

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/authorsrights>



Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmethReview of current methodologies to isolate and identify *Campylobacter* spp. from foodsGregory Gharst^a, Omar A. Oyarzabal^{b,*}, Syeda K. Hussain^c^a U.S. Food and Drug Administration, Bedford Park, IL, USA^b IEH Laboratories and Consulting Group, Lake Forest Park, WA, USA^c Department of Biological Sciences, Alabama State University, Montgomery, AL, USA

ARTICLE INFO

Article history:

Received 2 April 2013

Received in revised form 17 July 2013

Accepted 17 July 2013

Available online 27 July 2013

Keywords:

Aerobic enrichment

Broiler

*Campylobacter jejuni**Campylobacter coli*

Chicken

Isolation

ABSTRACT

This article summarizes the most effective protocols to isolate *Campylobacter* spp. (mainly *Campylobacter jejuni* and *Campylobacter coli*) from food, primarily poultry products, and includes a summary of the current methods recommended by the Food and Drug Administration and the U.S. Department of Agriculture in the USA, and ISO in Europe. The recommended temperature for incubation of the samples throughout the isolation procedure is 42 °C. The enrichment of the samples for 48 h, which can be performed under aerobic conditions, is recommended to achieve a detectable number of *Campylobacter* cells. Bolton broth or buffered peptone water supplemented with cefoperazone and amphotericin B is commonly used enrichment broths. The transfer of the enriched samples to plate media using membrane filters helps to obtain pure *Campylobacter* colonies. Charcoal cefoperazone deoxycholate (CCDA) is the best choice among all plate media. There is no need to add oxygen quenching substances or blood to enrichment broth for the isolation of *Campylobacter* spp. However, the addition of blood to plate media aids in differential identification of presumptive colonies. Phase contrast microscopy and latex agglutination tests are confirmatory tests for presumptive *Campylobacter* isolates. The use of multiplex polymerase chain reaction (mPCR) assays is the simplest and most rapid method to identify isolates to the species level. mPCR assays, or other methods assessing DNA sequence variations, will probably become the confirmation procedure of choice in the future. Recent work with retail broiler meat has revealed that the rinsing of meat is more sensitive for the recovery of naturally contaminated retail broiler meat than current reference methods and requires less time for preparation and processing of the samples. This protocol could be coupled with DNA-based methods for a fast screening of positive samples.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Campylobacteriosis is a highly prevalent foodborne disease in industrialized nations. In the US, campylobacteriosis is the third most frequent bacterial foodborne disease, with 0.8 million estimated cases that represent 8% of the overall estimated foodborne disease illnesses (Scallan et al., 2011). Besides the typical diarrheal syndrome, *Campylobacter* infection has been linked to Guillain–Barré syndrome, which is an autoimmune-mediated disorder of the peripheral nervous system that results in acute demyelinating polyneuropathy (Kaldor and Speed, 1984; Speed et al., 1984).

Although there are more than 20 *Campylobacter* spp., *Campylobacter jejuni* and *Campylobacter coli* are the two most important species associated to human disease (Man, 2011). *C. jejuni* accounts for 80–90% of the infections reported in the US (Gilliss et al., 2013). In the USA, a mean of 28 outbreaks per year has been reported for years 2003–2008 (Taylor

et al., 2013). The consumption of undercooked poultry is considered a major risk factor for sporadic infections (Friedman et al., 2004). However, the analysis of outbreak data in the last 10 years identifies raw milk as the main vehicle of transmission of campylobacteriosis, with 29% of the outbreaks associated with dairy products versus 11% associated with poultry and 5% associated with produce (Painter et al., 2013; Taylor et al., 2013).

C. jejuni has a relatively low infectious dose based on experimental infection studies. Robinson (1981) reported that approximately 500 organisms were enough to produce infection after the consumption of a *C. jejuni* strain isolated from a raw milk outbreak in 1979 (Robinson et al., 1979). A previous study reported an infectious dose of 10⁶ *Campylobacter* cells (Steele and McDermott, 1984), but the origin of the strain used in that study is unknown. The last report on experimental infections in humans was published in 1988, when disease was induced in volunteers who were given doses as low as 800 *Campylobacter* cells of a *C. jejuni* strain originated from a milk outbreak. These experiments corroborated that a low number of cells (less than 1000) can produce disease in humans (Black et al., 1988). It is accepted that all *C. jejuni* strains from all food sources are equally pathogenic, although none of

* Corresponding author at: 15300 Bothell Way NE, Lake Forest Park, WA 98155, USA. Tel.: +1 206 522 5432; fax: +1 206 306 8883.

E-mail addresses: oaoyarzal@gmail.com, omar@iehinc.com (O.A. Oyarzabal).

the strains isolated from poultry products or other foods have ever been tested for infectivity in humans. There is a great deal of data uncertainty when only the single dose–response model is used to calculate infectious doses, and in the future it is expected that the calculation of confidence intervals will provide a better understanding of the variability of infectious doses for *Campylobacter* spp. and other bacterial foodborne pathogens (Moon et al., 2013).

Campylobacter spp. colonize domestic animals (Friedman et al., 2004) and pets, especially puppies (Damborg et al., 2004; Tenkate and Stafford, 2001), and can be found in recreational waters. In the intestine of avian species, such as commercial chickens and turkeys, *Campylobacter* spp. establish a commensal relationship with the host that results in their occurrence in high numbers in colonized birds (Potturi-Venkata et al., 2007). Molecular techniques have helped the scientific community understand that *C. jejuni* and *C. coli* are the only species found in commercial chicken production worldwide (He et al., 2010; Oyarzabal et al., 1997; Suzuki and Yamamoto, 2009). Although *Campylobacter lari* strains have been isolated from live commercial turkeys (Smith et al., 2004), these strains have not survived storage and no further confirmation of the presence of *C. lari* in turkey flocks has been reported (S. Kathariou, personal communication, March 18, 2013). Therefore, it appears that turkey isolates are also primarily *C. coli* or *C. jejuni*, but *C. coli* is the most predominant species.

Contrary to sporadic cases associated with the consumption of chicken, the consumption of raw, unpasteurized milk has been the most important source of campylobacteriosis outbreaks in the USA, Canada and Europe in the last 15 years (Heuvelink et al., 2009; Jay-Russell et al., 2013; Lejeune and Rajala-Schultz, 2009; Oliver et al., 2009; Schildt et al., 2006). Feces from animals or infected humans may also contaminate waters, which in turn may become another source for campylobacteriosis. Overflow of sewage, polluted water run-off, and agriculture run-off are some of the ways water becomes contaminated with *Campylobacter* spp.

The protocols for isolation of *Campylobacter* spp. from foods were adapted originally from clinical microbiology protocols, and the methodology for isolation of *Campylobacter* from milk remains unchanged. However, the methods for isolation of *Campylobacter* spp. from poultry products have undergone several modifications. This review summarizes the protocols that are currently available for the isolation of *Campylobacter* spp. from poultry products, with emphasis on the improvements made in the last 10 years to the isolation and identification protocols targeting *C. jejuni* and *C. coli*.

2. Isolation of *Campylobacter* from foods

Campylobacter spp. are microaerobic bacteria with some species being thermotolerant. King (1957) noticed that *Campylobacter* strains isolated from humans grew well under microaerobic conditions at 42 °C and were serologically different from animal strains. In addition, the animal strains did not grow at 42 °C, suggesting that these strains were *Campylobacter fetus*. Soon after these findings were published, microaerobic conditions (5% O₂, 10% CO₂, and 85% N₂) and 42 °C became the standard incubation conditions to isolate *Campylobacter* spp. from clinical samples. Eventually, the use of microaerobic environments and a higher incubation temperature became the protocol of choice to isolate *Campylobacter* spp. from poultry and milk products. This higher incubation temperature inhibits some of the competing microflora present in food samples and allows for the isolation of *C. jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*, which are sometimes referred to as the “thermotolerant” *Campylobacter* species.

2.1. Enrichment conditions for isolation

During isolation from food samples, the presence of other bacteria with faster generation time always poses a limitation for the isolation of low number of *Campylobacter* cells. Therefore, the enrichment step

plays an essential role in facilitating the growth of low numbers of *Campylobacter* cells for later detection. It is unclear as to which condition selectively is in favor of the multiplication of *Campylobacter* cells, and not of the competing bacteria, during enrichment. All attempts to reduce the enrichment time from 48 to 24 h during the isolation of *Campylobacter* spp. from chicken meat have been unsuccessful (Oyarzabal et al., 2007; Liu et al., 2009). Therefore, the low numbers of cells (0.6–0.8 CFU per g) present in retail broiler meat, for instance (Oyarzabal et al., 2007), require incubation at 42 °C for 48 h.

