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# Prevalence and risk factors associated with campylobacter infections in broiler flocks in Shiraz, southern Iran

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ABSTRACT

*Campylobacter* species are among the most common bacterial causes of human gastroenteritis in many countries, and poultry meat is considered as a major source of human campylobacteriosis. The present study was conducted to determine the prevalence of infection by *Campylobacter jejuni* and *Campylobacter coli* in broiler flocks in Shiraz and to investigate the possible risk factors for the campylobacter infections in this area. For detection of campylobacter, multiplex polymerase chain reaction (mPCR) was used. Between August and September 2009, a total of 100 broiler flocks from 100 commercial broiler farms were selected at slaughter and campylobacter status was determined by mPCR on caecal samples. Data about farms and flocks were collected by questionnaires. Approximately 76% (95% CI: 67–84%) of the flocks were positive for *C. jejuni* or *C. coli*. Twenty two percent were positive for *C. jejuni*, 32% for *C. coli* and 22% for both species. Results of the statistical analysis using multivariable logistic regression showed that the odds of flock infection decreased when level of owner's education (years) increased (OR = 0.86, P = 0.04), also odds of infection was nearly five times higher when age at slaughter was ≥45 days compared with <45 days (OR = 5.3, P = 0.003) and use of antibiotic medications at early stage of production period was negatively associated with the infection status of the flock (OR = 0.33, OR = 0.059). We found no evidence of the effects of any other factors such as time interval between successive flocks, hygiene measures and number of broiler houses on the farm on the prevalence of campylobacter infection. Getting more attention to the health education issues and planning qualitative studies to reveal the behavioral aspects of the management policy, may be subjects of interest for future researches.



## 1. Introduction

*Campylobacter* species are found in the intestinal tracts of all types of domestic animals, usually without any harmful effects. However, they can cause severe disease in infected people and are recognized as one of the major causes of food-borne gastroenteritis in humans in many countries around the world (Humphrey et al., 2007). Human campylobacter illnesses are caused primarily by *Campylobacter jejuni* (~90%) and with a lesser extent (~10%) by *Campylobacter coli* (Lin, 2009). Classic symptoms of the campylobacter infection particularly *C. jejuni* and *C. coli* include diarrhea which sometimes is bloody, abdominal pain, and fever. Severe long-term complications such as reactive arthritis and peripheral neuropathies Guillain-Barré and Miller Fisher syndrome occur in a small percentage of the cases. Infection with *C. jejuni* is the most predisposing factor to neurological sequelae (McCarthy and Giesecke, 2001; Zia et al., 2003).

Consumption of poultry has been identified as a major source of campylobacter infection in human, although other types of foods also implicated for transmission of the infection (Humphrey et al., 2007). The reported prevalence of campylobacter in broiler flocks varies, ranging from 2.9% to 100% of flocks (Humphrey et al., 2007). After infection, chickens rapidly exhibit high levels of campylobacter in the caecal contents (Shanker et al., 1990). Once an infection becomes established in a poultry house, the bacteria spread rapidly through the flock, and most of the birds become colonized and remain so, until slaughter (Gregory et al., 1997; Evans and Sayers, 2000). The *Campylobacter* species on the carcasses originate mainly from the gastrointestinal tracts of live birds, as shown by studies in abattoirs (Ono and Yamamoto, 1999; Pearson et al, 2000). Efforts within the slaughterhouse to improve hygiene to reduce the campylobacter contamination have a limited effect and are likely to have little impact on risk to consumers (Mead et al., 1995). Although freezing can dramatically reduce the occurrence of campylobacter on carcasses, most consumers prefer fresh poultry products. Therefore, total prevention of campylobacter colonization of broilers at the farm level is the best way to prevent contamination of poultry products (Rivoal et al., 2005).

Many epidemiological studies have been carried out to determine the possible sources of campylobacter and related risk factors at the flock and farm level, but no definitive factors have been identified.

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Various factors including flock age (Evans and Sayers, 2000; Bouwknegt et al., 2004), presence of rodents (Kapperud et al., 1993), wild birds (Craven et al., 2000) and number of houses on the farm (Refregier-Petton et al., 2001; Bouwknegt et al., 2004) have been associated with the infection. It seems that the risk factors for campylobacter infection are likely to be different depending on local industry and farming practices as well as for wider geographically and climatic reasons (McDowell et al., 2008).

Several studies are available about campylobacter infection in Iran. In a recent study in children less than five years old with acute diarrhea in Tehran, campylobacter was found in 5.5% of patients, equal to 10.8% of all isolated bacteria (Jafari et al., 2009). In another study, campylobacter was isolated from a 36.5% of chicken and beef meat samples from retail outlets in Tehran (Taremi et al., 2006). Very recent investigation was carried out to evaluate the contamination of turkey carcasses to campylobacter species in Isfahan, Iran. Out of 216 campylobacter isolates from 348 samples, 175 (81.0%) were identified as *C. jejuni* and 41 (19.0%) *C. coli* (Rahimi et al., 2010). One study on 114 samples from patients with acute diarrhea aged 2–58 years showed that *C. jejuni* is a significant cause of gastroenteritis in Shiraz, Iran (Hassanzadeh and Motamedifar, 2007).

