Effect of Incubation Temperature on the Detection of Thermophilic
Campylobacter Species from Freshwater Beaches, and Nearby Wastewater
Effluents and Bird Fecal Droppings

Running title: Effect of incubation temperature on recovery of campylobacters

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This large-scale study compared incubation temperatures (37°C vs. 42°C) to study the detection of thermophilic *Campylobacter* species including *C. jejuni*, *C. coli*, and *C. lari* from various surface water samples and bird fecal droppings around Hamilton harbor, Lake Ontario. The putative culture isolates obtained from 37 and 42°C incubation temperatures were confirmed by *Campylobacter* genus and species-specific triplex PCR assays targeting the 16S rRNA gene and 16S-23S rDNA internal transcribed spacer (ITS) region. A total of 759 water, wastewater, and bird fecal dropping samples were tested. Positive amplification reactions for the genus *Campylobacter* were found for 454 (60%) samples incubated at 37°C compared to 258 (34%) samples incubated at 42°C. *C. jejuni* (16%) and *C. lari* (12%) were detected significantly more frequently at 42°C incubation temperature than at 37°C (8% and 5%), respectively. In contrast, a significantly higher rate of *C. coli* (14%) and other *Campylobacter* spp. (36%) were detected at 37°C than 42°C incubation temperature (8% and 7%), respectively. These results were consistent across surface water, wastewater and bird fecal dropping samples. At times *Campylobacter* spp. were recovered and detected at 37°C (*C. jejuni* 3%, *C. coli* 10% and *C. lari* 3%) when the same samples incubated at 42°C were negative. A significantly higher rate of other *Campylobacter* spp. were detected only at 37°C (32%) than only at 42°C (3%) incubation temperature. These results indicate that incubation temperature can significantly influence the culturability and detection of thermophilic and other fastidious *Campylobacter* spp. and a comprehensive characterization of the *Campylobacter* spp. in surface water, wastewaters, or bird fecal droppings will require incubation at both 37 and 42°C.
**INTRODUCTION**

*Campylobacter* species have been recognized as one of the leading causes of bacterial gastroenteritis in humans (1-3). The genus *Campylobacter* is currently comprised of 22 species and 8 subspecies (4), but from a public health perspective the *Campylobacter* species, *C. jejuni*, *C. coli*, and *C. lari* are the most frequently implicated in human infections (5). These species were first isolated on a selective agar incubated microaerobically at 43°C (6, 7), and that led to the name thermophilic campylobacters. These three species can commonly occur in the gastrointestinal tracts of humans and other animals including bovines, pigs and birds. Sea gulls and several other wild birds can be an important source of *Campylobacter* spp. such as *C. jejuni* and *C. lari* (3, 8, 9). Campylobacters from the feces of birds, domestic and wild animals, municipal sewage discharges, or agricultural runoff can contaminate water (3), and water is an established vehicle for the transmission of these species to human and domestic animals leading to outbreaks of waterborne disease.

Several *Campylobacter* isolation procedures have been developed for water including an ISO standard method (10) using concentration of cells by membrane filtration or centrifugation, and enrichment regimens by using different selective growth media. These procedures are being widely used to isolate these species from food and water (11, 12). There are numerous challenges in recovering of *Campylobacter* spp. in water such as the frequent small numbers of cells, slow
growth rate, intrinsic fastidious growth requirements, and the presence of a significant proportion of organisms that may be injured or have difficulty in adapting to in vitro conditions. Incubation temperature can play a vital role in the culturability and detection of thermophilic Campylobacter spp. (13-15).

