Investigation of Campylobacter in Reared Game Birds

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ABSTRACT

A total of 103 pooled samples of neck skin and meat from pigeons for the table and neck skin of pheasant were analyzed bacteriologically to determine the presence of Campylobacter. Colonies suspected of being Campylobacter were grown from 15.8% of pigeon neck skin samples, 12.5% of pigeon meat samples, and 50% of pheasant neck skin samples after culturing, and in 6.9% of pigeon neck skin samples (4 × 10^2 to 2 × 10^3 CFU/g) assessed quantitatively without preculturing. PCR confirmed the presence of Campylobacter spp. in 5.26 and 3.44% of samples of pigeon neck skin and meat, respectively. Species identified from pigeon neck skin samples by PCR were C. jejuni (3 of 3) and C. coli (1 of 3); no C. lari was identified. No species were identified by PCR in pheasant neck skin. We conclude that the small number of Campylobacter-positive pigeon samples presents a low risk of Campylobacter infection to Italian consumers, particularly since pigeon is always well cooked before consumption, although there is always the possibility of cross-contamination with raw or insufficiently cooked foods particularly during food preparation.

Campylobacter species are well known as causes of intestinal tract infections particularly in children (2, 21, 23). The number of cases of diarrheal disease because of these microorganisms has increased in recent years in all industrialized countries including Italy. In the United Kingdom, Campylobacter is more often isolated in enteric disease than is Salmonella, and in the United States Campylobacter is more often responsible for food poisoning than is Salmonella or Shigella (5, 21).

The Campylobacter species mainly causing enteric disease in humans are thermophilic, with optimum growth temperatures in the range of 42 to 45°C. C. jejuni is responsible for about 90% and C. coli for about 7 to 8% of enteric infections due to the genus, with 2 to 3% caused by C. lari and C. upsaliensis (12, 19).

Campylobacter infection in humans may arise from consumption of contaminated foods of animal origin such as raw milk, mollusks, and raw or lightly cooked meat or offal, and also by cross-contamination of raw or insufficiently cooked foods, particularly during food preparation. Infection may also occur from drinking contaminated water, and in 1.5 to 3% of cases from consumption of vegetables (12). The main source of infection is raw or poorly cooked chicken (21), consistent with data indicating that 20 to 98% of refrigerated poultry samples are contaminated (12). Ingestion of about 500 cells may cause infection (5). Other studies have found that Campylobacter was present in the intestines of between 11 and 80% of poultry specimens examined, and between 43 and 84% of poultry carcasses (1, 4, 7, 8, 11, 14, 17, 20, 22), suggesting that cross-contamination during slaughter may be an important means of dissemination (3, 9, 15, 18). A previous study on pigeons for the table also found that thermophilic campylobacteria were occasionally present (C. jejuni in 0.74% of cloacal plugs and 4% of giblet samples) (13), but at lower frequencies than those encountered in the giblets of chicken (23.5%), duck (19%), and turkey (14.5%) (10).

In view of such indications, the European Union recommendation (2004/24/EC) of December 2003 established a program to determine the extent of Campylobacter infection in poultry meat and other avian food products, with the aim of providing data for future regulation. As part of this initiative, the Italian Ministry of Education Universities and Research financed a program to investigate the extent of Campylobacter infection within Italian aviculture; the present study was carried out under this program, with the aim of investigating the presence of Campylobacter species in samples of reared pigeon and pheasant for the table from a single supplier in northern Italy, with a view to providing indications as to the risk to consumers.

MATERIALS AND METHODS

We examined 103 pooled samples from slaughtered pigeons and pheasants from a single farm in the province of Vercelli, northern Italy. The animals were slaughtered on the farm that was licensed to process 1,000 birds a week per Italian and European Union legislation. The animals were killed at 500-g live weight, generally at age 3 to 4 weeks. All operations (electrostunning, bleeding by internal jugular severing, crop removal, machine plucking, pneumatic gun evisceration, wax stripping, and manual finishing) were carried out dry to minimize contamination.

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TABLE 1. Results of microbiological analysis of pigeon and pheasant samples for Campylobacter spp., using CCDA media (after enrichment in Bolton broth for the only qualitative evaluation) and biochemical and Gram stain tests

<table>
<thead>
<tr>
<th></th>
<th>Qualitative evaluation</th>
<th>Quantitative evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples examined</td>
<td>Suspect(^a)</td>
</tr>
<tr>
<td>Pigeon neck skin</td>
<td>57</td>
<td>46</td>
</tr>
<tr>
<td>Pigeon meat</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Pheasant neck skin</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\) Samples that have shown colony growth on selective CCDA media.

\(^b\) Confirmed as catalase-positive, oxidase-positive, gram-negative cultures consisting of curved rod-shaped cells.

