Occurrence and Enumeration of Campylobacters Before and After Processing of Conventional Chickens in Egypt

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Abstract: Campylobacter is considered to be the most common cause of bacterial diarrheal illness and many cases are thought to be acquired from consumption of undercooked poultry or through cross-contamination of other foods during the preparation of poultry. The numbers of cases of human disease in Egypt with Campylobacter have increased but nothing is known about the specific source of infection in Egypt or about the incidence of Campylobacter in Egyptian reared poultry. The aim of this study was therefore to enumerate both campylobacters and total bacterial counts in four conventional chicken Egyptian flocks in North Sinai governorate, starting from the farm and progressing to the slaughterhouse. Samples were taken from chicken carcasses at six different stages during processing. The effect of post-slaughter chilling on the survival of campylobacters at 4°C and -20°C was also compared. All four flocks were infected with Campylobacter spp. before processing and these were also detected at the slaughterhouse on the chicken carcasses where C. jejuni was the dominant species. The highest Campylobacter counts, (mean 9.3 CFUg⁻¹), were detected in the intestinal contents of live birds, but high levels of contamination were also found on fresh cooled and frozen chicken meat samples (7.4 CFUg⁻¹ and 7.8 CFUg⁻¹ respectively). This high level and the continuous presence of Campylobacter in the slaughterhouse constitute a risk for transmission to negative carcasses. Our findings indicate that chilling and freezing processes have a limited effect in reducing contamination of final products. As in other parts of the world, chickens in Egypt that enter the slaughterhouse contaminated with Campylobacter represent a risk to consumers' health in cases of improper handling or cooking practices.

Key words: Campylobacter · Occurrence · Enumeration · Survival · Chicken · Processing

INTRODUCTION

Campylobacters are Gram-negative bacteria that are ubiquitous in the environment [1] and considered as a bacterial cause of enteric disease [2]. In developing countries Campylobacter jejuni enteritis usually occurs in infants whereas it is rare in adults [3]. Campylobacter isolation rates in developing countries range from 5 to 20% [4, 5]. In 2000, Wasfy et al. [6] isolated 146 campylobacters from the stools of 6278 Egyptian patients. In another study, Rao et al. [7] reported that most of Egyptian children aged less than 3 years are infected with Campylobacter at 0.6 episodes per child a year and they attributed this high rate to the presence of animals and birds in the house in addition to the poor hygienic practices. About 100 of 118 Campylobacter isolates from Egyptians during 1998-2005 were found to be resistant to fluoroquinolones [8].

Chickens are considered major reservoirs of Campylobacter infection [9] and significant relationship has been reported between sporadic human Campylobacter infection and the handling and preparation of chickens or consumption of undercooked chickens [10-11]. About 40% of tested chicken liver samples in Egypt were positive for Campylobacter [12]. In a recent study, Campylobacter was isolated from 39.17% of broiler carcass samples in Assiut city in Egypt [13]. Human can be infected with this bacterium with a relatively low dose (500 CFU) through cross-contamination of other food products or direct hand to mouth contact after handling raw chicken [14, 16].

Campylobacters are usually undetectable in intensively reared chickens until at least 10 days of age and most of chickens become colonized with campylobacters after 2 to 3 weeks [17]. This may be due to the protective activity of maternal antibodies against
Campylobacter colonization after hatching [18, 19]. Campylobacter appears to be so well adapted to the poultry gut that as few as 100 organisms can colonize [20]. Gastrointestinal tract; in particular the ceca, small intestine, large intestine and cloaca is the principle site of colonization with this microorganism [21]. Approximately three out of four live chickens and more than 80% of retail poultry meat are contaminated with Campylobacter [22, 23]. In one study of raw chicken meat sold in the UK, 80% was contaminated with Campylobacter [24] with 98 and 2% of isolates identified as C. jejuni and C. coli, respectively. C. jejuni is considered as the most important cause for foodborne gastroenteritis worldwide [25]. Therefore, large numbers of campylobacters can be dispersed during transport and abattoir processing causing a possible hygiene problem in abattoirs.

