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PII: S0956-7135(14)00513-1
DOI: 10.1016/j.foodcont.2014.09.007
Reference: JFCO 4056

To appear in: *Food Control*

Received Date: 21 February 2014
Revised Date: 27 August 2014
Accepted Date: 9 September 2014


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**ABSTRACT**

Wild birds are important to public health, and they carry emerging zoonotic pathogens as a reservoir host. They often cause acute gastroenteritis in humans. Many playgrounds in parks are natural habitats for wild birds. Children’s behavior, the frequent hand-mouth contact in this age group, is also likely to increase the risk of eating infective material. In this study, we looked into the likelihood that contamination by wild-birds of children’s playground is a source of human infection. We did that by estimating the prevalence of *Campylobacter spp.* in fresh and dried wild-bird fecal samples. From July to August, 2013, we collected 200 samples of fresh and dried feces from 5 publicly accessible children’s playgrounds in Mashhad, Iran. Our data by using a cultural method and a test, showed that 17.5% of feces samples contained *Campylobacter spp.* It was significantly (P < 0.05) more prevalent in the fresh samples (23.85%) than the dried samples (5.7%). We only detected one species of *Campylobacter spp.* from wild birds in this study. In conclusion, the results of this study showed wild bird species may set up a reservoir for *Campylobacter spp.* They could be of risk to humans. As far as we are aware, this is the first study in Iran to isolate *Campylobacter spp.* from children’s playgrounds.
Introduction:

*Campylobacter spp.*, is a widespread zoonotic multihost pathogen, which often causes acute gastroenteritis in humans (Zendehbad et al., 2013). It has been shown that *C. jejuni* infection may cause more cases of acute diarrhea yearly than infection with *Salmonella* spp. (Lillehaug et al., 2005; Messad et al., 2014). Infection with *Campylobacter spp.* is highest in children between 1 and 4 years old (French et al., 2009). Campylobacteriosis may last between 3 and 11 days. The diarrhea is typically watery or bloody and occurs 8 to 10 times a day at peak illness. The fever can persist for up to a week in this disease (Tracz et al., 2005). However, it is usually self-limiting and complications are rare (Lyhs et al., 2010). Guillain-Barre’s syndrome (Chen et al., 2010), Irritable bowel diseases and immunoproliferative small intestinal disease (Garin et al., 2012) are the numbers of diseases in associated with this infection. Campylobacteriosis was the most frequent zoonotic disease reported in 2009 in the European Union (Lahuerta et al., 2011).

Although poultry products are important transmission vehicles to humans, the bacterium is common in domestic and wild birds, which live in close contact with humans (Griekspoor et al., 2013; Vázquez et al., 2010). The occurrence of *Campylobacter spp.* in the gut of apparently healthy wild birds has frequently been reported (Abulreesh et al., 2007; Lillehaug et al., 2005; Waldenström et al., 2002). Transmission to humans can occur by aerosols, direct or indirect contact through food and *Campylobacter spp.* contamination (Vázquez et al., 2010). Many playgrounds are located in parks that are natural habitats for wild birds. Play equipment in them provides ideal perching sites for these birds, and overhanging trees provide ideal roosting areas. Wild birds carry a diversity of micro-organisms that are pathogens for humans. These micro-
organisms may be transmitted over long distances during migrations. They are potentially transmissible to people who handle and ring birds or children who play in playgrounds (Abulreesh et al., 2007). This creates the potential for establishment of a novel focuses on emerging or reemerging communicable diseases along bird migration routes (Altekruse and Tollefson, 2003). Children’s behavior, such as frequent hand-mouth contact in this age group, is also likely to increase the risk of eating infective material (Black et al., 2004; French et al., 2009).

In this study, we looked into the possibility that wild-bird contamination of children’s playgrounds is a source of human infection. We study that by estimating the prevalence of Campylobacter spp. in fresh and dried wild-bird fecal samples. According to our knowledge, this is the first study in Iran to isolate of Campylobacter spp. from children’s playgrounds.