Several types of enrichment broths have been developed for isolation of *Campylobacter* from foods (Corry et al., 1995). Usually, enrichment broths consist of a basal medium, such as Brucella broth or nutrient broth (Bolton and Robertson, 1982; Corry et al., 1995), supplemented with antimicrobials. Originally most enrichment media were supplemented with lysed horse or sheep blood, but it has been found that blood is not required to isolate *Campylobacter* spp. from poultry meat (Liu et al., 2009). Furthermore, formulations without blood may be more amenable to the coupling with molecular methods for faster detection and identification. The basal medium does not need to be rich for the isolation of *Campylobacter* spp. Bolton broth continues to be one of the best alternatives for enrichment (Baylis et al., 2000) but buffered peptone water, which has a similar composition as the basal component of Bolton broth, is equivalent to Bolton broth for the isolation of *Campylobacter* spp. from retail broiler meat (Oyarzabal et al., 2007). Table 1 describes the composition of the simplest broth and plate media for isolation of *Campylobacter* spp. from poultry products. The enrichment protocols for the isolation of *Campylobacter* spp. from retail broiler meat are based on a ratio of 25 g of meat in 225 ml of enrichment broth. However, an enrichment ratio of 1:9 (meat:broth) has been shown to perform similarly to the 1:9 ratio and reduces the amount of broth used during the isolation process (Oyarzabal et al., 2007).

2.2. Microaerobic environment

Campylobacter spp. have been traditionally isolated under microaerobic conditions. Various methods have been developed to create a microaerobic environment that would allow *Campylobacter* spp. to grow. A simple system is the use of sachets (Oxoid BR56, Oxoid, UK) that generate carbon dioxide from sodium bicarbonate and citric acid, or hydrogen from sodium borohydride with the use of palladium as a catalyst that converts hydrogen and oxygen to water (Sails et al., 1998). These sachets are used in a contained, sealable environment provided by jars traditionally used to create anaerobiosis. The sachets have been improved over the years and it is not longer necessary to add water to generate the microaerobic conditions. Newer sachets, for instance, generate carbon dioxide without the production of hydrogen (CampyGen, Oxoid).

Another system for generation of microaerobic conditions is the evacuation-replacement system, which uses a pump to evacuate the air within a jar and then replaces the air with a microaerobic mix. Microaerobic cylinders are easy to obtain from a gas manufacturer and the industrial microaerobic mix is adequate in providing the conditions necessary for the growth of *Campylobacter* spp. All the above systems can be employed in conjunction with plastic bags, such as Ziploc bags

Table 1

Comparison of Bolton broth with and without the addition of Oxyrase. Chi-square (as defined by McNemar) = 0.46. Statistical differences are found when the chi square vales are ≥ 3.8 .

		Bolton broth	
		Positive	Negative
Bolton + Oxyrase	Positive	34	16
	Negative	19	62

(SC Johnson, Racine, WI), that are used to freeze foods. These bags prevent the gas mix from leaking during incubation.

Microaerobic workstations (Don Whitley Scientific Ltd., West Yorkshire, UK) are also available and can generate environments with higher H₂ and/or different concentration of O₂ and CO₂ that improve the chances of isolating of non-*jejuni/coli* isolates. These workstations are usually reserved to large, research laboratories because of their high cost.

2.2.1. Oxygen quenching substances

Several oxygen-quenching agents have been used in media (broth and agar) for the isolation of *Campylobacter* spp. These agents include lysed horse blood, defibrinated blood from other animals or alkaline hematin (Corry et al., 1995; Border et al., 1974; Solomon and Hoover, 1999), iron salts, norepinephrine (Bowdre et al., 1976), ferrous sulfate, sodium metabisulfite, sodium pyruvate (George et al., 1978) and charcoal (Hutchinson and Bolton, 1984). These substances are added to growth media with the purpose of neutralizing the toxic effects of oxygen radicals and to help create a microaerobic environment. However, all of these agents, except blood in plates, are not consistently used in broth or plate media and their efficacy has not been fully studied. There is little evidence to date to support their use in the isolation process.

Oxyrase® (Oxyrase, Inc. Mansfield, Ohio), an enzyme system that selectively removes oxygen from its surrounding environment, can be added to both liquid and solid media to reduce oxygen (Adler et al., 1981). This enzyme is supplied commercially as a sterile suspension of *Escherichia coli* membrane fragments and it appears that the cytochrome electron transport system present in membrane fragments is responsible for oxygen-quenching activities (Schneitman, 1970; Adler et al., 1981). Although this enzyme was tested for the growth of *Campylobacter* spp. in inoculated food samples (Wonglumsom et al., 2001), there are no publications evaluating its efficacy in enrichment media for the isolation of naturally occurring *Campylobacter* spp. in retail broiler meat. We performed a study in which 131 commercial, retail broiler meat samples were tested for naturally occurring *Campylobacter* spp. using Bolton broth supplemented with 2% of Oxyrase for Broth™. From each meat package representing a sample, 25 g of meat was weighed twice. One 25 g portion was enriched with in 100 ml of Bolton plus Oxyrase under aerobic condition while the other 25-g portion (control group) was enriched in 100 ml of Bolton broth supplemented with 5% (v/v) lysed horse blood under microaerobic conditions (Airgas, Radnor, PA) using anaerobic jars gassed with a MACSmics Jar Gassing System (Microbiology International, Frederick, MD). Bolton alone and Bolton plus Oxyrase were supplemented with 33 mg of cefoperazone and 4 mg of amphotericin B per liter of medium. All samples were incubated at 42 °C for 48 h and then transferred to modified Campy-Cefex (Oyarzabal et al., 2005) agar plates. Presumptive *Campylobacter* spp. isolated on plates were identified to the species level with multiplex polymerase chain reaction assays as described elsewhere (Oyarzabal et al., 2005). The isolation rates from Bolton (microaerobic) and Bolton with Oxyrase (aerobic) were not significantly different (McNemar chi-square = 0.46) (Table 1). However, it is clear now that broth media naturally produce microaerobic environments suitable for the growth of *Campylobacter* spp. (Zhou et al., 2011) and therefore the addition of Oxyrase to broth would not provide any benefits for isolation of *Campylobacter* spp. It seems that Oxyrase may provide some benefits in the production of microaerobic environments during the incubation of agar plates, as long as the plates suggested by the manufacturer of this enzyme are used (Oxyrase, Inc., Mansfield, Ohio). However, the incorporation of these plates and this enzyme substantially increases the cost of the isolation procedure.

2.2.2. Microaerobic conditions in enrichment broths

Recent findings have revealed that the level of dissolved oxygen (DO) in enrichment broth is low enough that naturally occurring

Campylobacter spp. in retail broiler meat will multiply to detectable levels under aerobic incubation (Zhou et al., 2011). Using pulsed field gel electrophoresis (PFGE) with a cutoff of 90% DNA relatedness, the isolates that grew in enrichment broth under microaerobic conditions also grew in enrichment broth under aerobic conditions. In these experiments, only plastic bags (Whirl-Pak®, Nasco, Fort Atkinson, WI, and Ziploc) were tested. It would be interesting to know if other types of plastic bags and/or containers can be used to enrich samples under aerobic conditions while maintaining a low DO in the broth that allows for the isolation of *Campylobacter* spp.

Using denaturing gradient gel electrophoresis (DGGE) Zhou et al. (2011) also determined the microbial population of the enriched samples after incubation for 42 h at 42 °C under microaerobic or aerobic conditions. DGGE is a powerful technique to identify the total microbial population in complex samples. Although the phylogenetic data obtained from the amplification of a small segment of the 16S rDNA may limit the identification only to the genus level, the authors found that *Acinetobacter*, *Lactobacillus* and *Pseudomonas* were common genera present in the enriched samples regardless of the incubation condition (aerobic or microaerobic). Species from these genera are primarily facultative anaerobes or microaerobic organisms that have been reported in broiler meat (Oyarzabal et al., 2005) and make up several of the spoilage organisms in these food products (Vihavainen and Björkroth, 2010). A significant finding from these DGGE studies was the large variation in the microbial community and a lack of similar patterns at the intra- or inter-sample level. In other words, the bacterial populations after enrichment appeared to depend more on the original bacterial composition of the meat sample and less on the enrichment conditions (aerobic vs. microaerobic). The same set of samples tested by ribosomal intergenic spacer analysis (RISA), another DNA-based method for microbial population studies, corroborated the results from DGGE (Zhou et al., 2011).

