



Diagnostic Test Methods to Identify Campylobacter Species from Animal Samples

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Abstract

Campylobacter species are a leading cause of bacterial foodborne infections in both developed and developing countries. Considering the presence of the “emerging” and re-emerging of a human pathogenic Campylobacter species in the food and their production environment, it is prudent to include them when developing detection methods. Various diagnostic methods have been developed to detect Campylobacter in animal samples, including culture-based methods, Immunological assays, and molecular techniques. However, there is a lack of consensus on the most reliable and sensitive method for Campylobacter detection in animal samples. Different methods have limitations and advantages. So, Since Campylobacter species in the sample were desiccate and needs fast preparation time and process, the DNA-based methods for a fast screening of positive samples were recommended.

Keywords: Campylobacter, foodborne infections, Immunological assays, and molecular techniques

1. Introduction

Campylobacter spp. are a leading cause of bacterial foodborne infections in both developed and developing countries (Igwaran and Okoh, 2019; Kaakoush *et al.*, 2015). The annual incidence of foodborne campylobacteriosis has increased in the US, and Europe with 64.9 and 130 cases per 100,000 populations in these countries, respectively (EFSA *et al.*, 2019; Tack *et al.*, 2019).

The clinical symptoms of campylobacteriosis include gastrointestinal manifestations such as

bloody diarrhea, inflammatory bowel disease (IBD), esophageal diseases, periodontal diseases, celiac disease, colorectal cancer, and extra-gastrointestinal manifestations such as Guillain-Barre syndrome, Miller-Fisher syndrome, bacteremia and septicemia, cardiovascular complications, meningitis, and reactive arthritis (Kaakoush *et al.*, 2015). In a review article, Teunis *et al.* predicted the infectious dose to be <100 Colony Forming Units (CFU), depending on the *Campylobacter jejuni* strain (Teunis *et al.*, 2018).

Campylobacter species can colonize the mucosal surfaces of the gastrointestinal tracts of humans and a wide variety of wild and domesticated birds and mammals, including food animals. To date, the genus *Campylobacter* consists of 32 spp. and 9 subspecies (Costa and Iraola, 2019); most human infections are attributed to *C. jejuni* and *C. coli*. However, campylobacteriosis may also be caused by the “emerging” *Campylobacter* spp., including *C. concisus*, *C. sputorum*, *C. lari*, *C. upsaliensis*, *C. hyointestinalis*, *C. ureolyticus*, *C. curvus*, *C. fetus*, *C. gracilis*, *C. helveticus*, *C. insulaenigrae*, *C. mucosalis*, *C. rectus*, *C. showae*, *C. ureolyticus* and *C. volucris*, which have been associated with clinical presentations such as gastroenteritis and/or bacteremia (Costa and Iraola, 2019; Kaakoush *et al.*, 2015; Man, 2011).

The main sources attributed to foodborne campylobacteriosis outbreaks from 2015 through 2019 include raw milk (51.9%), chicken (31.1%), and seafood (4.9%) (Interagency Food Safety Analytics Collaboration, 2021). The recovery of *Campylobacter* isolates from poultry and beef that were genetically identical to isolates recovered from humans directly links this spp. to human illness, demonstrating their impact on public health (Duffy, 2010).

The predominant *Campylobacter* spp. found in fresh fruits and vegetables include *C. jejuni*, *C. coli*, and *C. fetus* (Chai *et al.*, 2007; Mohammadpour *et al.*, 2018; Wijnands *et al.*, 2014). Possible sources for *Campylobacter* contamination in fresh produce include irrigation water, composted animal manure, fecal contamination from wild or domestic animals, post-harvest processes, and cross-contamination at the retail level as well as at home (Keller and Shriver, 2014; Lawton *et al.*, 2018; Rodrigues *et al.*, 2019). Considering the presence of the “emerging” human pathogenic *Campylobacter* spp. in food and their production environment (Garcia-Sanchez *et al.*, 2018), it is prudent to include them when developing detection methods.

