International Food Research Journal 22(1): 41-45 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Occurrence of *Campylobacter* in chicken wings marketed in the northwest of Iran

Hosseinzadeh, S., Mardani, K., *Aliakbarlu, J. and Ghorbanzadehghan, M.

Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran

Article history

Received: 31 August 2014
Received in revised form: 2 July 2014
Accepted: 12 July 2014

Keywords

Campylobacter spp
Chicken
PCR

Abstract

Campylobacter spp. are significant causative agents of human diarrheal diseases. The common source of *Campylobacter* infection is contaminated food, particularly poultry meat. In the present study *Campylobacter* contamination was assessed in chicken's wings samples obtained from Urmia chicken retail shops. A total number of 96 chicken wings were examined for the presence of *Campylobacter* spp. using *Campylobacter* selective agar. The wings contaminated with *Campylobacter* spp. were further examined by polymerase chain reaction (PCR) for the confirmation of *Campylobacter*. Two primers were used for amplification of a 1004 bp fragment of 16s rRNA gene from *Campylobacter* spp. The results revealed that a number of 40 wings (41.66%) out of 96 chicken wings were contaminated with *Campylobacter* spp. using the *Campylobacter* selective agar. A number of 37 positive samples of detected *Campylobacter* spp. in biochemical assays were yielded a 1004 bp PCR product, confirming that at least 37 bacterial isolates were belonged to *Campylobacter* spp. It was concluded that the *Campylobacter* spp. was existed in chicken wings with high frequency. Further studies are needed for discriminating different strains of isolated *Campylobacter* spp. It was recommended that appropriate cooking of chicken meat will be an important approach for the control of Campylobacteriosis in human.

Introduction

Campylobacter spp. are gram-negative, slender, non-spore-forming microaerophilic bacteria, presently considered to be the leading bacterial etiological agent of acute gastroenteritis in the human population (Mead *et al*., 1999). Most *Campylobacter* spp. reside naturally in the intestine of birds and other warm-blooded animals, including seagulls and several other wild birds (Adams and Moss, 2000). A number of these bacteria are commensals, but many, particularly *Campylobacter jejuni* and its close relative, are enteric pathogens of humans and domestic animals. *C.jejuni* and *C. coli* are common cause of human acute bacterial enteritis worldwide (Notermans,1994).

C. jejuni is also responsible for other extra intestinal forms (meningitis, peritonitis, pancreatitis, urinary infection, neonatal sepsis, miscarriage) and some chronic immune-mediated disease (endocarditis, nodal fever, reactive arthritis) (Prencipe *et al.*, 2007). Human Campylobacteriosis is mainly food-borne and several case-control studies in different countries reported that undercooked or contaminated poultry meat and poultry products are the most important vehicles for human infection (Bryan and Doyle, 1995). During transport to the slaughterhouse (Berrang *et al*., 2004) and during processing cross-contamination of birds and carcasses may be caused by spillage of intestinal contents leading to surface contamination with *Campylobacter* (Berndtson *et al*., 1992). Other important sources for cross-contamination during slaughtering and processing are steps like defeathering, evisceration and cooling of carcasses (Rosenquist *et al*., 2006). Even contact to environmental surface, equipment and workers hands contributes to contamination with *Campylobacter* spp.(Bryan and Doyle, 1995). The formation of contaminated aerosols during the defeathering phase is the source of contamination of not only the carcasses (Hinton *et al*., 1996) but increase the risk of slaughterhouse workers contraction infection (European Food Safety Authority, 2005).

There have been many studies regarding isolation of *Campylobacter* spp. from food samples, especially in poultry meat. The reported incidence of *Campylobacter* spp. on poultry carcasses has ranged from less than 2% (Stern *et al*., 1984) to as high as 82% (Nannapaneni *et al*., 2005). Handling and consumption of poultry and poultry-related products account for up to 75% of all *Campylobacter* spp. infections (Griffiths and Park, 1990). In the USA, retail chickens have estimated contamination rates of 60–80% with counts averaging 10^6 cfu/g

*Corresponding author.
Email: *j.aliakbarlu@urmia.ac.ir*
Tel: +984412972620; Fax: +984412771926

for fresh chickens and 10^4 cfu/g for frozen chicken carcasses (Lam *et al.*, 1992; Altekruse *et al*., 1998). The aim of this study was to investigate the presence of *Campylobacter* spp. in broiler chicken wings (Khoshpokht and Morghe Khaneghi) available for the consumers at retail markets in Urmia, Iran.

Material and Methods

Preparation of specimens

A number of 24 pack of chicken wings (Khoshpokht and Morghe Khaneghi) presented in the retail stores of Urmia city were chosen randomly during 6 months from Jan to Jun 2011.The samples were put on a bag containing ice and transferred to laboratory of Food Hygiene and Quality Control of the Faculty of Veterinary Medicine, Urmia University.