**Sampling.** Between January and March 2005, 515 bird carcasses in six consignments were transported in a refrigerated truck (4 ± 2°C) to the laboratory. The carcasses had been placed in refrigerated containers (4 ± 2°C) and loaded onto the truck immediately after slaughter.

Samples from these animals were taken and pooled, five animals contributing to each pooling, to produce the 103 samples as follows: (i) 86 samples of pigeon neck skin, each sample weighing about 25 g; (ii) 15 samples of pigeon breast and leg meat, each sample weighing about 25 g; and (iii) 2 samples of pheasant neck skin, each sample weighing about 25 g.

**Microbiological analyses.** Fifty-seven of the pigeon skin samples, eight of the pigeon meat samples, and the two pheasant skin samples were analyzed qualitatively for Campylobacter spp. The remaining 29 pigeon skin and 7 pigeon meat samples were analyzed quantitatively for Campylobacter spp.

For qualitative microbiological evaluation, each 25-g sample was added to 225 ml of Bolton broth (Oxoid S.p.A., Milan, Italy), supplemented with Bolton broth selective supplement (Oxoid) and laked horse blood (Oxoid), in a screw-top sterile container and incubated for 4 h at 37°C, followed immediately by incubation for 44 h at 42°C. A 100-μl sample was removed and spread onto plates prepared with blood-free selective agar base (CCDA; Oxoid), supplemented with CCDA selective supplement (Oxoid; a selective Campylobacter medium). The plates were incubated at 42°C for 48 h, with the addition of CampyGen (Oxoid) to create microaerophilic conditions.

For quantitative microbiological evaluation, each 25-g sample was added to 100 ml of peptone water and homogenized in a blender. Samples of the resulting suspension were diluted 1:50 and 1:500 with peptone water, and spread onto plates containing CCDA selective medium (Oxoid) containing CCDA selective supplement. The plates were incubated at 42°C for 48 h in microaerophilic conditions, and the number of CFUs counted.

For both qualitative and quantitative evaluations the plates were read by using the following the manufacturer’s criteria for identifying Campylobacter: C. jejuni produce gray, wet colonies, although some are greenish and some are dry; they may or may not have a metallic sheen. Colonies of C. coli are creamy gray, wet, slightly raised, and typically well separated from each other. C. lari colonies take various forms although some resemble C. jejuni and C. coli. Suspicious colonies were tested with catalase, oxidase, and Gram stain. Catalase-positive, oxidase-positive, gram-negative cultures consisting of curved rod-shaped cells were transferred to slants in 2.5-liter jars in microaerophilic conditions and then incubated at 42°C for 24 h.

**PCR analyses.** The slant colonies were subjected to PCR to confirm Campylobacter by dispersing individual slants in 10 ml of Bolton broth, supplemented with Bolton broth selective supplement, and incubated in 2.5-liter jars at 42°C in microaerophilic conditions for 24 h. Equine blood was not added.

DNA was extracted (in duplicate) using GenomicPrep Cells and Tissue DNA Isolation Kits (Amersham Biosciences, Piscataway, N.J.), following the manufacturer’s instructions for gram-negative bacteria. The DNA present was assayed spectrophotometrically, and the duplicate with DNA of greatest purity was amplified. Generic amplification employed a pair of universal primers for Campylobacter (MD16S1 and MD16S2) that amplify the 16S rRNA gene. Cultures with a positive amplification then underwent species-specific PCR using primers specific for C. jejuni (MDmapA1 and MDmapA2), C. coli (COL3 and MDCOL2) (6), and C. lari (CL55 and CL632) (16) that amplify the genes mapA, cedu, and 16S rRNA, respectively.

The PuReTaq Ready-To-Go PCR Beads Kit (Amersham Biosciences) was used to amplify 2 μl of DNA, with 2 μl of each primer (concentration: 0.8 μM) in sterile water to a final volume of 25 μl per the manufacturer’s instructions. A 2400 Thermal Cycler (PerkinElmer, Wellesley, Mass.) was used, with denaturation at 95°C for 10 min, 35 cycles of 95°C for 30 s, 59°C for 1.5 min, and 72°C for 1 min, followed by a final extension phase at 72°C for 10 min.

The amplification products were separated by polyacrylamide gel electrophoresis (GeneGel Excel Kit, Amersham Biosciences) and stained with the DNA Silver Staining Kit (Amersham Biosciences).

**RESULTS**

The qualitative microbiological analyses found that 9 (15.8%) of 57 samples of pigeon neck skin, 1 (12.5%) of 8 samples of pigeon meat, and 1 (50%) of the 2 samples of pheasant skin were contaminated with microorganisms suspected to be Campylobacter. The quantitative analyses on different samples found that 6.9% (2 of 29) (i.e., plates with between 4 × 10^2 and 2 × 10^3 CFU/g) and 0% (0 of 7) of samples of pigeon skin and pigeon meat, respectively, were positive for microorganisms suspected to be Campylobacter (Table 1).