Therefore, a comprehensive review of the available diagnostic methods for *Campylobacter* identification in animal health settings is necessary to assess their effectiveness, accuracy, and practicality. Such a review will also help identify the limitations and challenges associated with each method and highlight areas for future research. Overall, this review manuscript aims to provide an up-to-date resource for researchers and practitioners in the animal health sector to improve the diagnosis and management of *Campylobacter* infections in animals,

2. Literature review

Various diagnostic methods have been developed to detect *Campylobacter* in animal samples, including culture-based methods, Immunological assays, and molecular techniques. However, there is a lack of consensus on the most reliable and sensitive method for *Campylobacter* detection in animal samples.

2.1 Culture-based methods

Culture-based methods are considered the gold standard for *Campylobacter* detection, but they are time-consuming, laborious, and require specialized media and incubation conditions (Seliwiorstow *et al.*, 2021).

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 hr, *Campylobacter* species 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* species from carcass rinses between charcoal based and blood-based media. However, Campy-Line plates appear to be selective against *Campylobacter* species 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.

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. 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 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).

2.2 Latex agglutination tests

Latex agglutination tests for rapid identification of *Campylobacter* species 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 immunoglobulin's 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 Bio-products 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.

2.3 Enzyme-linked immunosorbent (ELA) assays

Similarly to the latex test, there were several ELA assays commercially available for the identification of *Campylobacter* species in foods, but most of these tests have been discontinued. Currently, commercial tests include mainly the

VIDAS® Campylobacter (bioMérieux, Marcy l'Etoile, France), which is an automated ELA 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 ELA assays for clinical samples: the Premier™ CAMPY microplate ELA and the Immuno Card STAT! CAMPY by Meridian Bioscience, Inc. (Cincinnati, OH) (Granato *et al.*, 2010); the ProSpecT™ Campylobacter assay by Remel Inc. (Lenexa, KS) (Granato *et al.*, 2010; Tribble *et al.*, 2008); and the Ridascreen® Campylobacter by RBiopharm AG (Germany) (Bessede *et al.*, 2011). These assays have a sensitivity of 10⁵–6 CFU (colony forming units) per ml⁻¹ (Oyarzabal and Battie, 2012). A recent evaluation of these assays showed that ELA assays are quite variable and that these assays alone should not be used for direct identification of Campylobacter spp. in stool samples. In addition, ELA assays are not sufficient for confirmation and therefore laboratories should confirm positive ELA results by culture methods (Fitzgerald *et al.*, 2011).

2.4 Molecular techniques

Molecular techniques, such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP), offer rapid and sensitive detection of Campylobacter in animal samples (Karami *et al.*, 2019). Molecular identification methods are fast and specific for the identification of Campylobacter species. 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.

Polymerase chain reaction (PCR) and multiplex PCR (mPCR) assays, since the first polymerase chain reaction (PCR) assay for the identification of Campylobacter species 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 species 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 10³ 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 meats (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 species.

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.

3. Discussion and Recommendations

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. The samples collected are more sensitive for the recovery and requires less time for preparation and processing of the samples. The dark background of CCDA is a challenge for identification to the untrained scientist. But, this agar medium is one of the best choices based on simplicity and cost. Latex agglutination requires positive controls must be run with any latex agglutination test. A recent evaluation of Enzyme Linked immunosorbent Assay assays (ELA) are quite variable and that these assays alone should not be used for direct identification of Campylobacter spp. in stool samples. In addition, ELA assays are not sufficient for confirmation and therefore laboratories should confirm positive ELA results by culture methods. Several multiplex PCR assays have been successfully used for testing a large

number of and therefore these protocols assist in providing rapid identification of Campylobacter species. Analysis of 107 strains for the exclusivity studies resulted in no false positives using this qPCR assay. So, we recommend the DNA-based methods for a fast screening of positive samples.

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