MATERIALS AND METHODS

Sample Collection

From July 2013 to August 2013, 200 samples of fresh and dried feces collected from 5 publicly accessible children’s playgrounds of Mashhad, Iran. The maximum distance between playgrounds was 2 km. We placed all samples in separate sterile plastic bags to prevent spills and cross contamination and immediately transported to the laboratory in a cooler with ice packs.

Isolation and identification of Campylobacter spp.

We immediately placed samples in 45 ml of Preston’s enrichment broth base (HiMedia Laboratories, Mumbai, India, M899). It contains Campylobacter selective supplement IV
(HiMedia Laboratories, Mumbai, India, FD042) and 5% (v/v) defibrinated sheep blood. All samples incubated at 42 °C for 24 h in a microaerophilic condition (85% N2, 10% CO2 and 5% O2). Following that 0.1 ml of the enrichment was then streaked onto Campylobacter selective agar base (HiMedia Laboratories, Mumbai, India, M994). It contains an antibiotic supplement for the selective isolation of Campylobacter spp. (HiMedia Laboratories, Mumbai, India, FD006) and 5% (v/v) defibrinated sheep blood. Finally, we incubated that for 48 h, at 42 °C under the same conditions. One presumptive Campylobacter colony from each selective agar plate was subcultured. For identification, we used standard microbiological and biochemical procedures including Gram staining, production of catalase, oxidase, hippurate hydrolysis, urease activity, indoxyl acetate hydrolysis, and susceptibility to cephalotin (Rahimi and Ameri, 2011).

**DNA extraction and PCR conditions**

From Preston’s broth we extracted DNA from 200 samples after the enrichment step using a Genomic DNA purification kit (Fermentas, GmbH, Germany, K0512) according to the manufacturer’s protocol. We used the PCR procedures in this study which Denis et al. described previously (Denis et al., 1999). In this protocol, three genes were selected for the identification of the Campylobacter spp., C. jejuni, and C. coli. They were the 16S rRNA gene (Linton et al., 1997), the mapA gene (Stucki et al., 1995), and the ceuE gene (Gonzalez et al., 1997), respectively. The sequences of the primers used for gene amplification are presented in Table 1. Amplification reactions were performed in a 30 µl mixture. It was containing 0.6 U Taq polymerase (Fermentas, GmbH, Germany), 100 µmol L⁻¹ of each dNTP, 0.11 µmol L⁻¹ of MD16S1 and MD16S2 primers. It was also containing 0.42 µmol L⁻¹ of MDmapAl, MDmapA2, COL3 and MDCOL2 primers in the Fermentas buffer (Fermentas, GmbH, Germany).
Amplification reactions were carried out using a DNA thermal cycler (Master Cycle Gradiant, Eppendorf, Germany). They were one cycle of 10 min at 95 °C, 35 cycles each consisting of 30 s at 95 °C. Also, they were 1 min and 30 s at 59 °C, 1 min at 72 °C and a final extension step of 10 min at 72 °C. The amplification generated 857 bp, 589 bp, and 462 bp DNA fragments corresponding to the *Campylobacter* genus, *C. jejuni* and *C. coli*, respectively. We used *C. coli* (ATCC 33559) and *C. jejuni* (ATCC 33560) as the positive controls and DNAase free Campylobacter spp. as the negative control. The PCR products were stained with 1% solution of ethidium bromide and visualized under UV light after gel electrophoresis on 1.5% agarose.

**Statistical Analysis**

The data were transferred to Microsoft Excel spreadsheet (Microsoft Corp. Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc. Chicago, IL, USA), Chi-square test and Fisher’s exact Two-tailed test analysis were performed, and differences were considered significant at values of \( P < 0.05 \).

