV selective medium	[118]
C. volucris	42 °C. Microaerobic conditions. Campylobacter selective medium	[91]

*Species that have been associated with human infection are shown in bold.;
**Medium contains sheep blood 5% (v/v), and overlaid with a 0–65-µm pore size membrane filter.

surface water by wild birds contributes to clinical campylobacter infection has not been established to date.

Known reservoirs for the clinical-disease associated non-thermophilic Campylobacter species

As indicated in Table 1, it has been concluded that the oral cavity may be a natural reservoir of *C. concisus, C. curvus, C. gracilis, C. rectus,* and *C. sputorum* [84,85] as well as *C. showae* [84–86]. *C. ureolyticus* has also been associated with the oral cavity [84,85]. The sources for *C. fetus* have been reported to be mainly cattle and sheep [87]. *C. hyointestinalis* has been associated with cattle [88]. However, all the afore-mentioned non-thermophilic and thermophilic species have also been isolated from canine faeces [89]. *C. insulaenigrae* has been associated with marine mammals [90], and *C. volucris* with gulls [91]. The reservoirs of these emerging campylobacteria are incompletely understood.

Laboratory detection of Campylobacter spp

The viable but non-culturable state

Campylobacter has been shown to enter a state which has been termed Viable but Non-culturable (VBNC). It might be expected that if a bacterium does not grow then it must be dead but this is not necessarily the case. The VBNC state is seen when bacteria fail to grow on the usual bacteriological media used, but are still alive and capable of renewed metabolic activity [92]. After resuscitation, bacterial cells which have been in the VBNC state have been reported to become culturable again. Bacteria which do enter the VBNC state pose a major threat to food safety and ultimately public health as standard food and water testing methods do not detect them [93]. Ramamurthy et al. have stated that there are several criteria which may be used to determine whether bacteria in the VBNC state are alive or not, including cellular membrane integrity, uptake and incorporation of labelled amino acids, protection of the genomic DNA from DNAse digestion and global gene expression [94].

Optimal growth conditions of Campylobacter spp

Campylobacter species require strict anaerobic or microaerobic conditions for growth (i.e. approximately 5–10% O_2 and 5–10% CO_2). There are, however, some species which require a H_2 enriched atmosphere for growth. All species are shown in Table 2, along with their growth requirements. The optimum incubation temperature for growth of the majority of *Campylobacter* spp. is accepted as being between 37 and 42 °C, depending on the species of interest; where both 37 and 42 °C are listed together, either incubation temperature may be selected.

The development of media and conditions for the growth of the best-understood species is likely to have prevented the isolation of other species of the genus with some differing, but no less exacting requirements. To date, the approach by clinical and food testing laboratories in the case of campylobacter infection remains primarily culture-based. Allowing for this approach, a variety of methods might be expected to be needed for the detection and isolation of different *Campylobacter* species. One such method which was developed and shown to be successful for the isolation of *C. concisus, C. rectus, C. upsaliensis* and some strains of *C. jejuni* such as *C.jejuni* subspecies *jejuni* and *C. jejuni* subspecies *doylei* has been the Cape Town Protocol [119], developed in 1990, as an alternative to using comparatively expensive antibiotic-containing campylobacter media. This method uses membrane filtration onto antibiotic free blood agar plates and incubation in a H_2-enriched microaerobic atmosphere. The advantage of using this method is that it allows the detection of antibiotic-sensitive strains of campylobacter. Furthermore, this allows for the growth of species previously mentioned which require H_2 in the growth atmosphere. However, this method has not been widely adopted, owing to its labour intensity and prolonged time to clinical diagnosis of campylobacter enteritis.

The most common conventional methods for the detection of *Campylobacter* species in food stuffs comprise selective enrichment followed by plating onto selective media. This is then followed by biochemical confirmation, if required [120]. In 2006, The International Standardization Organisation (ISO) recommended a standard method (ISO 10272-1:2006) for the detection of *Campylobacter* spp. in food which involves enrichment in Bolton Broth, followed by plating on modified Charcoal Cefoperazone Desoxycholate Agar (mCCDA) and one other alternative agar plate. Campy Food Agar (CFA), which is a chromogenic medium, is used as an alternative type of agar plate and Preston broth is often used as an alternative to Bolton Broth. (Ugarte-Ruiz, et al. 2012). A study by Ugarte-Ruiz et al. compared several different methods, and suggested that Preston Broth is more successful than Bolton Broth as Bolton Broth allows for the growth of some strains of *Escherichia coli* which can mask campylobacter growth and cause false-negative results [121]. Their study also suggested that CFA was easier to use than mCCDA as it was easier to distinguish colonies with the former medium. Finally, the biggest finding in the study was that a culture independent approach based on DNA amplification had more advantages than bacteriological methods. PCR was a quicker method, it had lower detection limits and it also enabled the detection of VBNC [121]. In the UK, however, Public Health England recently recommended using Bolton Broth followed by plating on mCCDA for the detection method and also using mCCDA plates for the enumeration method for campylobacter [122].