Traditionally, procedures to isolate these organisms from food, water and feces have most commonly been conducted at 42°C incubation temperature (16-18). Many investigations of Campylobacter spp. in water have used a 42°C incubation temperature (9, 19-25). However, other water studies have used a 37°C incubation temperature (26-28). Several studies have used the Cape Town method with an incubation temperature of 37°C for detection of Campylobacter from food and human feces, suggesting this method as better for detecting a wide range of Campylobacter spp. (14, 29-32). Although the Cape Town method has been considered as a useful tool for the isolation of Campylobacter spp. from clinical samples (31, 32), other studies have reported it as insufficient for the recovery and detection of thermophilic Campylobacter spp. in aquatic environments where species such as C. jejuni and C. lari could be present in low numbers (26, 27). Another approach in water studies has been to have a 2-4 hour pre-enrichment step at 37°C before subsequent enrichment at 42°C (33-38). However, studies that used a pre-enrichment step of 37°C followed by an enrichment at 42°C have often observed a low detection of C. coli suggesting that a 42°C incubation temperature might not support the growth of stressed and injured cells of C. coli present in low numbers in water. Phillips (39) suggested that more than one incubation temperature might substantially improve isolation of thermophilic Campylobacter spp. without diminishing isolation of other fastidious Campylobacter spp.
To our knowledge, no large-scale study has previously compared *Campylobacter* detection rates at 37 and 42°C across diverse environmental matrices such as surface water samples, municipal wastewater samples, and bird fecal droppings common to urban environments. It is important to assess the limitations of different incubation temperatures on the culturability and detection of thermophilic and other fastidious *Campylobacter* spp. from various environmental matrices. This study compared 37 vs. 42°C incubation temperatures for detecting *Campylobacter* spp. including *C. jejuni*, *C. coli*, and *C. lari* in water samples collected from two freshwater beaches and offshore harbour water, as well as municipal wastewater and bird fecal dropping samples from around Hamilton Harbour, Lake Ontario. We refer to campylobacters other than *C. jejuni*, *C. coli*, and *C. lari* as other *Campylobacter* spp.

**MATERIALS AND METHODS**

**Collection of surface water and bird fecal dropping samples**

Surface water samples were collected from two freshwater beaches (Bayfront Park and Pier4 Park) in Hamilton Harbour at Lake Ontario from 2007-2009. Sampling at the beaches was carried out along a single transect at each beach at three depth zones including sand pore water, ankle and chest depth waters. Sand pore water was collected by digging a hole in the wet foreshore sand about one meter inland from the water’s edge, and collecting the water that seeped into the hole. Offshore surface water samples were collected by boat from the middle of the harbor, near a wastewater treatment plant offshore outfall. Municipal wastewater samples were obtained from the final effluents of four municipal sewage treatment plants (STPs) that discharge into the harbor area, and a combined sewer overflow (CSO) storage tank located at...
Bayfront and Pier4 Park beaches that occasionally overflowed during storm events. Fresh bird fecal dropping samples from ring-billed gulls (*Larus delawarensis*) and Canada geese (*Branta canadensis*) were collected on the beaches within 2m of the waterline. All water, wastewater and bird fecal samples were collected on a bi-weekly basis over three years (2007-2009) between April and December. Water and wastewater samples were collected in 2L sterile bottles, whereas fecal samples were collected in sterile tubes containing 2 ml phosphate buffer saline (1x PBS) solution. The water, wastewater, and fecal samples were returned on ice to the laboratory and processed on the same day of their collection.

**Isolation and culture conditions**

Samples were processed following a protocol described by Khan et al. (12). Briefly, one liter of each water or wastewater sample was centrifuged at 14,000xg for 20 min (Beckman, Indianapolis, IN, USA), and the pellet was resuspended in 3 ml saline (0.85%) solution for the concentration of *Campylobacter* cells. Bird fecal samples collected in PBS solution were homogenized by vortex and analyzed to detect the presence/absence of thermophilic *Campylobacter* spp. To estimate the number of *Campylobacter* cells per liter, the resuspended pellet obtained from 1L of centrifuged water was analyzed by a minimum probable number (MPN) method using a 10-fold serial dilution approach for a semi-quantitative analysis of *Campylobacter* occurrence. 1 ml of each suspended pellet and fecal sample was inoculated in two sets of Bolton broth (Oxoid) tubes containing a selective antibiotic (Cefoperazone, Cyclohexamide, Trimethoprim and Vancomycin) supplement. The inoculum was serially diluted in the two sets of tubes and incubated at 37 and 42°C, respectively, under microaerophilic conditions (5% O2, 85% N2 and 10% CO2) for 48 h in an MCO-18M multi-gas incubator (Sanyo,
Tokyo, Japan). The semi-quantitative enumeration was carried out by assessing turbidity and subculture confirmation. The cultures from each tube were further streaked with a sterile loop on Modified Karmali Agar (MKA) (Oxoid, USA) containing a selective supplement including antibacterial and antifungal (Amphotericin B, Cefoperazone, Sodium Pyruvate and Vancomycin) agents, and plates were incubated at 37 and 42°C, respectively, under microaerophilic conditions for 24-48 h. The putative *Campylobacter* cultures were selected based on their growth characteristics and colony morphology i.e. smooth, shiny and convex with defined or flat edges, transparent or translucent colorless to grayish or light cream in color (24). The plates that contained such colonies were further analyzed by DNA extraction and PCR assays.