**RESULTS**

Prevalence rates of *Campylobacter* spp. isolated from 5 publicly accessible children’s playgrounds in Mashhad, Iran are present in Table 2. Overall, 35 of 200 samples (17.5%) were positive for *C. jejuni*, including 31 fresh (23.85%) and 4 dried (5.7%) samples. The PCR assay could identify one *Campylobacter*-containing fecal sample that was negative using the cultural method. Positive samples were found on wood, concrete, soil, bark, plastic, and grass surfaces. Many species of birds were saw at the time of sampling. The main species of them were the
Corvus corone cornix, house sparrow (Passer domesticus), Spilopelia senegalensis, Columba livia, pigeons and Pied Wagtail (Motacilla alba).

**DISCUSSION**

The overall prevalence of *Campylobacter* spp. of all wild birds sampled in this study was 17.5%. *Campylobacter* colonizes in high concentrations in the cecum and colon of birds. The higher metabolic temperatures (42 °C) found in bird species may prepare them to be a prominent reservoir for thermotolerant *Campylobacter*. This is due to this fact that the maximum growth rate of *Campylobacter* is at this temperature (Horrocks et al., 2009). The increased temperature may allow the thermophilic species to regulate gene expression related to motility and energy regulation based on specific growth requirements within a particular environmental temperature (Rahimi et al., 2011; Stintzi, 2003). The results of our study were comparable with the results of other studies describing *Campylobacter* spp. in wild bird populations. They reported prevalence ranging from 2% to 50% (Griekspoor et al., 2013; Hughes et al., 2009; Ito et al., 1988; Keller and Gregory, 2014; Keller et al., 2011; Waldenström et al., 2002). Prevalence estimates are likely to vary between studies due to the use of different sampling regimens and culture methods, which vary in sensitivity. Furthermore, clearly the ecology of *Campylobacter* infection in wild birds is complex. It is involving many intrinsic and extrinsic factors, and unless such factors are carefully considered, comparisons of prevalence values between studies may be misleading. High prevalence of *Campylobacter* spp. in wild birds have often been interpreted as evidence that there is nonpathogenic coexistence of *Campylobacter* spp. and wild bird hosts (Hughes et al., 2009). Our findings of a higher rate of *Campylobacter* in fresh feces compared to dried feces are comparable with previous studies (Adhikari et al., 2004; Chuma et al., 2000). There is
evidence that the survival of *Campylobacter spp.* in fecal samples from different wild bird species is variable (Hughes, Bennett et al. 2009). We only detected one species of *Campylobacter* (*C. jejuni*) from wild birds in this study. This finding is consistent with previous reports by researchers who have shown that *C. jejuni* is widespread in nature. However the carriage rates in wild birds have been found to be 2% to 84% (Altekruse and Tollefson, 2003; Griekspoor et al., 2013; Petersen et al., 2001). However, throughout the world, researchers have found different *Campylobacter* species in wild birds. They isolated specifically *C. jejuni*, *C. lari*, and *C. coli* (Horrocks et al., 2009; Hughes et al., 2009; Rahimi et al., 2011; Waldenström et al., 2002).