Campylobacter-positive chickens entering the slaughter line can cause extensive cross-contamination to non-infected carcasses during slaughtering and processing and even after chilling and cutting of poultry products and represents a significant challenge for the poultry industry [26]. The surface of the chickens can be contaminated with this microorganism after scalding and defeathering and these numbers are reported to be in the region of 5.9 log CFU per carcass [27]. Most of chicken flocks become contaminated by Campylobacter spp. on the day of slaughter. It has been reported that refrigeration of poultry meat is very useful to control the growth of pathogenic organisms such as Campylobacter [28]. Previous studies have shown that freezing to -20°C and cooling to 4°C reduce viable counts of campylobacters by causing lethal or sub lethal injuries [29, 30]. However, this will not significantly reduce the numbers of campylobacters associated with the chicken skin [30].

In this study we described the enumeration of both Campylobacter and total bacteria isolated during the rearing cycle of conventional chickens and also during processing. We also studied the effect of cooling and freezing in a domestic refrigerator on the survival characteristics of Campylobacter.

MATERIALS AND METHODS

Source of Samples: Four conventional chicken farms were randomly selected at different geographic locations in El-Arish city, North Sinai governorate. All birds in this study were Ross broilers from a commercial hatchery. Birds were reared on single species farms with up to 5,000 birds kept in a single shed. Soil and fresh fecal samples were collected from the farm at the end of the trial. At the slaughterhouse, samples from specimens during slaughtering were obtained using the standard method. The slaughterhouse was divided into 7 areas according to the ongoing activity: arrival, scalding, defeathering, evisceration, final wash, refrigeration and freezing stages. Three birds from each flock were investigated at the different processing stages from arrival and onwards. For the defeathering, final wash, refrigeration and freezing stages, an area of at least 100 cm² of the surface of each of 5 carcasses per stage was cut, aseptically transferred to a plastic bag. In addition, at evisceration, 10 whole ceca were collected from each flock under study, transferred to sterile plastic bag to the laboratory.

Isolation and Enumeration of Campylobacter from Conventional Chickens: Three birds were selected at random from the conventional flocks during abattoir processing. Fresh soil and excreta samples were collected from chickens before slaughtering. Birds were removed from the flock and transported directly to the abattoir for slaughtering and for commercial processing. After slaughtering, the ceca from sample birds were removed by sterile dissection and the contents were collected for Campylobacter and total bacterial isolation. During abattoir processing chicken samples were removed after defeathering, eviscerating, after final wash and also after refrigeration (after 3 days of storage) and freezing (after 20 days of storage) for Campylobacter isolation. All samples were transported to the Food Microbiology Laboratory (Faculty of Environmental Agricultural Sciences, Suez Canal University, Egypt) under refrigerated conditions. Serial dilutions were made by using the Maximum Recovery diluent as described before [31] (catalogue no. CM733; Oxoid, Basingstoke, United Kingdom). Volumes (100 µl) of each diluted sample were then spread on the surface of the modified cefoperazone charcoal deoxycholate agar (mCCDA) selective medium (CM739 and selective supplement SR155; Oxoid). The plates were then incubated at 42°C for 48 h under microaerobic conditions (5% O₂, 5% H₂, 10% CO₂ and 80% N₂). Ten or more typical Campylobacter colonies were examined by Gram stain and wet mount and sub cultured on blood agar [blood agar base number 2 (CM 271; Oxoid) with horse blood]. Oxidase and catalase tests were performed and the identification of the isolates was
confirmed by using the hippurate test. A loopful of a fresh Campylobacter culture was emulsified in 500 µl of 5% (w/v) hippuric acid (Sigma: H-9380) and incubated aerobically at 37°C for 2 h. After incubation, the suspension was overlaid with 200 µl of 3.5% (w/v) ninhydrin solution (BioMerieux: REF 70 490) for a further 10 minutes. A positive result is indicated by the development of a deep blue colour whereas a colourless or light to medium purple coloration is considered negative [32]. Some of the isolates were identified by species specific PCR with primers specific to hipO and putative aspartokinase gene and flanking ORF target genes described by Linton et al. [33]. Campylobacter DNA for PCR analysis was extracted using the protocol determined by Pitcher et al. [34].