Molecular genetic methods for detection of campylobacter

Molecular genetic methods of detection and identification for campylobacter include genus-specific and species-specific PCR, ribosomal RNA analysis and whole genome sequencing. In recent years, the isolation of campylobacter has in some cases followed primary detection using a PCR-based approach. In some cases the necessary isolation strategy was developed only after PCR-based detection of certain species, as shown in the papers of O'Doherty et al., and Koziel et al. [98,118].

The continuing quest to understand the reasons for the continued high incidence of diagnosed campylobacter enteric infection

It is evident that food-related measures to reduce the incidence of clinical campylobacter infection have been partially effective, at best.

Frequent exposure to thermophilic campylobacter has been demonstrated in the human population in a Dutch study which reported that by age 20, >95% of the population studied had antibodies to thermophilic species of campylobacter [123]. The IgA (mucosal) response increased at a low level, and IgM increased steadily; however, levels of IgG continued to rise in an almost linear fashion [123]. These authors also pointed out that the half-life of IgG is estimated to be two years, and that there was evidence of frequent asymptomatic infection. Such a study of seroprevalence is also valuable in that it avoids the problem of under-detection of thermophilic campylobacter associated with fastidiousness of this genus of bacteria when conducting cultivation.

Another Dutch study, in this case of campylobacter in patients with GBS, campylobacter enteritis and healthy controls has shown that, among 30% of healthy persons, circulating human peripheral mononuclear cells contained campylobacter DNA, (detecting two independent campylobacter-specific genes) [124]. The results were not significantly different for healthy controls than for the GBS or enteritis patients. Furthermore, this remained the case for 1–2 years after the initial detection. This finding remained incompletely explained, although the authors pointed out in their discussion of the possible basis for this that the observed long-term persistence of the DNA seems incompatible with the limited life span of the cells, unless it is assumed that the viable bacteria can lengthen the life of the host cells, as previously shown for certain other pathogens.

Nakajima et al. have found that some urease-positive thermophilic campylobacter isolates exhibited catalase-activity sufficient to protect from oxygen stress, and they have suggested that this might provide them with some protection in host and natural environments [125]. Furthermore, Askoura et al. have reported that in *C. jejuni* the ferric uptake promoter not only protects against acidity, but that this promotor also cross-protects against oxidative stress [126].

Whether there is commonly a reservoir in the human body from which campylobacter can cause re-infection of the host, or person-to-person infection has not been established. A study by Perez et al. [127], reported that human breast milk contains a limited number of viable bacteria but a range of bacterial DNA signatures, as also found in maternal peripheral blood mononuclear cells; they concluded also that their results suggest that intestinally derived bacterial components are transported to the lactating breast within mononuclear cells. It may be fruitful to evaluate the possibility that certain campylobacter strains or species may be present in a latent non-virulent form within a human host for long periods of time. The possibility exists that such pathogens may be transmitted maternally in one way or another in the early months and years of life.

In light of the acknowledged tendency for other curved or spiral bacterial genera associated with various types of infection to persist after human colonisation or infection, it would be surprising, perhaps, not to have a similar pattern for at least some strains of campylobacter, if not for entire species. *Helicobacter pylori*, for example, shows a life-long persistence in the human stomach, which suggests that the host response fails to clear the infection, although it induces a mixed immune response characterised by T helper (Th) 1, Th17 and regulatory T cell (Treg) responses [128]. Species of *Borrelia* and *Treponema* show a similar agility in evading annihilation by the host immune response, which facilitates long-term survival by these pathogens, from which resurgence or triggering of infection can occur periodically in the host.

Several European countries have been reported to have shown very consistent seasonal patterns from year to year, with peaks around week 22 in Wales, week 26 in Scotland, week 32 in Denmark, week 30 in Finland and week 33 in Sweden [72]. A New Zealand paper [129], which examined age- and season-related incidence of campylobacterosis, also found that, in New Zealand and Canada, as in other temperate countries, including, for example Ireland and the UK, when all notifications are examined as a single group, a strong seasonal pattern, typically an early winter low and an early summer high rate of incidence, occurs. This is accompanied by peak incidences among those under five years of age and among persons in their 20s.