Isolation and identification of Campylobacter *spp.*

Campylobacter specific medium (Quelab, Canada) was used for isolating *Campylobacter* spp. Following preparation of the medium, it was sterilized for 30 min in autoclave at 120°C. Then the temperature was lowered to 45°C. The *Campylobacter* skirrow supplement (Quelab, Canada) containing vancomycin, trimethoprim and polymyxin was added to the medium in concentration of 1%. Four wings were selected from each pack (two from sides, one from top and one from bottom of each pack) and a swab was taken from medial aspect of each wing. The swab was cultured on the medium and the cultured plates along with type C Gaspack (Merck, Germany) and 6 ml distilled water were incubated anaerobically for 24–48 h at 42°C. Gram negative colonies and curved bacteria underwent further biochemical tests including catalase, oxidase and motility for precise identification of *Campylobacter* spp. To purify the colonies, they were subcultured 2-3 times on the specific medium. When all microscopic fields contained gram negative and curved bacteria in gram staining, the purification was confirmed (Rodrigo *et al*., 2005). For definite identification of the bacteria, PCR technique was employed using a segment with 1004 pair of base from 16S gene in rRNA.

Genomic DNA extraction

Genomic DNA extraction from isolated *Campylobacter* spp. was performed using boiling method described by Liu (2008). In brief, a single colony of isolated *Campylobacter* spp. was grown in 5 ml broth and amount of 1.5 ml of grown bacteria in broth was centrifuged at 10000 rpm for 3 minutes. An amount of 100 µl distilled water was added to the bacterial pellet and the bacterial pellet was resuspended in distilled water. Resuspended bacterial pellet was boiled in 100° C for 5 min and centrifuged at 10000 rpm for 5 min. An amount of 5 µl of the supernatant containing released DNA of the bacterial cells was used in PCR reaction.

Amplification of 16s rRNA gene

For amplification of the 16s rRNA gene of isolated *Campylobacter* spp., two primers targeting 1004 bp comprising a part of 16s rRNA gene were used. Forward primer (5' AAT ACA TGC AAG TCG AAC GA 3') and reverse primer (5' TTA ACC CAA CAT CTC ACG AC3') which described by Marshall *et al*. (1999) were used. The PCR reaction was carried out in 25 µl mixtures containing 50 mM each of dATP, dTTP, dGTP and dCTP, 0.5 mM each primers 2.5 µl of 10 X PCR buffer (Cinnagen, Iran), 2 mM magnesium chloride, 2.5 U Taq DNA polymerase (Cinnagen) and 50-100 ng extracted DNA as template. The cycling condition for amplification was performed using an initial incubation at 95°Cfor 2 min followed by 30 cycles of amplification, each consisting of 94°C for 30 s, 52°C for 30 s, and 72°C for 90 s. A final primer extension at 72°C for10 min was included (Marshall *et al*. 1999). The resultant PCR products were separated in a 1.5% agarose gel and the gel photographed using ultraviolet transillumination.

Results and Discussion

Isolation of Campylobacter *spp.*

Campylobacter spp. was isolated from 12 out of 24 chicken wing packs using specific culture medium and identified using biochemical tests. In eight wing packs all of selected wings were contaminated with *Campylobacter*. In the rest of packs, one to three of the selected wings were contaminated with *Campylobacter.* Overall, 40 (41.66%) out of 96 examined wings were contaminated with *Campylobacter* and 56 (58.34%) wings were free of *Campylobacter* contamination. The highest rate of contamination was observed in January and the lowest rate was observed in April (Figure 1).

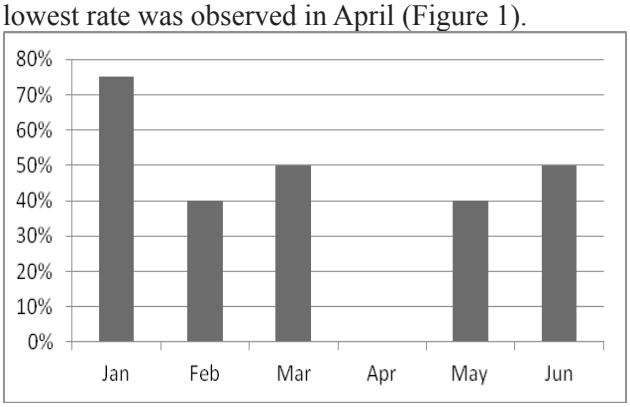

Figure 1. The percentage of *Campylobacter* spp. contamination of chicken wings during Jan-Jun 2011