**DNA extraction and genus-specific PCR amplification**

The DNA extractions from scrapping multiple isolates recovered from a MKA growth media plate were carried out using a boiling protocol as previously described (40). Briefly, putative *Campylobacter* colonies were resuspended in 75µl 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The suspended solution was boiled for 10 min and centrifuged. The supernatant containing DNA was transferred to a 1.5 ml sterile microfuge tube and kept at -20°C for further analysis. For the confirmation of putative cultures to genus-level identification, a DNA-based PCR amplification assay was performed using *Campylobacter* genus-specific oligonucleotide primers (41). The 25 µl reaction mixture contained 50-70 ng of template DNA, 0.15 units of *Ex Taq* DNA polymerase (Takara, Shiga, Japan), 1X *Ex Taq* buffer with MgCl₂, 200 µM of each of the dNTPs, and 50 ng of the each forward (5´-GGA TGA CAC TTT TC G GAG C- 3´) and reverse (5´-CAT TGT AGC ACG TGT GTC- 3´) primers. The amplification was performed by an initial template denaturation step at 94°C for 3 min, followed by 30 cycles of amplification by...
repeating denaturation at 94°C for 60 sec, annealing at 55°C for 60 sec, and extension at 72°C for 60 sec with a final 5 min incubation at 72°C using a Mastercycler Gradient PCR system (Eppendorf, Hamburg, Germany). The PCR amplicons were electrophoresed on a 1% agarose gel matrix (Fisher Scientific, NJ, USA) with 1x TAE buffer using a 100 bp DNA size marker (PGC Scientific, MD, USA). The gels were stained in ethidium bromide (0.5 µg/ml), visualized on a UV transilluminator, and photographed using an Ingenius Syngene Bioimaging gel documentation system.

Species-specific PCR amplification

A species-specific PCR amplification was further performed to identify Campylobacter spp. by a triplex PCR assay using oligonucleotide primer pairs for three thermophilic Campylobacter spp. (C. jejuni, C. coli and C. lari) as described by Khan and Edge (42). The triplex PCR amplification reaction was carried out in the Mastercycler Gradient PCR system (Eppendorf) with a 25 µl reaction mixture containing 50-70 ng of template DNA, 1.25 units of Ex Taq DNA polymerase (Takara), 1X Ex Taq buffer with MgCl₂, 200 µM of each of the dNTPs, and 80 ng of the forward and reverse primer pairs for each target Campylobacter spp. including C. jejuni (forward- ACT AAA TGA TTT AGT CTC A) and (reverse- CTT AGA TTT ATT TTT ATC TTT AAC T); C. lari (forward- AAA TAT ATA CTT GCT TTA GAT T) and (reverse- CAA TAA AAC CTT ACT ATC TC); and C. coli (forward- GAA GTA TCA ATC TTA AAA AGA TAA), (reverse- CTT ACT TTA GGT TTT AAG ACC). The final volume (25 µl) was adjusted with filtered, sterile distilled water. The PCR reaction was performed using an initial template denaturation step at 94°C for 3 min followed by 30 cycles of amplification (denaturation at 94°C for 30 sec, annealing at 46°C for 45 sec and extension at 72°C for 30 sec) ending with a 5 min
extension at 72°C. Due to expected small amplicon fragment size, the PCR amplicons were
electrophoresed on a 2% agarose gel matrix, stained and scanned as described in the preceding
section.

Statistical analysis

Statistical analyses were performed using STATISTICA 10.0 (StatSoft Inc., 2011; USA). Mc Nemar Chi-square contingency tests were applied to test for significant differences in the
recovery and detection of thermophilic and other Campylobacter spp. between 37 and 42°C. Differences were considered significant at p < 0.05.