### RESULTS

In this study, quantitative examination for both total bacteria and Campylobacter in the investigated samples (total 188) before and after processing of the conventional chickens (Table 1) was performed. The present study showed high prevalence of Campylobacter in chicken samples during slaughtering process. Overall, Campylobacter contamination, ranged from 7.4 log_{10} up to 9.3 log_{10} CFUg^{-1}. Both soil and fecal samples were highly contaminated with Campylobacter and the observed mean counts were 8.3 log_{10} and 8.6 log_{10} CFUg^{-1}, respectively. The isolation rate at the pre-evisceration stage was quite high (mean counts 8.0 log_{10}, CFUg^{-1}) due to feathers and skin contamination with feces from transportation through the defeathering stage.

As expected, the highest Campylobacter counts mean (9.3 CFUg^{-1}) was in intestinal contents. Also, the carcasses were contaminated with higher numbers of Campylobacter after evisceration (8.4 CFUg^{-1}). The mean count of Campylobacter after final washing was quite similar to the mean count after evisceration (8.4 CFUg^{-1}). The Campylobacter mean values detected in cooled and frozen carcasses were significantly lower (P < 0.05) than those detected in intestinal contents (Table 1). All the 40 fresh cooled and frozen chicken meat samples examined were Campylobacter positive and the observed counts were (7.4 CFUg^{-1}) and (7.8 CFUg^{-1}) respectively.

The results of this study showed that the therмотolerant Campylobacter contamination was prevalent in caecal contents and the poultry carcasses and that all four broiler flocks were colonized with Campylobacter (100%) (Illustrated in Table 1). Both conventional hippurate test and polymerase chain reaction (PCR) differentiation of the Campylobacter isolates revealed that C. jejuni was the dominant species among isolates from all flocks before and after processing of the conventional chickens (Fig. 1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of samples tested (n)</th>
<th>Total bacterial counts mean log CFUg^{-1}(SD)</th>
<th>Campylobacter counts mean log CFUg^{-1} (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>24</td>
<td>7.9 (± 0.07)</td>
<td>8.3 (± 0.06)</td>
</tr>
<tr>
<td>Faecal</td>
<td>24</td>
<td>9.3 (± 0.20)</td>
<td>8.6 (± 0.19)</td>
</tr>
<tr>
<td>Intestine</td>
<td>40</td>
<td>9.9 (± 1.74)</td>
<td>9.3 (± 1.78)</td>
</tr>
<tr>
<td>After defeathering</td>
<td>20</td>
<td>8.7 (± 1.69)</td>
<td>8.0 (± 2.44)</td>
</tr>
<tr>
<td>After evisceration</td>
<td>20</td>
<td>9.4 (± 1.47)</td>
<td>8.4 (± 2.04)</td>
</tr>
<tr>
<td>After final wash</td>
<td>20</td>
<td>9.1 (± 1.93)</td>
<td>8.4 (± 1.57)</td>
</tr>
<tr>
<td>After refrigeration</td>
<td>20</td>
<td>8.1 (± 1.63)</td>
<td>7.4 (± 2.17)</td>
</tr>
<tr>
<td>After freezing</td>
<td>20</td>
<td>7.3 (± 1.46)</td>
<td>7.8 (± 2.05)</td>
</tr>
<tr>
<td>Total</td>
<td>188</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1: Agarose gel showing PCR products obtained from genomic DNAs of conventional chicken isolates using a primer specific to hipO target gene. A) Lane 1 DNA marker 1 kbp; Lanes 2 to 5 C. jejuni isolate DNA that amplified with hipO gene primers

DISCUSSION

Campylobacteriosis is considered as a serious foodborne disease worldwide [35] and poultry meat contaminated from intestinal contents of colonized chickens is the major source of infection with Campylobacter. Most of Egyptians like poultry meat and they enjoyed chicken the most. The commercial poultry sector in Egypt became more dynamic. Most of Egyptians prefer live birds which can be slaughtered immediately at sale to consumers and it constitute about 70% of chicken production and the remaining 30 percent is sold frozen [36]. The Egyptian chicken sector is subdivided into commercial (international breeds) and Balady (traditional breeds). The fate of Campylobacter populations following chicken slaughter in Egypt is almost unknown. Handling of raw chicken meat at home bears the risk of cross-contamination, particularly in the case of unsatisfactory kitchen hygiene [14]. However, campylobacters are almost susceptible to environmental stresses such as oxygen and heat and as a result their viable numbers decline in the environment.