It appears to be a matter of time for DNA-based techniques to clarify the diversity of the microbial population present in retail broiler meat. However, the already identified genera, primarily *Pseudomonas* and *Acinetobacter*, are well-adapted bacteria that are best controlled by the addition of antimicrobials during enrichment. The use of membrane filters when transferring enrichment broth onto plates certainly helps in the isolation of *Campylobacter* spp. by reducing the use of antimicrobials, especially vancomycin, during enrichment and plating (refer to Section 2.4. Use of membrane filters).

Table 2

Composition of different enrichment and plate media for isolation of *Campylobacter* spp.

Medium	Basal medium ^a	Supplements ^b
Enrichments ^c		
Bolton broth	Basal medium (27.6 g) Deionized water (1 l)	Amphotericin B (4 mg) ^d Cefoperazone (33 mg)
Buffered peptone water	Basal medium (20 g) Deionized water (1 l)	Amphotericin B (4 mg) Cefoperazone (33 mg)
Plates ^e		
CCDA	Basal medium (45.5 g) Deionized water (1 l)	Amphotericin B (10 mg) Cefoperazone (33 mg)
Blood agar plates	Basal medium, such as Brucella agar, Columbia blood agar, etc Deionized water (1 l)	Amphotericin B (4 mg) Cefoperazone (33 mg) Lysed horse (sheep) blood (50 ml)

^a Basal media can be purchased from Acumedia (Lansing, MI), Oxoid (Thermo-Fisher), etc. Some formulations (Bolton broth, CCDA, for example) some manufacturers provide a set of antimicrobial supplements.

^b All antimicrobial supplements can be purchased from Sigma-Aldrich (St. Louis, MO).

^c From references Oyarzabal et al., 2007; Zhou et al., 2011.

^d It can be replaced by cycloheximide at 50–200 mg per liter.

^e Oyarzabal et al., 2005; Zhou et al., 2011.

2.3. *Campylobacter* plating media

Table 2 shows the most common plate media used to isolate *C. jejuni* and *C. coli* from foods. Media can be divided into blood-based, charcoal-based and others. Charcoal cefoperazone deoxycholate (CCDA) is the most commonly used selective plate medium worldwide (Bolton and Coates, 1983; Bolton and Robertson, 1982). Although *Campylobacter* colonies have unique characteristics on charcoal plate, the dark background is a challenge for identification to the untrained scientist. However, for those with sufficient experience, CCDA offers an easy identification of *Campylobacter* spp. and contaminants. On CCDA incubated at 42 °C for 36–48 h, *Campylobacter* spp. usually appear as gray, flat, swarming colonies. We particularly consider this agar medium as one of the best choices based on simplicity and cost.

In general, there are no differences in the isolation rate of naturally occurring *Campylobacter* spp. from carcass rinses between charcoal-based and blood-based media. However, Campy-Line plates appear to be selective against *Campylobacter* spp. and yield a lower isolation rate from carcass rinses and poultry fecal material (Oyarzabal et al., 2005; Potturi-Venkata et al., 2007). Few other media have been developed and marketed in the last 10 years. One of the newer plates is CampyFood ID agar (bioMerieux, Marcy l'Etoile, France). Habib et al. (2008) found that CampyFood ID performed similarly to CCDA when testing artificially contaminated samples. However, CampyFood ID was less effective for the detection of naturally contaminated samples, where non-*Campylobacter* colonies had similar color and were undistinguishable from *Campylobacter* colonies. The same authors later reported that this plate performed similarly to CCDA for direct counting of *Campylobacter* spp. in naturally contaminated chicken meat samples (Habib et al., 2011). Recently, a *C. jejuni*/*C. coli* Chromogenic Plating Medium has been introduced into the market (R&F Laboratories, Downers Grove, IL). However, there are no scientific publications on this new medium and therefore its incorporation in food microbiology laboratories will depend on validation studies and cost.

Most selective plate media have several antimicrobial agents, such as cefoperazone and vancomycin, as the primary inhibitor of enteric bacterial flora. However, the incorporation of filter membranes (see Section 2.4. Use of membrane filters below for an explanation of the role of membrane filters) has allowed us to reduce the number and amount of antimicrobials. Currently, the addition of 33 mg of cefoperazone and 4 mg of amphotericin B per liter of medium appears to be the best combination to isolate *Campylobacter* spp. from retail broiler meat (Williams and Oyarzabal, 2012). Amphotericin B performs similarly to cycloheximide for the isolation of *Campylobacter* spp. from poultry products and can be used in enrichment and plate media (Oyarzabal et al., 2005). The incorporation of either of these compounds appears to be a matter of cost. The use of filters also improves the performance of blood media, such as Campy-Cefex, which appears to have low selectivity and isolation rate because of the growth of competing microorganisms (Chon et al., 2012).

2.4. Use of membrane filters

Membrane filters were first tested for the isolation of *Campylobacter* spp. from animal samples more than 50 years ago. This filtration technique was first used to isolate *C. fetus* from bulls (Plumer et al., 1962). In the 1980s and 1990s, a few studies mentioned the placement of drops of stool on a membrane filter, whose pore size was between 0.45 and 0.85 µm, for the isolation of *Campylobacter* from stool samples. The filter was then placed on the surface of a blood agar and removed during incubation (Steele and McDermott, 1984; Le Roux and Lastovica, 1998). While larger microorganisms get trapped in the filter, the high motility and the slender shape of *Campylobacter* spp. aid in the passage of the cells through the small pores of the membrane filter. Le Roux and Lastovica (1998) were able to isolate

Campylobacter spp. directly from stool samples on plate media without the addition of any selective antimicrobial agent.

The membrane filter method has been successfully used for isolation of *Campylobacter* from foods as well. In one study, 0.45 µm size membrane filters were used to prevent accidental passing of competitive flora (Baggerman and Koster, 1992). A comparison of different filters showed the highest isolation rate of *Campylobacter* when drops of a 24 h enriched broth culture were placed on a cellulose nitrate filter with pore size of 0.65 µm (Baggerman and Koster, 1992). In another study, 0.45 µm size membrane filters were used to prevent accidental passing of competitive flora (Baggerman and Koster, 1992). In a more recent study, scanning electron microscope was used to determine the rate of the passage of *Campylobacter* cells and to calculate the amount of time that filters should be left on plates for optimal recovery of cells (Speegle et al., 2009). These studies showed that 100 µl of enriched broth distributed as drops on top of 0.65 µm size membrane filter for 15 min was sufficient for the recovery of *Campylobacter* cells. When using membrane filters for isolation of *Campylobacter* on plate media, the addition of an enrichment step is recommended to allow for the multiplication of *Campylobacter* cells to approximately 2–3 log CFU per ml of enriched broth (Speegle et al., 2009). The main advantage of the use of membrane filters is that pure *Campylobacter* cultures are obtained in 36–48 h. Because some other contaminating bacteria may also pass through the filters, it is advisable to include cefoperazone and amphotericin B to the plate media used in combination with the filters. There is no need to add vancomycin when using membrane filters. Membrane filters can also help to obtain pure *Campylobacter* colonies from mix cultures and therefore appear to improve the isolation on agar plates.

3. Identification of *Campylobacter* spp.

3.1. Latex agglutination tests

Latex agglutination tests for rapid identification of *Campylobacter* spp. have been in use for approximately 20 years. The principle behind this test is the use of polyclonal antibodies to detect flagellar or outer membrane proteins. The latex particles are coated with immunoglobulins that are raised against antigen from several *Campylobacter* species, primarily *C. jejuni*, *C. coli* and *C. lari*. Several studies have assessed previous latex tests, but as of 2013 there are only two commercial latex tests available in the US market: Microgen M46 *Campylobacter* (Microgen Bioproducts Ltd., Camberley, Surrey, United Kingdom), which reacts with most of the *Campylobacter* spp., and SCIMEDXCAMPY (jcl)TM (Scimedx Corp., Denville, NJ), which reacts only with *C. jejuni*, *C. coli* and *C. lari* (Miller et al., 2008). Although several studies have assessed previous latex tests, only one commercial latex test is available in the market. Of present importance, a latex agglutination immunoassay, and/or the use of phase contrast microscopy are considered confirmatory tests in the suggested methodology for isolation, identification and enumeration of *Campylobacter* spp. from poultry rinses and sponge samples by the U.S. Department of Agriculture (Anonymous, 2013a). It is important to remember that positive controls must be run with any latex agglutination test.

