Prevalence of *Campylobacter spp.* in broiler chickens in Croatia

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Campylobacteriosis represent an important public health problem in Croatia, especially in children. Broilers are primarily colonized with *C. jejuni*, less often with *C. coli* and rarely with other species (Newel and Wagenaar, 2000). Poultry meat is a major source of campylobacteriosis and flock prevalence varies between EU countries, ranging from 5% to more than 90% (EU, 2003). Previous investigations regarding *Campylobacter spp.* in poultry in Croatia performed on feces of different poultry species revealed 11.69% positive broiler samples (Krstulović et al., 2003). This investigation was conducted on selective media, and recently modern molecular diagnostic methods weren’t used.

To detect the prevalence of *Campylobacter spp.* in the northern part of Croatia, where the most of the broiler farms are located, cloacal swabs were randomly taken from 91 broiler chickens of different ages situated at three different farms. After pre-enrichment, cultures was streaked on modified charcoal cefoperazone desoxycholate agar (mCCDA) plates and incubated for 48 hours at 42ºC, in microaerobic atmosphere. All suspected colonies were presumptively identified as *Campylobacter* on the basis of macroscopic and microscopic appearance of colonies and positive catalase and oxidase reaction. The DNA was extracted from a 48 h cultures prepared in Nutrient Broth No. 2 as described by Jackson *et al.* (1993). The identification of strains was obtained by the multiplex PCR (mPCR) as described by Manfreda *et al.* (2003). The expected PCR amplicons were at 857, 589 and 462 by corresponding to the genus *Campylobacter* and to the species *C. coli* and *C. jejuni*, respectively. Altogether 35 (38.46%) samples were positive, whereas 18 (51.42%) of them were detected as *C. coli* and 17 (48.58%) as *C. jejuni*.

Further investigation will be conducted on larger number of chickens to reveal the real prevalence of both Campylobacter strains. The mPCR, for the first time used in our laboratory, based on the use of different sets of primers is simple and time-saving method, can be performed for the simultaneous identification of different *Campylobacter* species and could contribute to the interpretation of results of epidemiological studies and determine whether particular *Campylobacter* types persist along the food chain or establish themselves as house flora in farms and slaughterhouses, what will be the goal of future investigations.

**Keywords:** *Campylobacter spp.*; broiler chicken; multiplex PCR

**Introduction**

Campylobacteriosis represent an important public health problem in most areas of the world. According to the EU reporting of zoonoses, the incidence of campylobacteriosis is significant throughout EU and there has been a general upward in the incidence over the last decade (EC, 2004). Thermophilic *Campylobacter* species, particularly *C. jejuni* and *C. coli* are extremely important because of harmful impact of these microorganisms, as zoonotic agents, on human (Altekruise *et al.*, 1999; Lastovica and Skirrow, 2000; Park, 2002).
Broilers are primarily colonized with *C. jejuni*, less often with *C. coli* and rarely with other species (Newel and Wagenaar, 2000). Poultry meat is a major source of campylobacteriosis and flock prevalence varies between EU countries, ranging from 5% to more than 90% (EU, 2003).

Regarding Croatia, campylobacteriosis is the second reported food-borne infection and represent an important public health problem, especially in children. Previous investigations regarding *Campylobacter* spp. in poultry in Croatia, conducted only by using different selective media, performed on feces of different poultry species revealed 11.69 % positive broiler samples (Krstulović *et al.*, 2003). Epidemiological investigations that will determine differences or similarities between poultry and human isolates haven’t been conducted in Croatia yet and also, modern molecular diagnostic methods weren’t used to detect *Campylobacter* spp.

**Materials and methods**

From altogether 91 broiler chickens of different ages situated at three different farms (Farm A, B, C), cloacal swabs were taken to detect the prevalence of *Campylobacter* spp already on the farm. Samples were randomly taken from broilers raised in the northern part of Croatia, where the most of the broiler farms are located.

Sterile swabs were pre-moistened in physiological saline (0.9% NaCl) before sampling. In laboratory were aseptically transferred to Campylobacter Selective Enrichment Broth (Oxoid, Unipath, UK) and incubated at 42°C for 48 hours in a microaerobic atmosphere (85% N₂, 10% CO₂, 5% O₂). One loopful of the 48 hour cultures was streaked on modified Charcoal Cefoperazone Desoxycholate Agar (mCCDA) plates (Oxoid, Unipath, UK) and incubated for 48 hours at 42°C, in microaerobic atmosphere. All suspected colonies were first presumptively identified as *Campylobacter* on the basis of macroscopic appearance of colonies, Gram-negative staining and positive catalase and oxidase reaction.

The DNA to be used as target in the multiplex PCR (mPCR) was extracted from a 48 h cultures prepared in Nutrient Broth No. 2, as described by Jackson *et al.* (1993). The identification of strains was obtained by the multiplex PCR as described by Manfreda *et al.* (2003). Following the amplification, 20 µl of PCR product were electrophoresed in 1 % TAE buffer on 1% agarose gel stained with ethidium bromide and visualized under UV light. The expected PCR amplicons were at 857, 589 and 462 by corresponding to the genus *Campylobacter* and to the species *C. coli* and *C. jejuni*, respectively. *C. jejuni* ATCC 33560 and *C. coli* RM 2228 were used as positive controls. Our own strain of *E. coli* 223/05 was used as negative control.

**Results and discussion**

Out of 91 cloacal swabs taken from broiler chickens, from 45 (49.45%) of them isolated bacteria could be classified as *Campylobacter* spp, on the basis of microbiological examination. All of positive isolates were subjected to mPCR, and after visualization of the products, 35 (38.46%) isolates were found PCR positive, whereas 18 (51.42%) of them were detected as *C. coli* and 17 (48.57%) as *C. jejuni*. Four colonies presumptively identified as *Campylobacter* were found PCR negative, so further investigation will be conducted to reveal real *Campylobacter* genotype. All samples from Farm C were found negative (Table 1), presumably because of long antimicrobial treatment.
Table 1. Source and identification of Campylobacter strains and the results of genotyping.

<table>
<thead>
<tr>
<th>Source (cloacal swabs)</th>
<th>Results</th>
<th>Multiplex PCR of Campylobacter spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Campylobacter spp.</td>
<td>Negative</td>
</tr>
<tr>
<td>Farm A n = 31</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(80.64%)</td>
<td>(19.36%)</td>
</tr>
<tr>
<td>Farm B n = 20</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>(70%)</td>
<td>(30%)</td>
</tr>
<tr>
<td>Farm C n = 40</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>(0%)</td>
<td>(100%)</td>
</tr>
</tbody>
</table>

According to current legislatives, program for surveillance of campylobacteriosis doesn’t exist in Croatia, and epidemiological studies weren’t conducted to reveal the real prevalence of these food-borne zoonoses. Recent investigation conducted on 325 broiler fecal samples reveal 38 (11.69%) positive isolates, two different selective media (Bolton’s and Karmali’s) were used for the isolation of Campylobacter spp. and genotype weren’t examined (Krstulović et al., 2003). The result of 38.46% positive isolates in this investigation points that the approach to the control of Campylobacter in Croatia should be changed. The microbiological and serological diagnostic currently used methods are not suitable enough to establish differences and similarities among isolates. Developed different molecular methods can be appropriate for comparison of Campylobacter strains.

Multiplex PCR, based on the use of different sets of primers appears to be simple and time-saving method for the simultaneous identification of different Campylobacter species (Manfreda et al., 2003). It is also suitable for epidemiological studies to determine Campylobacter types in poultry on the farms and slaughterhouses.

References