RESULTS

A total of 759 surface water, wastewater, and bird fecal dropping samples were collected around
Hamilton Harbour between 2007 and 2009. These samples included 288 beach water samples, 89
offshore water samples, 220 wastewater samples, and 162 bird fecal dropping samples. All
putative Campylobacter culture isolates observed at 37 and 42°C incubation temperatures on
MKA media showed typical growth patterns, and were consistently confirmed by the
Campylobacter genus-specific 16S rRNA gene PCR assay with an expected amplicon size of
816 bp. Species-specific detection of Campylobacter spp. in water and fecal samples was further
confirmed using the triplex PCR assay with expected amplicon sizes of 349, 279 and 72 bp for
C. jejuni, C. lari, and C. coli, respectively.
Analysis of all 759 beach water, wastewater, offshore and bird fecal samples indicated that Campylobacter was detected significantly more frequently at 37°C (60%) than at 42°C (34%) incubation temperature (p < 0.05). These findings were consistent across beach, wastewater, offshore water, and bird fecal dropping samples. Campylobacter recovery from beach water (63%), wastewater (63%), offshore water (61%) and bird fecal (50%) samples was significantly higher (p < 0.05) at 37°C than at 42°C incubation temperature (49%, 15%, 24% and 39%), respectively (Fig. 1).

A comparison of the two incubation temperatures revealed differences in the ability to recover and detect Campylobacter spp. across all 759 beach and offshore water, wastewater, and bird fecal samples (Table 1). Thermophilic Campylobacter spp. including C. jejuni (16%) and C. lari (12%) were detected significantly more often using 42°C incubation temperature, whereas, C. coli (14%) and other campylobacters (36%) were detected significantly more often using 37°C incubation temperature (p < 0.05).

These differences between incubation temperatures in the ability to recover and detect Campylobacter spp. were consistent across all beach water, offshore water, wastewater, and bird fecal samples (Fig. 2A-D). Based on the number of samples collected from each sampling location and type, C. jejuni and C. lari were always more commonly detected at 42°C than at 37°C, and they were more common in beach water samples (24 and 18%) and bird fecal droppings (26 and 20%) than offshore (7 and 11%) and wastewater (3 and 1%) samples, respectively (Fig. 2A&B). On the other hand, C. coli and other Campylobacter spp. were always more commonly detected at 37°C than at 42°C, and they were more common in beach water
samples (20 and 30%), wastewater (17 and 45%) and offshore (11 and 35%) samples than bird fecal droppings (2 and 32%), respectively (Fig. 2C&D). Interestingly, no *C. coli* were detected in bird fecal dropping at 42°C incubation temperature.

Further analysis on the recovery and detection of multiple thermophilic *Campylobacter* spp. including *C. jejuni*, *C. coli*, and *C. lari* in a single sample revealed an overall low frequency of co-occurrence of *Campylobacter* spp. in water and bird fecal samples. *C. jejuni* and *C. lari* were more commonly detected together (6%) at 42°C than at 37°C (2%) incubation temperature (Table 2). On the other hand, *C. coli* was rarely recovered together with other *Campylobacter* spp. (≤ 1%) at both incubation temperatures (Table 2). A higher frequency of the samples yielded only *C. coli* (13%) compared to only *C. jejuni* (5%) and *C. lari* (3%) at 37°C.

From 759 water, wastewater, and bird fecal dropping samples, a significantly higher frequency of campylobacters (35%; *p* < 0.05) was detected only at 37°C than only at 42°C (9%), whereas 25% of samples were positive for *Campylobacter* spp. at both incubation temperatures (Table 3A). A significantly higher frequency (p < 0.05) of *C. jejuni* (11%) and *C. lari* (10%) were only detected at 42°C compared to only at 37°C (3% for each species). In contrast, a higher frequency of *C. coli* (10%) and other *Campylobacter* spp. (32%) were only detected at 37°C, compared to only at 42°C (Table 3A).

An analysis of the frequency of detection of *Campylobacter* among different sampling locations at 37°C and/or 42°C was performed, and the results showed that the highest frequency of *C. jejuni* and *C. lari* were detected at 42°C in beach water (17% and 16%) and bird fecal
dropping (16% and 15%) samples (Table 3B). In contrast, *C. coli* and other *Campylobacter* spp. were most frequently detected at 37°C in wastewater (15% and 43%) (Table 3B).

The concentration of *Campylobacter* cells/ L was estimated in water samples at both incubation temperatures using a minimum probable number (MPN) method (Fig. 3). The highest *Campylobacter* cell concentrations (≥10,000 cells/ L) were commonly obtained from wastewater samples where most of the samples with very high MPN values were *Campylobacter* negative at 42°C incubation temperature, but were found to contain *C. coli* or other *Campylobacter* spp. at 37°C. The lowest *Campylobacter* cell concentrations ranging from 10 to 1000 cells/ L were commonly obtained in beach and offshore water samples that were positive for *C. jejuni* and *C. lari* at 42°C.