3.2. Enzyme-linked immunosorbent (EIA) assays

Similarly to the latex test, there were several EIA assays commercially available for the identification of *Campylobacter* spp. in foods, but most of these tests have been discontinued. Currently, commercial tests include mainly the VIDAS® *Campylobacter* (bioMerieux, Marcy l'Etoile, France), which is an automated EIA system that has undergone several validations and has been in use in several countries for numerous years (Liu et al., 2009; Reiter et al., 2010). There are currently four non-automated EIA assays for clinical samples: the PremierTM CAMPY microplate EIA and the ImmunoCard STAT! CAMPY by Meridian

Bioscience, Inc. (Cincinnati, OH) (Granato et al., 2010); the ProSpec™ *Campylobacter* assay by Remel Inc. (Lenexa, KS) (Granato et al., 2010; Tribble et al., 2008); and the Ridascreen® *Campylobacter* by R-Biopharm AG (Germany) (Bessede et al., 2011). These assays have a sensitivity of 10^{5-6} CFU (colony forming units) per ml^{-1} (Oyarzabal and Battie, 2012). A recent evaluation of these assays showed that EIA assays are quite variable and that these assays alone should not be used for direct identification of *Campylobacter* spp. in stool samples. In addition, EIA assays are not sufficient for confirmation and therefore laboratories should confirm positive EIA results by culture methods (Fitzgerald et al., 2011).

3.3. Molecular identification methods

Molecular identification methods are fast and specific for the identification of *Campylobacter* spp. Besides detecting specific segments of DNA or RNA, sequencing protocols are now providing a fast way to detect specific segments of the DNA that are unique for identification to the species and sometimes subspecies level. Currently, these methods provide reliable tools for rapid screening of presumptive positive samples. However, the two regulatory agencies in charge of food safety in the USA, the Food and Drug Administration of the Department of Health and Human Services, and the Food Safety and Inspection Services of the U.S. Department of Agriculture, do not consider these molecular tests “confirmatory” and therefore the actual culture has to be obtained from presumptive positive samples for confirmation purposes.

3.3.1. Polymerase chain reaction (PCR) and multiplex PCR (mPCR) assays

Since the first polymerase chain reaction (PCR) assay for the identification of *Campylobacter* spp. more than 20 years ago (Oyofe et al., 1992), PCR has become one of the most commonly used platforms for the identification of these foodborne pathogens. Genes unique to *Campylobacter* spp. have been used as targets for PCR assays to identify different *Campylobacter* spp. Manufacturers of PCR-based methods include enrichment protocols to recover injured or stressed cells and to increase the number of cells to detection level. The sensitivity of PCR assays is in the range of 10^3 CFU per ml in pure cultures, but the sensitivity is reduced considerably when testing food matrices. The enrichment step also serves as a calibration to increase the probability of confirming a presumptive positive by cultural methods.

Until 8–10 years ago, PCR assays were single PCR reactions, meaning that they detected only one bacterial species. But in recent years a series of multiplex PCR (mPCR) assays have been designed to detect the presence of two or more species in the same sample. For instance, PCR assays have helped us understand that both *C. jejuni* and *C. coli* multiply to detection numbers during the enrichment of naturally contaminated retail broiler meat (Oyarzabal et al., 2007). Although the presence of both *Campylobacter* spp. was suggested in enriched poultry meat, it is extremely difficult to isolate both species from the enrichment with current plate media. Several multiplex PCR assays have been successfully used for testing a large number of commercial retail broiler meat (Cloak and Fratamico, 2002; Linton et al., 1997; Oyarzabal et al., 2005, 2007; Persson and Olsen, 2005; Zhou et al., 2011) and therefore these protocols assist in providing rapid identification of *Campylobacter* spp.

The platform for the detection of amplified product has already moved to real time detection protocols. There are two commercial real time PCR (qPCR) assays in the market: BAX® System for *C. jejuni/coli/lari* (DuPont, Qualicon, Wilmington, DE) and iQ-Check™ *Campylobacter* (Bio-Rad, Hercules, CA). More assays based on real time platforms, and its variations, will continue to appear in the market in the near future.

Recently, a multiplex qPCR assay that targets the *ceuE* enterochelin gene for iron acquisition in *C. jejuni* (accession X82427) and *C. coli* (accession X88849) was developed and a total of 166 strains were tested in inclusivity and exclusivity studies (Gharst et al., 2013). This qPCR protocol was able to detect 100% of 59 *Campylobacter* strains examined, which included 34 *C. jejuni* strains and 25 *C. coli* strains.

Analysis of 107 strains for the exclusivity studies resulted in no false positives using this qPCR assay.

4. Current detection methodologies in the USA

4.1. USDA Food Safety Inspection Service (FSIS), Office of Public Health Science (OPHS) *C. jejuni/coli* detection methodology

The methodology suggested by FSIS is described in Microbiology Laboratory Guidebook (MLG) 41.02 (Anonymous, 2013a). This methodology focuses on the isolation of *Campylobacter* spp. from carcass rinses and sponge samples from poultry. Carcass rinses are collected by the use of 400 ml of peptone water and gently shaking the collected carcass for 1 min to ensure even distribution.

The quantitative analysis from the direct plating of 1.2 ml of the rinse on selective agar plates is the only value that FSIS USDA use for reporting results. FSIS USDA does not endorse any particular product and acknowledges that equivalent products are available for *Campylobacter* isolation and identification. Although a blood-based plate medium is suggested in MLG 41.02, similar results for direct count of *Campylobacter* spp. from poultry carcass rinses were found using different charcoal and blood plate media in a large study (Oyarzabal et al., 2005). Therefore, charcoal-based plates, such as CCDA, are the best alternative to blood-based plates. It is important to mention that the addition of more plates and/or the enrichment of the carcass rinse result in a high number of *Campylobacter* positive samples. These findings highlight the fact that the number of *Campylobacter* cells in processed carcass rinses is slightly lower than the limit of detection achieved by direct plating (Fig. 1).

Quantitative examination uses 1 ml of the 400 ml peptone water carcass rinse distributed in 250 μl aliquots across four Campy-Cefex plates. Because of the large amount of liquid applied to the plates, it is important to properly dry the plates before use. The drying of the plates is still an empirical process and laboratories should identify the best practices for their own environments. Plates are incubated at 42 °C for 48 h under microaerobic conditions. Qualitative analysis of the sample was suggested by enriching 30 ml carcass rinse in 30 ml of 2 × Blood-free Bolton enrichment broth (BF-BEB) and then transferring 0.1 ml of the enriched sample to agar plates for isolation. However, scientists at FSIS USDA have recently compared the results from the quantitative (1 ml) and the qualitative (30 ml) portions for 248 sets from young chicken establishments and have decided to discontinue the qualitative portion in future tests (Anonymous, 2013b).

The turkey sampling protocol uses a single sponge to sample 50 cm^2 of the back and 50 cm^2 of the thigh for a total area of 100 cm^2 of one turkey carcass sampled. The resulting sponge is then placed into 25 ml of peptone water. The sponge is squeezed multiple times before quantitative or qualitative examination. Quantitative testing uses 1 ml of the 25 ml distributed equally in 250 μl aliquots across four Campy-Cefex plates. Dilutions are prepared accordingly to obtain a countable range of suspected *C. jejuni/coli* CFU per plate. Qualitative results are obtained by enriching the rest of the diluent and sponge using an equivalent volume of 2 × BF-BEB. A 100 μl portion is plated onto each of two Campy-Cefex agar plates and incubated at 42 °C for 48 h under microaerobic conditions.