PCR analyses of colonies suspected to be Campylobacter confirmed the presence of this genus in 5.26% of samples of pigeon skin subjected to qualitative analysis, and in 3.44% of samples of pigeon skin subjected to quantitative analysis (expressed as percentages of total samples analyzed qualitatively and quantitatively, respectively). None of the pigeon muscle samples, and 50% of the pheasant skin samples were positive (Table 2).

Subsequent species-specific PCR on colonies shown to contain Campylobacter by generic PCR found that in the
TABLE 2. Results of molecular analysis for Campylobacter spp., using PCR method

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples examined</th>
<th>No. (%) of samples positive</th>
<th>No. positive/total</th>
<th>Species</th>
<th>No. of samples examined</th>
<th>No. (%) of samples positive</th>
<th>No. positive/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigeon neck skin</td>
<td>9</td>
<td>3 (33)</td>
<td>3/57 (5.26)</td>
<td>C. jejuni</td>
<td>3/3</td>
<td>1/3 C. coli</td>
<td>0/3 C. lari</td>
</tr>
<tr>
<td>Pigeon meat</td>
<td>1</td>
<td>0</td>
<td>0 (0)</td>
<td></td>
<td>0/1 C. jejuni</td>
<td>0/1 C. coli</td>
<td>0/1 C. lari</td>
</tr>
<tr>
<td>Pheasant neck skin</td>
<td>1</td>
<td>1 (100)</td>
<td>1/2 (50)</td>
<td></td>
<td>0/1 C. jejuni</td>
<td>0/1 C. coli</td>
<td>0/1 C. lari</td>
</tr>
</tbody>
</table>

three skin samples from pigeon neck that were positive by qualitative analysis, C. jejuni was present, and in one of these samples C. coli was also present. The single pigeon skin sample that was positive by quantitative analysis contained only C. jejuni. The single pheasant neck sample (from qualitative analysis) that was positive for generic Campylobacter was negative for C. jejuni, C. coli, and C. lari (Table 2).

DISCUSSION

The aim of this study was to determine the presence of Campylobacter infection in pigeons for the table from a single supplier in northern Italy, with a view to providing indications as to the risk to consumers. Campylobacter was present in some samples of pigeon skin and muscle. However, the overall risk must be considered low, as only two samples were positive by quantitative analysis (which measured the number of CFUs in the sample without prior culturing). The concentration of viable units in these samples was in the range of $10^2$ to $10^3$ CFU/g. Such levels are able to cause infection, but still represent a small risk since pigeons are always well cooked before consumption (5, 19). Clearly, however, there is always the possibility of cross-contamination with contaminated raw or insufficiently cooked foods, particularly during food preparation.

We also assessed two samples of pheasant neck skin for Campylobacter, finding that an unknown Campylobacter species was present in one of the samples. This finding is not significant since only two samples were analyzed, but it is consistent with findings that Campylobacter frequently contaminates game birds (10), and suggests the utility of a specific study on pheasants.

It is noteworthy that the microbiological analyses using CCDA were not totally specific for Campylobacter, since we found 46 suspect colonies but confirmed only 9 (catalase-positive, oxidase-positive, gram-negative cultures consisting of curved rod-shaped cells) by the qualitative method, and 13 suspect versus 2 confirmed colonies by the quantitative method. It is noteworthy also that the PCR results were markedly different from the microbiological results. For example, only three of the nine microbiologically confirmed colonies were confirmed as Campylobacter by PCR. Our provisional observations suggest that this discrepancy is due to the fact that certain strains of Escherichia coli can mimic Campylobacter microbiologically and biochemically.

The low levels of C. jejuni and C. coli contamination identified by species-specific PCR (both present in a single sample of pigeon skin) is consistent with previous findings in poultry carcasses (1, 8, 11, 17, 18, 22). The lack of identification of C. jejuni, C. coli, or C. lari in the pheasant skin sample, notwithstanding confirmation by generic PCR of Campylobacter spp., indicates the presence of other species.

Our previous study (22) on young cockerels found a much greater frequency of Campylobacter in neck skin samples (73.6%) than was found in the current study (15.8% of pigeon neck skin samples), using the same qualitative assay method. A probable reason for this is that the pigeons were slaughtered and processed dry, whereas the poultry were processed by traditional methods.

To conclude, our data suggest that the risk of human infection by Campylobacter from pigeon meat is low, not only because of the low percentages of samples found contaminated, but also because Campylobacter infection is only likely to occur from the consumption of raw or partially cooked meat—pigeons are always consumed cooked, and Campylobacter does not survive normal cooking temperatures (5). However, there is always the possibility of cross-contamination with contaminated raw or insufficiently cooked foods, particularly during food preparation.

ACKNOWLEDGMENT

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REFERENCES