**DISCUSSION**

Method comparative studies often require intensive and long-term sampling in order to capture the variation and perform comprehensive comparative analyses as well as assessment of different parameters used in the study. This allows researchers to improve understanding and recommend optimal parameters that can be used for future applications. Several comparison studies have been conducted for enhanced recovery and isolation of thermophilic *Campylobacter* spp. like *C. jejuni* from food, feces and water using different cell concentration approaches (filtration vs. centrifugation), enrichment and growth media, and incubation duration (24 vs. 48 hrs.). However, the samples processed for the isolation of thermophilic *Campylobacter* spp. have been typically incubated at 42°C (12, 43, 44). Pre-enrichment at 37°C for 2-4 hrs. before exposure to
selective agents and incubation at 42°C has also been reported as a recovery method that resulted in an increased isolation of *Campylobacter* spp. from natural waters (33-35, 37). Many previous studies on the occurrence of *Campylobacter* spp. from various waters ranging from drinking to river, lake, pond, urban and agricultural watersheds used a 42°C incubation temperature (9, 18-21, 23, 24, 45). These studies detected *C. jejuni* more commonly than other thermophilic species such as *C. coli*. These results could reflect actual differences in species occurrence, or they could reflect that a 42°C incubation temperature may not provide a comprehensive recovery of all campylobacters including *C. coli* and other *Campylobacter* spp. Since *C. coli* has also been significantly implicated in human infections, it is important to consider the growth temperature requirements of *C. coli* and other *Campylobacter* spp. at 37°C. Therefore, this large-scale three year study was designed with a goal to compare two incubation temperatures (37 vs. 42°C) to determine if incubation temperature significantly influenced the detection of thermophilic *Campylobacter* spp. (*C. jejuni*, *C. coli* and *C. lari*) that are often present in low numbers, with slow growth rates, and with cells that may be stressed as well as sub-lethally injured. The comparative analysis was conducted on environmental samples collected from 597 water and wastewater samples and 162 bird fecal droppings using an initial enrichment step with Bolton broth and incubation for 48hrs at 37 and 42°C, respectively.

*Campylobacter* was detected significantly more frequently from water samples (including beaches, wastewater, offshore) and bird fecal droppings at 37°C compared to 42°C incubation temperature. In addition, the water samples collected from two different beaches showed a similar trend of significantly higher recovery of campylobacters at 37°C than 42°C incubation temperature. Similar results were reported by Bolton et al. (46), where the majority of
Campylobacters showed better recovery and culturability at 37°C than 42°C incubation temperature.

Interestingly, *C. jejuni* and *C. lari* were found to be more commonly detected at 42°C in our water and bird fecal dropping samples than at the 37°C incubation temperature. In contrast, *C. coli* and other *Campylobacter* spp. were significantly more commonly detected at 37°C than 42°C. Studies of *C. jejuni* and *C. fetus* subsp. *jejuni* in human fecal samples using 35 or 37 and 42°C incubation temperatures by Gee et al. (47) and Janssen and Helstad (48) found that 42°C resulted in recovery of more isolates of *C. jejuni* and *C. fetus* subsp. *jejuni*. Conversely, Bolton et al. (46) showed that *C. fetus* subsp. *fetus* grew better at 37°C than 42°C incubation temperature.

In our Hamilton Harbour study, the most notable difference between the two incubation temperatures was that 37°C recovered more other *Campylobacter* spp. than 42°C. Other *Campylobacter* spp. were only recovered from 52 (7%) of water and bird fecal dropping samples at 42°C compared to 270 (36%) that were recovered and detected at 37°C incubation temperature. Although our environmental samples may have amplified the problem of recovery due to sublethal injury, cell stress, and low numbers of campylobacters, it is possible that these results are also applicable for clinical samples. For example, occurrence of *Campylobacter* spp. like *C. coli* may be underestimated in stool samples by only using 42°C incubation temperature for recovering thermophilic *Campylobacter* spp.