Currently, the FSIS recommends the use of phase contrast microscopy or a latex agglutination kit for the confirmation of presumptive *Campylobacter* positive isolates. The confirmation by phase contrast microscopy requires the use of a 100× immersion oil objective to observe the spiral morphology and high motility. In 24–36 h cultures, *Campylobacter* cells are highly motile and therefore it is difficult to clearly see their morphology under high magnification. In these cases, the slide can be left at 42 °C for 2–3 h or slightly heated to reduce the motility of the cells and allow for a better visualization of the spiral shape. Lower magnification phase contrast objectives,

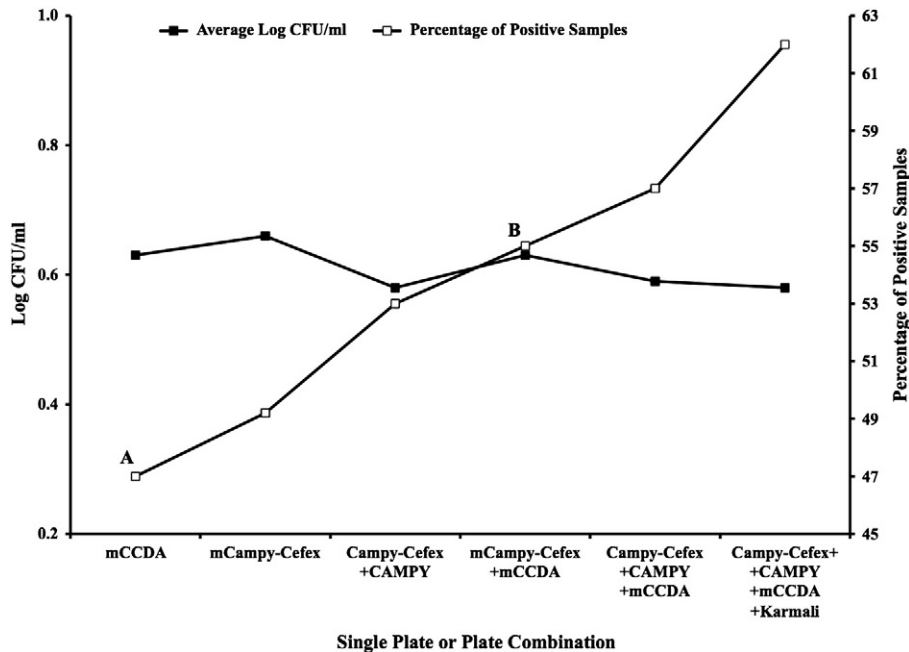


Fig. 1. Percentage of positive samples for *Campylobacter* spp. after plating carcass rinses on six different plates. A and B are statistically different ($P < 0.05$) with the Fisher exact test calculated using R (Anonymous, 2013c). The analysis was done on data from reference Oyarzabal et al. (2005).

such as 40×, can result in the erroneous confirmation of isolates that are not *Campylobacter* spp. but have high motility. Confirmation can also be done by the use of a latex agglutination test.

4.2. New methodology from the U.S. Food and Drug Administration (FDA)

The current *Campylobacter* detection method from food and water is described in Chapter 7 of the Bacteriological Analytical Manual (BAM) (Hunt et al., 2001). This protocol includes a pre-enrichment step in which 25 g of the suspected food matrix is combined with 100 ml of Bolton broth supplemented with lysed horse blood and the corresponding selective antibiotics (vancomycin, trimethoprim lactate, amphotericin B or cyclohexamide, cefoperazone) at 37 °C for 4 h. Then the temperature of incubation is raised to 42 °C for 20–44 h followed by plating on charcoal based modified CCDA or Abeyta–Hunt–Bark. Presumptive colonies are examined by phase contrast microscopy for characteristic shape and motility. A gas tank system (“bubbler”), shaking flasks, or bag system are the suggested conventional method used to achieve the desired microaerobic conditions for incubation. Typical colonies are confirmed by biochemical testing such as oxidase, catalase, TSI reaction, glucose utilization, and hippurate hydrolysis (Hunt et al., 2001). However, biochemical tests, specially the use of the hippurate test and antimicrobial resistance, are unreliable and very few, reference laboratories use these tests (Morris et al., 1985; Oyarzabal et al., 1997).

Many variants are listed for this protocol depending upon the food matrix. For example, raw milk has a centrifugation step at 12,000 ×g for 40 min with the adjustment of the pH to 7.5 ± 0.2 using sterile NaOH. This pH adjustment will inactivate the lactoperoxidase system, toxic to *Campylobacter*. Detection of *Campylobacter* from water typically uses 2–4 L and requires filtration with a pore size of 45 μm before enrichment (Hunt et al., 2001).

The scientific community has made numerous advances in the detection of *Campylobacter* in the past few years, including media, detection equipment, and molecular procedures. To emphasize these advancements and overcome some of the drawbacks of the reference culturing methods, an updated methodology has been developed for rapid isolation and detection of *C. jejuni* and *C. coli* (Fig. 2). A key component of this revised protocol was developing a confirmation method

based on real-time PCR (qPCR) targeting the enterochelin *ceuE* gene for iron acquisition (Gharst et al., 2013). This revised method will hopefully allow consistency in the confirmation among many agencies and private industry, provide higher throughput of sample analysis, simplify specific identification from suspect foods, and reduce the detection time.

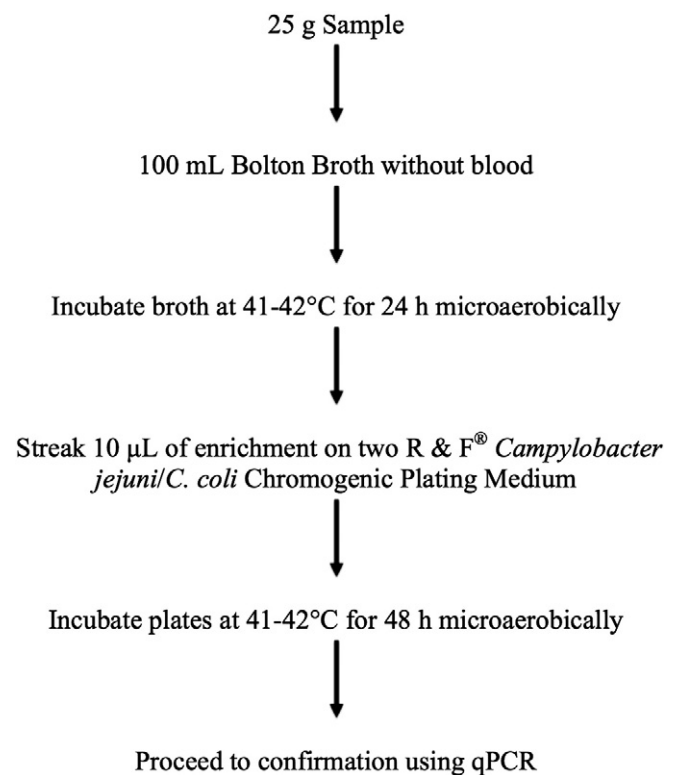


Fig. 2. Diagram summary of proposed updated FDA method. CCPM = R & F® *Campylobacter jejuni/C. coli* Chromogenic Plating Medium.

5. Review of International Organization for Standardization (ISO)

In 2009 the International Organization for Standardization (ISO) revised 10272. This revision described numerous new updates to the protocol for the detection and enumeration of *Campylobacter* from food and animal feeding stuffs (Anonymous, 2006). Currently 10272 states that no single enrichment is optimal for *Campylobacter* detection in all foods and that selectivity is greater using Preston broth. The revised version suggests the use of Bolton broth incubated microaerobically for 4–6 h at 37 °C then 40–48 h at 41.5 °C in foods with low background or stressed *Campylobacter* organisms (Anonymous, 2009). The detection of *Campylobacter* from foods with high background count such as raw chicken products, raw meats, and raw milk uses Preston broth and microaerobic incubation for 24 h at 41.5 °C then isolation using mCCDA. All plating revisions to the standard use mCCDA as primary selective medium in combination with a medium not containing cefoperazone like Preston agar. As depicted above, incubation is done under microaerobic conditions using a variety of apparatus. Four options to obtain microaerobic conditions are outlined to include a cabinet that automatically controls the gaseous atmosphere, gas filled jars, jars using gas-generating kits, and screw capped bottles with limited headspace. The addition of blood, charcoal, hemin, ferrous sulfate, sodium metabisulfite, and sodium pyruvate is indicated as desired additives to neutralize any toxic effects from O₂, H₂O₂, and superoxide ions (Anonymous, 2009). Enumeration is obtained by plating the initial suspect suspension using decimal dilutions (Anonymous, 2007). A rather interesting revision when expecting low counts is to plate 1 ml of the initial suspension over three agar plates, in duplicate.