There is no report concerning the prevalence and risk factors for campylobacter infection in broiler farms in Iran. The present study was conducted to determine the prevalence of infection by *C. jejuni* and *C. coli* in broiler flocks in Shiraz, southern Iran, also to investigate the possible risk factors for the campylobacter infection in this area. For detection of both species of the bacteria, multiplex polymerase chain reaction on caecal samples (mPCR) was used.

## 2. Materials and methods

### 2.1. Study population

The current investigation was a cross-sectional study conducted in Shiraz, southern Iran. In this region like other parts of the country, commercial broilers flocks are from industrial breeds like Ross, Cobb and Arbor Acres. Feed starter, grower and finisher diets are primarily based on corn and soybeans, which contains vitamins and minerals, and in some instances growth promotants, antibiotics, and anticoccidials. The production system is based on all-in-all-out system in confined houses. Farm size is very variable ranging from farms with only one house to larger farms with five or more houses. The capacity of each house usually varied from 5000 to 15,000 birds. It is noteworthy that in the recent years, there is a great tendency for expanding the poultry industry and replacing the small farms with larger ones all over the country.

The target population was the total number of commercial broiler flocks in Shiraz, slaughtered during August to September 2009. The study population consisted of all broiler flocks slaughtered in four largest slaughterhouses in the region during the study period. The slaughterhouses were visited six consecutive days in each week and at each visit, 2–5 flocks were selected whenever possible based on practical consideration. A flock was defined as a group of birds at the end of rearing period, raised in the same broiler house in a farm. From each flock, caecal samples from 21 birds were collected after completion of the evisceration stage. Only one flock per farm was allowed for selection in the study. Therefore, a total of 100 broiler flocks from 100 commercial broiler farms were selected.

### 2.2. Data collection

For the collection of data, a structured questionnaire related to general information of the farm, biosecurity, hygiene and management practices as well as general information of the selected flock was prepared and completed by telephone interview with the farm owners. All owners participated in the study. Most of the questions were compiled from the related literature, and directed to evaluate the possible risk factors associated with increased prevalence and were important in the transmission of campylobacter. A brief description of the questions is provided in Table 1.

### 2.3. Extraction of DNA and PCR assay

Caecal samples were collected immediately after evisceration and brought cooled to the laboratory in less than 8 h. Caecal contents of each seven birds were pooled in TSB broth, so that for each flock three pooled sample were provided. For eliminating the other bacteria, 0.8 μm membrane filter was used. Thereafter, 250 μl of filtered samples was cultured in an enriched broth media [TSB (30 g/L), dextrose (2.5 g/L), sodium thioglycolate (0.5 g/L), Rifampicin (10 mg/L), Trimethoprim (10 mg/L), Vancomycin (10 mg/L), Ceftriaxone (10 mg/L), Amphotracin-B (10 mg/L)], incubated in a microaerophilic atmosphere (Anaerocult C, Merck) and at 37 °C for 4 hour, followed by incubation at 42 °C for 44 h.

For detection of campylobacter, 1.5 ml of the culture media was taken and stored at −20 °C up to the time of investigation, DNA extraction was carried out using the phenol–chloroform techniques. Briefly, the samples were centrifuged at 10,000×*g*, the supernatants were discarded before adding 250 μl of buffer 1 (resuspension solution contained 100 μg/ml RNase) and 250 μl buffer 2 (Lysis buffer), a 550 μl saturated phenol was then added, mixed thoroughly and centrifuged at 8000×*g*. The supernatant was collected into a new Eppendorf; the same volume of the phenol was then added, centrifuged at the same speed. The clear phase was collected into a new tube, before adding sodium acetate (2 M, pH 5.2, 0.1× volume of each aliquot). The aliquot was mixed with 1.5 ml 100 % ethanol, kept at −20 °C for 1 h, centrifuged at 12,000×*g*, the supernatant was then

**Table 1**
Description of data collected by questionnaire in a study of risk factors for campylobacter infection in 100 broiler flocks from Shiraz, southern Iran based on PCR on caecal samples (2009).

| Variables | Examples |
|---|---|
| Owner information | Age, education |
| Farm description | Age, number of houses, distance between houses, bird capacity, surface area, proximity to other animal rearing units, presence of other livestock on the farm |
| Flock information | Number of birds, age and weight at slaughter, age and commercial breed of parent flock, time between slaughter of previous flock and repopulation, use of antibiotics, clinical disease, total mortality |
| Farm staff | Number of workers, family workers, working in other animal rearing units |
| Ventilation | Type, size and number of ventilation |
| Water supply | Source of water, disinfection procedures, type of drinker |
| Feed supply | Type of feeder system, use of animal source feed, use of disinfectant in feed, use of probiotics |
| Litter | Type of litter, litter quality, litter treatment |
| Biosecurity and hygiene practice | Use of foot dip, type of product and frequency of replenishment, presence of fence around the farm, presence of rodents and wild birds, house cleaning method, type of disinfectants used, fumigation, manure disposal site, |