It was observed that using a 37°C incubation temperature lead to detecting water samples with high numbers of campylobacters (MPNs greater than ≥10,000-1000,000 cells/ L) in 104 samples which were negative for campylobacters at 42°C. Most of the water samples with very high
MPNs were found to contain *C. coli* or other *Campylobacter* spp. A 37°C incubation temperature may facilitate detection of cells of *C. coli* and other *Campylobacter* spp. in water samples, by allowing growth of stressed and sub-lethally injured cells. It is also possible that some of the putative campylobacters detected at 37°C are as yet undescribed and potentially novel species within the *Campylobacter* genus or may also be closely related *Campylobacter*-like species from genera such as *Arcobacter* spp. (24). However, Humphrey (33, 34) reported that pre-enrichment at 37°C may also increase isolation rate of *C. jejuni* from food and water, and we found that *C. jejuni* (3%) and *C. lari* (3%) cells can also be recovered at 37°C when the same samples were negative at 42°C. These results suggest that 37°C may facilitate the recovery and culturability of classical thermophilic *Campylobacter* spp. such as *C. coli*, *C. jejuni* and *C. lari*, and also enhance the isolation of a wider range of other fastidious *Campylobacter* spp. that could not grow at 42°C.

Similarly, the growth of other fastidious *Campylobacter* spp. can be facilitated and recovered at 42°C incubation temperature that may not be recovered at 37°C. The study suggests that the 37°C could have advantages over the 42°C in providing an optimum environment for enhancing culturability of *C. coli* and the diversity of *Campylobacter* spp. present in water, wastewater and bird fecal dropping samples. This would appear to be particularly the case when investigating *Campylobacter* occurrence in municipal wastewaters where an incubation temperature of 42°C may not detect many *C. coli*. From a human health prospective, the objective of many previous studies was mainly focused on detecting clinically important species such as *C. jejuni*, therefore, the 42°C incubation temperature has been widely used for the recovery and isolation of thermophilic *Campylobacter* spp. However, the presence of *C. coli* and multiple types of...
Campylobacter spp. in human infections has been recognized and is seen as a significant epidemiological problem, since diagnostic laboratories may only isolate and characterize a single colony. This has led to some studies suggesting that to obtain a wide range of Campylobacter spp., it may be necessary to enrich water, food and fecal samples at both 37 and 42°C incubation temperatures (15, 49).

In conclusion, this large-scale long-term study results show that both 37 and 42°C incubation temperatures influence the ability to recover thermophilic and other Campylobacter spp. from water, wastewater and bird fecal droppings, especially if unusual fastidious campylobacters are believed to be significant problem and possible threat to human health. It is recommended that for a comprehensive characterization of the Campylobacter spp. in surface waters, wastewaters, or bird fecal droppings, incubation at both 37 and 42°C will likely be needed. Application of molecular methods would also be recommended to provide a more comprehensive characterization of campylobacters, including those that may occur in a viable but nonculturable condition. Further research on subtyping of thermophilic Campylobacter spp. isolates recovered from 37 and 42°C is needed in order to compare the similarity of strains recovered at these two temperatures from water samples. This subtyping might help in assessing strain diversity and in identifying the source of contamination of campylobacters in fresh water and bird fecal droppings.

**ACKNOWLEDGEMENTS**
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REFERENCES


Table 1: Number (percent) of beach water, wastewater, offshore water, and bird fecal dropping samples (n= 759) positive for thermophilic *Campylobacter* spp. using two different incubation temperature conditions

<table>
<thead>
<tr>
<th>Campylobacters</th>
<th>37°C (N, %)</th>
<th>42°C (N, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>454 (60)</td>
<td>258 (34)</td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>61 (8)</td>
<td>124 (16)</td>
</tr>
<tr>
<td><em>C. coli</em></td>
<td>107 (14)</td>
<td>60 (8)</td>
</tr>
<tr>
<td><em>C. lari</em></td>
<td>40 (5)</td>
<td>94 (12)</td>
</tr>
<tr>
<td>Other <em>Campylobacter</em> spp.</td>
<td>270 (36)</td>
<td>52 (7)</td>
</tr>
</tbody>
</table>

Table 2: Number (percent) recovery and detection of multiple *Campylobacter* species in beach water, wastewater, offshore water, and bird fecal dropping samples (N= 759) at two different incubation temperature conditions