Confirmation/identification of suspect isolated colonies is traditionally ascertained using biochemical tests such as oxidase, catalase, hippurate, and indoxyl acetate. Interestingly, the revision to this standard advises removing antibiotic sensitivity tests for nalidixic acid and cephalothin and aerobic growth at 41.5 °C concerning identification. Additional confirmation tests recommended in the standard include latex agglutination kits. Other tests to confirm/identify suspect colonies are PCR, microarray, immunological tests, and O.B.I.S. campy kit (Gram-lysis and L-alanyl aminopeptidase) (Oxoid).

6. Updates on protocols for sample collection

The spatial appearance of bacteria in foods is explained by a contagious distribution, where the variance is significantly greater than the mean. Although several mathematical models have been proposed to explain this distribution, the binomial or negative binomial models are commonly used in food microbiology (Jarvis, 2008). In 2-class attributes sampling plan (presence/absence), the testing for the presence of a bacterial foodborne pathogen is done under the premise that the pathogen is not present in the sample. From the statistical standpoint, this premise (null hypothesis) signifies that the failure to find a pathogen when it is present is a Type II error and represents a false negative result (Ferrier and Buzby, 2013). Because the probability of finding a positive sample under this scheme is very low, and assuming that there are no methodological errors or artifacts, a Type I error (false positive) is reserved for the status of a sample after the first screening and from which no bacterial pathogen has been isolated after attempting a cultural confirmation. In the case of a positive, the recommendation is not to argue the results because re-testing will probably yield negative results and therefore a false sense of safety. From the public health stand point, Type II errors (false negative results) are the most dangerous and, probabilistically speaking, very difficult to predict. If increasing in sample size reduces the probability of incurring into a Type II error, it would be logical to think that more sampling protocol should have been tried over the years to understand how to reduce Type II errors and improve public health outcomes. But this is not the case with the testing of *Campylobacter* spp. in foods for the last 40 years, in which the actual sampling protocols have been unchallenged.

The incorporation of HACCP in 1996 brought about the increase in the amount of liquid to 400 ml for the collection of rinses from processed carcass in commercial poultry processing plants. But the actual sampling protocol, the rinse of a carcass, dates back to the 1970s (Smith and Muldoon, 1974). The testing for the performance standard for *Campylobacter* spp. in poultry is performed on chilled, processed broiler carcasses, before carcasses undergo further processing to portioning (Anonymous, 2011). The only incorporation of an original sampling method is the use of sponges to sample the large carcasses of turkeys.

In the USA, most of the retail broiler meat is sold as boneless, skinless products in tray packs. Yet, there is no performance standard for *Campylobacter* spp. at the retail level. The current testing at retail entails the enrichment of 25-g of poultry meat in nine times the volume of enrichment (225 ml). However, a ratio of 1:4 of meat:enrichment has been shown to produce similar results (Oyarzabal et al., 2007). The latest modification to the sampling techniques for retail samples involve the rinse of chicken parts in 100 ml of buffered peptone water with a subsequence incubation at 42 °C for 48 h under aerobic conditions (Oyarzabal et al., 2013). This protocol has shown to be more sensitive for the recovery of naturally contaminated retail broiler meat, it requires less time for preparation and processing of the samples, and it is perhaps more amenable to coupling with DNA-based methods for a fast screening of positive samples. The increase in the number of positive samples with this new sampling method also translated in a more diverse number of types based on pulsed field gel electrophoresis. Therefore, new sampling protocols should be tested at the retail level to determine if the strains that have more epidemiological relevance to humans can be quickly identified, and to test the efficacy of interventions to reduce human exposure to *Campylobacter* spp.

7. Conclusions

Campylobacter is a significant foodborne pathogen associated with the consumption of undercooked poultry or raw milk. In poultry products, the only *Campylobacter* spp. are *C. jejuni* and *C. coli*. In samples with low number of cells, the enrichment for 48 h under aerobic conditions is recommended to achieve a detectable number of *Campylobacter* cells. Bolton broth and even buffered peptone water can be used as enrichment broths, and CCDA is the best choice in plate media. The incubation of samples at 42 °C throughout the isolation process is still preferred. The confirmation of presumptive *Campylobacter* isolates suggested by FSIS USDA is based on phase contrast microscopy and latex agglutination test. It is important, however, to use a 100× immersion oil objective to avoid mistakes during the confirmation by phase contrast microscopy. Although not accepted by food regulatory agencies in the USA, DNA assays are simple and rapid methods to confirm *Campylobacter* isolates and identify them to the species level at the same time. Finally, the recent development in sampling procedures highlights that the rinsing of retail broiler meat is more sensitive for the recovery of naturally contaminated retail broiler meat; requires less time for preparation and processing of the samples. This protocol could be coupled with DNA-based methods for a fast screening of positive samples.

Acknowledgment

The authors would like to thank Dr. Nancy M. Gell for her critical review of the manuscript.

References

- Adler, H.I., Carrasco, A., Crow, W., Gill, J.S., 1981. Cytoplasmic membrane fraction that promotes septation in an *Escherichia coli* lon mutant. *J. Bacteriol.* 147, 326–332.
- Anonymous, 2006. Microbiology of food and animal feeding stuffs – horizontal method for detection and enumeration of *Campylobacter* spp. Part 1: Detection Methods. ISO 10272-1:2006.