Isolation and identification of *Campylobacter spp.* have traditionally involved the use of selective culture media combined with biochemical tests. This method is expensive, laborious and time consuming whereas PCR assay is fast and cost-effective (Boonmar et al., 2007; Rahimi and Ameri, 2011). In this study, *Campylobacter* was more detected by PCR assay than the cultural method. This could be due to the higher analytical and diagnostic sensitivities of PCR assay. Similarly, Boonmar et al. (2007) found a higher prevalence rate of *Campylobacter spp.* in duck meat samples using PCR assay, compared to culturing methods. However, care must be taken to avoid false-positive results arising from DNA contamination, as well as false-negative results caused by inhibitory substances in foods or enrichment broths. As the possibilities for amplicon contamination were minimized by separation of the preparation and amplification/detection laboratories, and the blank controls were negative, false-positive results due to amplicon contamination are unlikely to have occurred in our study (Rahimi and Ameri, 2011).
In this study, the main species of birds observed in the playgrounds at the time of sampling were *Corvus corone cornix*, house sparrow (*Passer domesticus*), *Spilopelia senegalensis*, *Columba Livia*, pigeons and Pied Wagtail (*Motacilla alba*). The fact that these birds are carriers of *Campylobacter* spp. and may have been involved in prevalence of *Campylobacter* and human infection, was reported by other investigators. The reported prevalence rate of *Campylobacter jejuni* in *Corvus corone cornix* was 13% to 89.8% (Ferrazzi et al., 2010; Heuvelink et al., 2008; Ito et al., 1988; Kapperud et al., 2003). This rate in the house sparrow (*Passer domesticus*) was 0% to 45.5% (Adhikari et al., 2004; Altekruse et al., 1999; Chuma et al., 2000; Ellis-Iversen et al., 2012; Glünder and Petermann, 1989; Kapperud and Rosef, 1983; Sippy et al., 2012). In *Columba Livia*, was 4.2% to 79% (Catroxo et al., 2011; Kapperud and Rosef, 1983; Vázquez et al., 2010). These were 1.9% to 69.1% in pigeons (Ito et al., 1988; Lillehaug et al., 2005; Rahimi et al., 2011; Vázquez et al., 2010). Rate of that in Pied Wagtail (*Motacilla alba*) was 0% to 6.2% (Broman et al., 2004; Pearson et al., 1993; Skov et al., 2008; Tsiodras et al., 2008), and 3% in *Spilopelia senegalensis* (Ito et al., 1988).

**Conclusion:**

The findings of this study suggest that wild bird species may constitute a reservoir for *Campylobacter* that could be of risk to other birds, farm animals and humans. Fecal material deposited by wild birds may contain *C. jejuni* that is associated with human disease. Therefore, hand washing after playing in playgrounds is a careful step to prevent zoonotic transmission of *Campylobacter* in household settings.

**References:**


Chuma, T., Hashimoto, S., and Okamoto, K. (2000). Detection of thermophilic Campylobacter from...


Screening for several potential pathogens in feral pigeons (Columba livia) in Madrid. Acta Vet Scand, 52, 45.


**Table 1.** Primers for Polymerase Chain Reaction (PCR) amplification for identification of *Campylobacter* isolates

<table>
<thead>
<tr>
<th>Organism</th>
<th>Primer</th>
<th>PCR product (bp)</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em> spp.</td>
<td>16SrRNA</td>
<td>857</td>
<td>F: 5´ ATC TAA TGG CTT AAC CAT TAA AC 3´</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: 5´ GGA CGG TAA CTA GTT TAG TAT T3´</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td><em>mapA</em></td>
<td>589</td>
<td>F: 5´ CTA TTT TAT TTT TGA GTG CTT GTG 3´</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: 5´ GCT TTA TTT GCC ATT TGT TTT ATT A3´</td>
</tr>
<tr>
<td><em>Campylobacter coli</em></td>
<td><em>ceuE</em></td>
<td>462</td>
<td>F: 5´ AAT TGA AAA TTG CTC CAA CTA TG 3´</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: 5´ TGA TTT TAT TAT TTG TAG CAG CG 3´</td>
</tr>
</tbody>
</table>

F = forward; R = reverse.
Table 2. Prevalence of *Campylobacter* spp. isolated from 5 publicly accessible children’s playgrounds in Mashhad, Iran.

<table>
<thead>
<tr>
<th>Samples</th>
<th>No. of samples</th>
<th><em>Campylobacter</em> spp. positive</th>
<th>C. jejuni</th>
<th>C. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>130</td>
<td>31 (23.85%)</td>
<td>31 (23.85%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Dried</td>
<td>70</td>
<td>4 (5.7%)</td>
<td>4 (5.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>35 (17.5%)</td>
<td>35 (17.5%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>
• The objective was to determine the prevalence of *Campylobacter* spp. isolated from wild-bird fecal samples in Mashhad, Iran.
• *Campylobacter* spp. were found in 17.5% of feces samples.
• We only detected one species of *Campylobacter* (*C. jejune*) from wild birds in this study.
• *Campylobacter* spp. were significantly (*P* < 0.05) more prevalent in the fresh samples (23.85%) than the dried samples (5.7%).