Amplification of 16s rRNA gene

To confirm the identification of isolated *Campylobacter* spp., a primer pair was used for amplification of a 1004 bp fragment 16s rRNA. All 40 *Campylobacter* spp. isolates examined using PCR reaction and a number of 37 (92.5%) isolates yielded the expected PCR product (Figure 2). Prevalence of *Campylobacter* spp. in raw chicken on retail sale has been reported in several studies (Hong *et al*., 2007; Meldrum and Wilson, 2007; Madden *et al*., 2011) which can be a public health risk. In the present study, the contamination of chicken wings on retail sale was investigated. From January to June 2011, a total number of 96 wings were examined and 40 (41.66%) wings were confirmed to be contaminated with *Campylobacter* spp. In a study the contamination rate of *C. jejuni* and *C. coli* in packaged and unpackaged chickens were 41.8 and 54.1%, respectively (Soltan Dallal *et al*. 2009). In a survey on chicks performed in Switzerland the contamination rate of packaged and unpackaged chickens with *C. jejuni* and *C. coli* were 21.4% and 23.4%, respectively (Ledergerber *et al.*, 2003). In South Africa using culture method, 32.36% of the chicken carcasses were found to be contaminated with *Campylobacter* spp.(Van Nierop *et al.*, 2005). These results show that contamination with *campylobacter* in chickens is high in different countries.

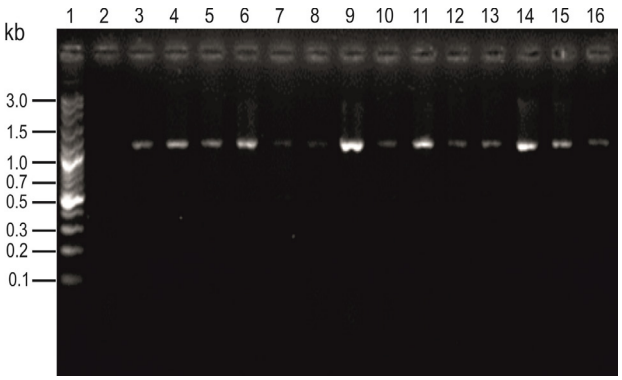

Figure 2. Amplified PCR products of 16s rRNA of *Campylobacter* spp. isolated from chicken wings. Lane 1: 100bp molecular marker (Cinnagen, Iran). Lane 2: Negative control, Lanes 3-16: Amplified PCR products from 16S rRNA gene

Chicken wings were selected for *Campylobacter* contamination examining in this study because *campylobacter* contamination occurs with high frequency in chicken wings and high *Campylobacter* load in chicken wings could increase the probability of pathogen transfer to other surfaces through cross-contamination and inappropriate handling during meal preparation and cooking (Nauta *et al.*, 2007). During laboratory assays, it was notable that traces of feathers or feather shafts were commonly still connected to wing samples. *Campylobacter* originally associated with feathers might be transferred to the skin through the action of the picker's rubber fingers during mechanical feather removal in the slaughterhouse (Buhr *et al.*, 2003). Feathers can be contaminated with feces during transport, and *Campylobacter* originally associated with feathers can be transferred to the skin during the plucking process (Berrang *et al*., 2000). In addition, the high *Campylobacter* count in chicken wings might be attributed to imperfect scalding, post scalding contamination, or due to the combination of both (Cason *et al.*, 2004).

It is generally believed that this *Campylobacter* spp. is not spread through hatcheries; however, chickens are contaminated with feces. Cecum is the main part of formation of colonies and naturally it is not pathogenic in adult birds. When this bacterium is spread in the farm, over the time most of the flock is contaminated. Researchers have concluded that once it has been detected in a chicken it will spread throughout the flock during a week (Smitherman *et al*., 1984). In a study, 7 out of 90 people with acute diarrhea were contaminated with *Campylobacter jejuni* (7.8 %) (Rahimi *et al.*, 2009). In other study, 40 out of 400 children (8%) with diarrhea were contaminated with *Campylobacter jejuni* (Feizabadi *et al*. 2007) which was consistent with Rahimi's work. They conclude that *Campylobacter* is the main cause of diarrheas in Iranian children. While the *Campylobacter* is a thermophile bacterium, the prevalence of Campylobacteriosis in poultry farms is higher in winter than spring. Soltan Dallal *et al*. (2009) concluded that the turning on the heating system of farms due to cold seasons, fail to follow biosecurity programs and delay in exclusion of wastes are the reasons for increasing the cases of Campylobacteriosis in winter compared to spring.

Conclusion

Our study suggests that an improvement of control measures at farm and retail level is necessary to reduce the risk of infection with *Campylobacter* spp. for consumers. Further, public education of consumers on proper handling of poultry products and cooking may help to minimize the risk of infection with *Campylobacter* spp. Further studies are needed to differentiate isolated Campylobacter spp. from poultry farms.

Acknowledgments

Authors would like to thank the dean of research

of Urmia University for funding this project.

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