- Anonymous, 2007. Microbiology of Food and Animal Feeding Stuffs – General Requirements and Guidance for Microbiological Examinations. ISO 7218:2007.
- Anonymous, 2009. Microbiology of Food and Animal Feed – Horizontal Method for Detection and Enumeration of *Campylobacter* – Part 1: Detection Method. ISO 10272-1A:2009.
- Anonymous, 2011. New performance standards for *Salmonella* and *Campylobacter* in young chicken and turkey slaughter establishments: response to comments and announcement of implementation schedule. Fed. Reg. 76, 15282–15290.
- Anonymous, 2013a. Isolation, identification, and enumeration of method for the enumeration of *Campylobacter jejuni/coli/lari* from poultry rinse, sponge and raw poultry product samples. USDA FSIS Microbiology Laboratory Guidebook, Chapter 41.02. (Available at <http://www.fsis.usda.gov/wps/wcm/connect/0273bc3d-2363-45b3-befb-1190c25f3c8b/MLG-41.pdf?MOD=AJPERES>).
- Anonymous, 2013b. Report on *Campylobacter* Testing of Poultry Products – Decision to Suspend the Qualitative Test (30 mL). Food Safety and Inspection Services, U.S. Department of Agriculture (Available at http://www.fsis.usda.gov/wps/wcm/connect/0c4adb75-51a4-4b77-9f13-07d61589812e/Campylobacter_Methods_Comparison_Report.pdf?MOD=AJPERES).
- Anonymous, 2013c. R: a language and environment for statistical computing. <http://www.R-project.org>.
- Baggerman, W.L., Koster, T., 1992. A comparison of enrichment and membrane filtration methods for the isolation of *Campylobacter* from fresh and frozen foods. Food Microbiol. 9, 87–94.
- Baylis, C.L., MacPhee, S., Martin, K.W., Humphrey, T.J., Betts, R.P., 2000. Comparison of three enrichment media for the isolation of *Campylobacter* spp. from foods. J. Appl. Microbiol. 89, 884–891.
- Bessede, E., Delcamp, A., Sifre, E., Buissonniere, A., Megraud, F., 2011. New methods for detection of campylobacters in stool samples in comparison to culture. J. Clin. Microbiol. 49, 941–944.
- Black, R.E., Levine, M.M., Clements, M.L., Hughes, T.P., Blaser, M.J., 1988. Experimental *Campylobacter jejuni* infection in humans. J. Infect. Dis. 157, 472–479.
- Bolton, F.J., Coates, D., 1983. Development of a blood-free *Campylobacter* medium: screening tests on basal media and supplements, and the ability of selected supplement to facilitate aerotolerance. J. Appl. Bacteriol. 54, 115–125.
- Bolton, F.J., Robertson, L., 1982. A selective medium for isolating *Campylobacter jejuni/coli*. J. Clin. Pathol. 35, 462–467.
- Border, M.M., Firehammer, B.D., Myers, L.L., 1974. Tube culture method for viable counts of *Campylobacter fetus* (*Vibrio fetus*). Appl. Microbiol. 28, 730–732.
- Bowdre, J.H., Krieg, N.R., Hoffman, P.S., Smibert, R.M., 1976. Stimulatory effect of dihydroxyphenyl compounds on the aerotolerance of *Spirillum volutans* and *Campylobacter fetus* subspecies *jejuni*. Appl. Environ. Microbiol. 31, 127–133.
- Chon, J.W., Hyeon, J.Y., Park, J.H., Song, K.Y., Seo, K.H., 2012. Comparison of 2 types of broths and 3 selective agars for the detection of *Campylobacter* species in whole-chicken carcass-rinse samples. Poult. Sci. 2012 (91), 2382–2385.
- Cloak, O.M., Fratamico, P.M., 2002. A multiplex PCR for the differentiation of *Campylobacter jejuni* and *C. coli* from a swine processing facility and characterization of isolates by PFGE and antibiotic resistance profiles. J. Food Prot. 65, 266–273.
- Corry, J.E.L., Post, D.E., Colin, P., Laisney, M.J., 1995. Culture media for isolation of campylobacters. Int. J. Food Microbiol. 26, 43–76.
- Damborg, P., Olsen, K.E., Moller-Nielsen, E., Guardabassi, L., 2004. Occurrence of *Campylobacter jejuni* in pets living with human patients infected with *C. jejuni*. J. Clin. Microbiol. 42, 1363–1364.
- Ferrier, P.M., Buzby, J.C., 2013. The economic efficiency of sampling size: the case of beef trim. Risk Anal. 33, 368–384.
- Fitzgerald, C., Patrick, M., Jerris, R., Watson, R., Tobin-D'Angelo, M., Gonzalez, A., Polage, C., Wymore, K., Gillim-Ross, L., Sadlowski, J., Monahan, J., Hurd, S., Dahlberg, S., DeMartino, M., Pentella, M., Razeq, J., Leonard, C., Jung, C., Juni, B., Robinson, T., Gittelman, R., Garrigan, C., Nachamkin, I., *Campylobacter* diagnostics working group, 2011. Multicenter study to evaluate diagnostic methods for detection and isolation of *Campylobacter* from stool. Annual Meeting of the American Society for Microbiology, New Orleans, LA., (n.d.), 20 Sept.
- Friedman, C.R., Hoekstra, R.M., Samuel, M., Marcus, R., Bender, Shiferaw, B., Reddy, S., Ahuja, S.D., Helfrick, D.L., Hardnett, F., Carter, M., Anderson, B., Tauxe, R.V., Emerging Infections Program FoodNet Working Group, 2004. Risk factors for sporadic *Campylobacter* infection in the United States: a case-control study in FoodNet sites. Clin. Infect. Dis. 38 (Suppl. 3), S285–S296.
- Gharst, G., Bark, D.H., Newkirk, D., Guillen, L., Wang, Q., Abeyta, C., 2013. Evaluation and single laboratory verification of a proposed modification to the U.S. Food and Drug Administration method for the detection and identification of *Campylobacter jejuni* or *Campylobacter coli* from raw silo milk. J. AOAC Int. (accepted for publication).
- Gilliss, D., et al., 2013. Incidence and trends of infection with pathogens transmitted commonly through food – foodborne diseases active surveillance network, 10 U.S. sites, 1996–2012. MMWR Morb. Mortal. Wkly Rep. 62, 283–287.
- George, H.A., Hoffman, P.S., Smibert, R.M., Krieg, N.R., 1978. Improved media for growth and aerotolerance of *Campylobacter fetus*. J. Clin. Microbiol. 8, 36–41.
- Granato, P., Chen, L., Holiday, I., Rawling, R., Novak-Weekley, S., Quinlan, T., Musser, K., 2010. Comparison of premier CAMPY enzyme immunoassay (EIA), ProSpecT *Campylobacter* EIA, and ImmunoCard STAT! CAMPY tests with culture for laboratory diagnosis of *Campylobacter* enteric infections. J. Clin. Microbiol. 48, 4022–4027.
- Habib, I., Sampers, I., Uyttendaele, M., Berkvens, D., De Zutter, L., 2008. Performance characteristics and estimation of measurement uncertainty of three plating procedures for *Campylobacter* enumeration in chicken meat. Food Microbiol. 25, 65–74.
- Habib, I., Uyttendaele, M., De Zutter, L., 2011. Evaluation of ISO 10272:2006 standard versus alternative enrichment and plating combinations for enumeration and detection of *Campylobacter* in chicken meat. Food Microbiol. 28, 1117–1123.
- He, Y., Yao, X., Gunther IV, N.W., Xie, Y., Tu, S.-I., Shi, X., 2010. Simultaneous detection and differentiation of *Campylobacter jejuni*, *C. coli*, and *C. lari* in chickens using a multiplex real-time PCR assay. Food Anal. Methods 3, 321–329.
- Heuvelink, A.E., van Heerwaarden, C., Zwartkruis-Nahuis, A., Tilburg, J.J., Bos, M.H., Heilmann, F.G., Hofhuis, A., Hoekstra, T., de Boer, E., 2009. Two outbreaks of campylobacteriosis associated with the consumption of raw cow's milk. Int. J. Food Microbiol. 134, 70–74.
- Hunt, J.M., Abeyta, C., Tran, T., 2001. Bacteriological Analytical Manual, Chapter 7 *Campylobacter*. FDA (<http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm072616.htm>).
- Hutchinson, D.N., Bolton, F.J., 1984. Improved blood free selective medium for the isolation of *Campylobacter jejuni* from faecal specimens. J. Clin. Pathol. 37, 956–957.
- Jarvis, B., 2008. The distribution of microorganisms in foods in relation to sampling, Chapter 4. In: Jarvis, B. (Ed.), Statistical Aspects of the Microbiological Examination of Foods, 2nd Ed. Academic Press, Elsevier, pp. 45–277.
- Jay-Russell, M.T., Mandrell, R.E., Yuan, J., Bates, A., Manalac, R., Mohle-Boetani, J., Kimura, A., Lidgard, J., Miller, W.G., 2013. Using MOMP typing as an epidemiologic tool to investigate milkborne *Campylobacter jejuni* outbreaks in California. J. Clin. Microbiol. 51, 195–201.
- Kaldor, J., Speed, B.R., 1984. GBS and *Campylobacter jejuni*: a serological study. Br. Med. J. 288, 1867–1870.
- King, E.O., 1957. Human infections with *Vibrio fetus* and a closely related vibrio. J. Infect. Dis. 101, 119–128.
- Le Roux, E., Lastovica, A.J., 1998. The Cape Town protocol: how to isolate the most campylobacters for your dollar, pound, franc, yen, etc. In: Lastovica, A.J., Newell, D.G., Lastovica, E.E. (Eds.), *Campylobacter, Helicobacter and related organisms*. The Rustica Press, Pinelands, South Africa, pp. 30–33.
- Lejeune, J.T., Rajala-Schultz, P.J., 2009. Food safety: unpasteurized milk: a continued public health threat. Clin. Infect. Dis. 48, 93–100.
- Linton, D., Lawson, A.J., Owen, R.J., Stanley, J., 1997. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrhetic samples. J. Clin. Microbiol. 35, 2568–2572.
- Liu, L., Hussain, S.K., Miller, R.S., Oyarzabal, O.A., 2009. Research note: efficacy of mini VIDAS for the detection of *Campylobacter* spp. from retail broiler meat enriched in Bolton broth with or without the supplementation of blood. J. Food Prot. 72, 2428–2432.
- Man, S.M., 2011. The clinical importance of emerging *Campylobacter* species. Nat. Rev. Gastroenterol. Hepatol. 8, 669–685.
- Miller, R.S., Speegle, L., Lastovica, A.J., Oyarzabal, O.A., 2008. Letter to the Editor. An evaluation of three commercial latex agglutination tests for the identification of *Campylobacter* spp. J. Clin. Microbiol. 46, 3546–3547.
- Moon, H., Kim, S.B., Chen, J.J., George, N.I., Kodell, R.L., 2013. Model uncertainty and model averaging in the estimation of infectious doses for microbial pathogens. Risk Anal. 33, 220–231.
- Morris, G.K., el Sherbeeny, M.R., Patton, C.M., Kodaka, H., Lombard, G.L., Edmonds, P., Hollis, D.G., Brenner, D.J., 1985. Comparison of four hippurate hydrolysis methods for identification of thermophilic *Campylobacter* spp. J. Clin. Microbiol. 22, 714–718.
- Oliver, S.P., Boor, K.J., Murphy, S.C., Murinda, S.E., 2009. Food safety hazards associated with consumption of raw milk. Foodborne Pathog. Dis. 6, 793–806.
- Oyarzabal, O.A., Battie, C., 2012. Immunological methods for the detection of *Campylobacter* spp. – current applications and potential use in biosensors. In: Abuelzein, E. (Ed.), Trends in Immunolabelled and Related Techniques. InTech. ISBN: 978-953-51-0570-1, pp. 203–226.
- Oyarzabal, O.A., Wesley, I.V., Barbaree, J.M., Lauerman, L.H., Conner, D.E., 1997. Specific detection of *Campylobacter lari* by PCR. J. Microbiol. Meth. 29, 97–102.
- Oyarzabal, O.A., Macklin, K.S., Barbaree, J.M., Miller, R.S., 2005. Evaluation of agar plates for direct enumeration of *Campylobacter* spp. from poultry carcass rinses. Appl. Environ. Microbiol. 71, 3351–3354.
- Oyarzabal, O.A., Backert, S., Nagaraj, M., Miller, R.S., Hussain, S.K., Oyarzabal, E.A., 2007. Efficacy of supplemented buffered peptone water for the isolation of *Campylobacter jejuni* and *C. coli* from broiler retail products. J. Microbiol. Meth. 69, 129–136.
- Oyarzabal, O.A., Williams, A., Zhou, P., Samadpour, M., 2013. Improved protocol for isolation of *Campylobacter* spp. from retail broiler meat and use of pulsed field gel electrophoresis for the typing of isolates. J. Microbiol. Meth. (Available online 29 March 2013 (<http://www.sciencedirect.com/science/article/pii/S0167701213001024>)).
- Oyoyo, B.A., Thornton, S.A., Burr, D.H., Trust, T.J., Pavlovskis, O.R., Guerry, P., 1992. Specific detection of *Campylobacter jejuni* and *Campylobacter coli* by using polymerase chain reaction. J. Clin. Microbiol. 30, 2613–2619.
- Painter, J.A., Hoekstra, R.M., Ayers, T., Tauxe, R.V., Braden, C.R., Angulo, F.J., Griffin, P.A., 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg. Infect. 19, 407–415.
- Persson, S., Olsen, K.E.P., 2005. Multiplex PCR for identification of *Campylobacter coli* and *Campylobacter jejuni* from pure cultures and directly on stool samples. J. Med. Microbiol. 54, 1043–1047.
- Plumer, G.L., Duvall, W.C., Shepler, V.M., 1962. A preliminary report on a new technique for isolation of *Vibrio fetus* from carrier bulls. Gen. Vet. 52, 110–122.
- Potturi-Venkata, L.P., Backert, S., Lastovica, A.J., Vieira, S.L., Norton, R.A., Miller, R.S., Pierce, S.J., Oyarzabal, O.A., 2007. Evaluation of different plate media for direct cultivation of *Campylobacter* spp. from live broilers. Poult. Sci. 86, 1304–1311.
- Reiter, M.G., López, C., Jordano, R., Medina, L.M., 2010. Comparative study of alternative methods for food safety control in poultry slaughterhouses. Food Anal. Meth. 3, 253–260.
- Robinson, D.A., Edgar, W.M., Gibson, G.L., Matchett, A.A., Robertson, L., 1979. *Campylobacter* enteritis associated with consumption of unpasteurized milk. Br. Med. J. 1, 1171–1173.
- Robinson, D.A., 1981. Infective dose of *Campylobacter jejuni* in milk. Br. Med. J. 282, 1584.