The results of this study showed that the thermotolerant Campylobacter contamination was prevalent in caecal contents and the poultry carcasses that all four broiler flocks were colonized with Campylobacter (100%) and this could be regarded as high, compared with other countries with proportions varying from about 10 to 90% [1]. This result is not surprising because cross contamination with Campylobacter in the farm and during processing is very common and unavoidable. Our study showed that C. jejuni was the dominant species and this result is in agreement with previous studies with intensively reared birds which stated that 80 to 90% of isolates were C. jejuni and the remainder was C. coli [24, 38].

Since slaughtering process involved many steps Campylobacter cross contamination is very likely to occur. The contamination of carcasses with Campylobacter increased during evisceration steps. The evisceration process contributes significantly to Campylobacter contamination [39] and this increase in contaminated carcasses is due to the rupture of intestine which leads to the contamination of equipment and working surfaces and increase the opportunities for cross contamination of Campylobacter-free carcasses during processing [22, 40]. Once the surface of a carcass is contaminated with Campylobacter, such contamination has the potential to persist through the rest of the carcass processing [41, 42]. Accordingly, special attention should be given to control the potential contamination with Campylobacter during processing, such as the plucking and evisceration steps so as to reduce cross contamination with Campylobacter during following processes.

Good practices during poultry processing such as decontamination of equipment, the use of counter flow multistage water systems during scalding and disinfecting the carcasses might reduce the poultry contamination with campylobacters [22]. The changes in bacterial viability during processing of poultry carcasses are due to different stresses including nutrient deprivation and changes in temperature, oxygen tension and the osmolarity [37]. Insufficient cleaning and disinfection will support the survival of Campylobacter, up to 1 week, which is most often found in humid and wet places in the slaughterhouse [26]. This survival in the environment through washing and disinfection may be due to Campylobacter remaining in biofilm layers [43].

Since chicken meat is a perishable food it must be stored either refrigerated or frozen throughout the food chain in order to avoid and reduce microbial contamination and growth. Previous studies showed that Campylobacter can survive during refrigerated (4°C) and frozen (-20°C) storage on various chicken meat [30, 44]. The results of this study showed that cooled and frozen chicken meat samples from the slaughterhouse were positive for Campylobacter and the numbers of Campylobacter were decreased to 7.4 and 7.8 log_{10} compared to the numbers of Campylobacter after evisceration (8.4 CFUg^{-1}). On the other hand,
El-Shibiny et al. [30] and Sampers et al. [45] stated that 0.9 to 3.2 log10 reductions were observed in viable Campylobacter counts on chicken skin after storage at refrigeration and frozen temperature. Contamination of carcasses at the slaughterhouse may be due to the leakage of the gastrointestinal tract after its rupture which has high numbers of Campylobacter, with levels up to 10⁶ CFU/g of intestinal content. Consequently, cross-contamination during processing at the slaughterhouse is considered the main risk factors for the presence of Campylobacter spp. on poultry carcasses [46, 47]. Seeing that Campylobacter is able to grow between 30 and 46°C and require micro aerobic conditions, it is unlikely that numbers of the organism will increase on contaminated carcasses during storage. However, Campylobacter might persist in refrigerated and frozen stored chicken meat long enough to recover then pose a health risk to humans [30].

In conclusion, reducing contamination on the farm and monitoring Campylobacter contamination at different stages of chicken production are more likely to be effective than chilling or freezing.

ACKNOWLEDGMENT

This work was supported by Food and Dairy Sciences and Technology department, Faculty of Environmental Agricultural Sciences, Suez Canal University, El-Arish, North Sinai. The authors would like to thank all chicken producers and slaughterhouses owners for their helping to achieve this work.

REFERENCES