<table>
<thead>
<tr>
<th>Campylobacter spp.</th>
<th>37°C (N, %)</th>
<th>42°C (N, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. jejuni</em></td>
<td>40 (5)</td>
<td>63 (8)</td>
</tr>
<tr>
<td><em>C. coli</em></td>
<td>98 (13)</td>
<td>46 (6)</td>
</tr>
<tr>
<td><em>C. lari</em></td>
<td>24 (3)</td>
<td>37 (5)</td>
</tr>
<tr>
<td><em>C. jejuni</em> and <em>C. coli</em></td>
<td>7 (1)</td>
<td>5 (1)</td>
</tr>
<tr>
<td><em>C. jejuni</em> and <em>C. lari</em></td>
<td>14 (2)</td>
<td>48 (6)</td>
</tr>
<tr>
<td><em>C. coli</em> and <em>C. lari</em></td>
<td>2 (&lt;1)</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td><em>C. jejuni</em>, <em>C. coli</em> and <em>C. lari</em></td>
<td>0 (0)</td>
<td>8 (1)</td>
</tr>
</tbody>
</table>
Table 3A: Number (percent) of *Campylobacter* spp. recovered from water and bird fecal dropping samples (N = 759) at 37 and/or 42°C incubation temperature

<table>
<thead>
<tr>
<th>Campylobacters</th>
<th>Both temp. +ve N (%)</th>
<th>Only 37°C +ve</th>
<th>Only 42°C +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>191 (25)</td>
<td>263 (35)</td>
<td>67 (9)</td>
</tr>
<tr>
<td>C. <em>jejuni</em></td>
<td>40 (5)</td>
<td>21 (3)</td>
<td>84 (11)</td>
</tr>
<tr>
<td>C. <em>coli</em></td>
<td>29 (4)</td>
<td>78 (10)</td>
<td>31 (4)</td>
</tr>
<tr>
<td>C. <em>lari</em></td>
<td>16 (2)</td>
<td>24 (3)</td>
<td>78 (10)</td>
</tr>
<tr>
<td>Other <em>Campy</em> spp.</td>
<td>26 (3)</td>
<td>244 (32)</td>
<td>26 (3)</td>
</tr>
</tbody>
</table>
Table 3B: Number (percent) of thermophilic and other *Campylobacter* spp. recovered from beaches, wastewater and offshore water samples and bird fecal droppings at 37 and/or 42°C incubation temperature

<table>
<thead>
<tr>
<th>Campylobacters</th>
<th>Beaches n= 288*</th>
<th>Wastewater n= 220</th>
<th>Offshore n= 89</th>
<th>Bird fecal droppings n= 162</th>
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<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td>Both temp. +ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only 37°C +ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only 42°C +ve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td>107 (37)</td>
<td>22 (10)</td>
<td>18 (20)</td>
<td>44 (27)</td>
</tr>
<tr>
<td>Other <em>C. jejuni</em></td>
<td>73 (25)</td>
<td>116 (52)</td>
<td>37 (41)</td>
<td>37 (23)</td>
</tr>
<tr>
<td><em>C. coli</em></td>
<td>33 (11)</td>
<td>11 (5)</td>
<td>4 (4)</td>
<td>19 (12)</td>
</tr>
<tr>
<td><em>C. coli</em></td>
<td>14 (5)</td>
<td>0 (0)</td>
<td>5 (2)</td>
<td>16 (10)</td>
</tr>
<tr>
<td><em>C. lari</em></td>
<td>48 (17)</td>
<td>5 (2)</td>
<td>1 (1)</td>
<td>4 (2)</td>
</tr>
<tr>
<td><em>C. lari</em></td>
<td>1 (≤1)</td>
<td>5 (2)</td>
<td>3 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Other Campy spp.</td>
<td>11 (4)</td>
<td>6 (3)</td>
<td>30 (34)</td>
<td>5 (3)</td>
</tr>
<tr>
<td></td>
<td>73 (25)</td>
<td>94 (43)</td>
<td>47 (29)</td>
<td></td>
</tr>
</tbody>
</table>

* Total number of collected samples
Fig. 1: Percent of beach water, wastewater, offshore water, and bird fecal dropping samples positive for campylobacters using two different incubation temperatures (n= total number of collected samples). The number of positive samples is presented above each bar.
Fig. 2: Percent of recovery of *C. jejuni*, *C. lari*, *C. coli* and other *Campylobacter* spp. (Panels A-D) from various water and bird fecal dropping samples at two different incubation temperatures. The number of positive samples is presented above each bar.
Fig. 3: Effect of incubation temperature (37 and/or 42°C) on the percentage of *Campylobacter* spp. positive beach water, wastewater and offshore samples at various minimum probable numbers (MPNs). The number of positive samples is presented above each bar.