- Sails, A.D., Wareing, D.R.A., Bolton, F.J., 1998. Evaluation of three microaerobic systems for the growth and recovery of *Campylobacter* spp. In: Lastovica, A.J., Newell, D.G., Lastovica, E.E. (Eds.), *Campylobacter, Helicobacter and related organisms*. The Rustica Press, Pinelands, South Africa, pp. 39–42.
- Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L., Griffin, P.M., 2011. Foodborne illness acquired in the United States – major pathogens. *Emerg. Infect. Dis.* 17, 7–15.
- Schnaitman, C.A., 1970. Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli*. *J. Bacteriol.* 104, 890–901.
- Schildt, M., Savolainen, S., Hanninen, M.L., 2006. Long-lasting *Campylobacter jejuni* contamination of milk associated with gastrointestinal illness in a farming family. *Epidemiol. Infect.* 134, 401–405.
- Smith, M.V., Muldoon, P.J., 1974. *Campylobacter fetus* subsp. *jejuni* (*Vibrio fetus*) from commercially processed poultry. *Appl. Microbiol.* 27, 995–996.
- Smith, K., Reimers, N., Barnes, H.J., Lee, B.C., Siletzky, R., Kathariou, S., 2004. *Campylobacter* colonization of sibling turkey flocks reared under different management conditions. *J. Food Prot.* 67, 1463–1468.
- Solomon, E.B., Hoover, D.G., 1999. *Campylobacter jejuni*: a bacterial paradox. *J. Food Safety* 19, 121–136.
- Speed, B., Kaldor, J., Cavanagh, P., 1984. Guillain–Barre syndrome associated with *Campylobacter jejuni* enteritis. *J. Infect.* 8, 85–86.
- Speegle, L., Miller, M.E., Backert, S., Oyarzabal, O.A., 2009. Research note: use of cellulose filters to isolate *Campylobacter* spp. from naturally contaminated retail broiler meat. *J. Food Prot.* 72, 2592–2596.
- Steele, T.W., McDermott, S.N., 1984. The use of the membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from feces. *Pathology* 16, 263–265.
- Suzuki, H., Yamamoto, S., 2009. *Campylobacter* contamination in retail poultry meats and by-products in the world: a literature survey. *J. Vet. Med. Sci.* 71, 255–261.
- Taylor, E.V., Herman, K.M., Ailes, E.C., Fitzgerald, C., Yoder, J.S., Mahon, B.E., Tauxe, R.V., 2013. Common source outbreaks of *Campylobacter* infection in the USA, 1997–2008. *Epidemiol. Inf.* 141, 987–996.
- Tenkate, T.D., Stafford, R.J., 2001. Risk factors for *Campylobacter* infection in infants and young children: a matched case–control study. *Epidemiol. Infect.* 127, 399–404.
- Tribble, D., Baqar, S., Pang, L., Mason, C., Houg, H., Pitarangsi, C., Lebron, C., Armstrong, A., Sethabutr, O., Sanders, J., 2008. Diagnostic approach to acute diarrheal illness in a military population on training exercises in Thailand, a region of *Campylobacter* hyperendemicity. *J. Clin. Microbiol.* 46, 1418–1425.
- Vihavainen, E.J., Björkroth, J., 2010. Microbial ecology and spoilage of poultry meat and poultry meat products. In: Guerrero-Legarreta (Ed.), *Handbook of Poultry Science and Technology, Secondary Processing*, vol. 2. Blackwell-Wiley, New York, NY, pp. 485–493.
- Williams, A., Oyarzabal, O.A., 2012. Prevalence of *Campylobacter* spp. in skinless, boneless retail broiler meat from 2005 through 2011 in Alabama, USA. *BMC Microbiol.* 12, 184.
- Wonglumsom, W., Vishnubhatl, A., Kim, J.M., Fung, D.Y., 2001. Enrichment media for isolation of *Campylobacter jejuni* from inoculated ground beef and chicken skin under normal atmosphere. *J. Food Prot.* 64, 630–634.
- Zhou, P., Hussain, S.K., Liles, M.R., Arias, C.R., Backert, S., Kieninger, J.R., Oyarzabal, O.A., 2011. A simplified and cost-effective enrichment protocol for the isolation of *Campylobacter* spp. from retail broiler meat without microaerobic incubation. *BMC Microbiol.* 11, 175.