Another paper, by Dowell at the Centers for Disease Control and Prevention, (CDC), Atlanta, in 2001, describes how seasonal cycles of infectious diseases have been variously attributed to changes in atmospheric conditions, the prevalence or virulence of the pathogen, or the behaviour of the host [130]. The predictably seasonal incidence of campylobacter enteritis among most age groups has so far proven impossible to explain definitively on the basis of survivability of the pathogen in the environment or on food, or on prevalence, for example, among poultry destined for human consumption, particularly when considering the widespread and varied potential sources of this pathogen. The regular mounting of IgG anti-campylobacter immune response to campylobacter demonstrating a challenge by this organism is interesting in combination with the findings of a large UK study from 2008, which demonstrated that incidence was higher in males from birth until the late teens and in females from 20 to 36 years. Age- and gender-specific differences in campylobacter incidence were observed among different ethnic and socioeconomic groups [131]. The authors remarked on particular differences in incidence of campylobacter infection during the peak years of male puberty and the main childbearing years for females and the known positive effects of the associated hormones on increased campylobacter growth [131]. Therefore, host susceptibility to campylobacter infection varies between individuals and at different stages of the human lifecycle.

A study of immune system-based and hormonal host factors might be useful in the cohort of the human population who may experience a noticeable decrease in wellness for short periods at specific times of the year, (such as the spring/early summer peak in campylobacter infection,) but who are not so unwell as to seek medical assistance, along with a group who remain symptom-free at these times, in an effort to better understand all the drivers for clinical campylobacter infection.

A study by Dopico et al. [132], of more than 4,000 protein-coding mRNAs in a human population has shown a remarkable seasonality in up- and down-regulation among these genes, for which they believe that daylight and ambient temperature are likely candidate environmental cues. These regulate seasonal hormonal and immune system variations. The authors suggested that a seasonal human immune system may contribute to host-mediated pathology and morbidity after infection. Their findings may suggest the need for further expression studies of combinations of genes influencing immunity.

The thermophilic campylobacter strains vary greatly in their capacity to colonise host species and in their virulence in humans, as evidenced by a study by Zautner et al. [133]. The authors tested for the presence of certain genetic markers in a population of *C. jejuni* isolates, and found that the presence of some of these markers and the absence of others to be associated with a higher prevalence in human campylobacteriosis, and also with bloody diarrhoea and hospitalisation. The markers studied were diversely distributed among individual MLST types.

Conclusion

The detection rates of the campylobacter genus in clinical and other samples remain likely to have poor negative predictive values when primary detection methods are culture-based. For this and other reasons, most campylobacter infections in humans remain undiagnosed. In particular, for the non-thermophilic species, careful documentation of case studies describing infections caused by these less-commonly detected species is needed to further our combined understanding of their significance, both in normally sterile and in normally non-sterile sites. While it has been reported that fewer than 1% of individuals are asymptomatic gut carriers of thermophilic campylobacter based on culture methods, the possible persistence of campylobacter in a viable but non culturable state in monocytes [124] suggests a higher prevalence of campylobacter in asymptomatic individuals than had been previously thought. Winstanley et al. conducted a large study of two populations of *C. jejuni* and *C. coli* that had been isolated from clinical infections in the mid-1990s and the period 2008–2009, respectively [134]. Using MLST and Whole Genome Sequencing, the investigators conducted an epidemiological analysis of their collection and stated that there were no clear variations of note between the two periods with respect to MLST clonal complex. When comparing their data to previous worldwide studies, the authors concluded that their findings replicated the results of previous studies elsewhere. These findings do not help to exclude the possibility of endogenous persistence and reactivation in the human population.

Further host factors, including age and gender, have a bearing on the risk of acquiring campylobacter infection. The seasonality of campylobacter infection may be influenced by the seasonality of the human host immune system, and further research is needed in this field. Campylobacter has a diverse distribution in the environment and within animals and birds, so exposure to this genus may be frequent from exogenous sources. In the final analysis, of course, the occurrence of severe clinical campylobacter infection depends on a combination of host factors, and on having acquired a suitably virulent strain with a tropism for human epithelium.

Disclosure statement

No potential conflict of interest was reported by the authors.

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